Development and characterization of segmented polyurethanes based on L-amino acid based chain extenders

A Thesis submitted for the degree of Doctor of Philosophy

By

Swati Sharma
(M.Sc. Biochemistry)

Faculty of Life and Social Sciences
Swinburne University of Technology
Melbourne

Australia

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Abstract

The acid catalysed Fischer and alkali metal salt based esterification reactions were used to synthesise a series of diamine ester and dihydroxy ester compounds respectively from various naturally occurring L-amino acids. L-leucine, L-isoleucine, L-phenylalanine, L-tyrosine amino acid were used to synthesise diamine diester compounds whilst amine group protected L-Z-serine, L-Z-threonine and L-Z-tyrosine amino acids were used to synthesise novel dihydroxy diester compounds. For the dihydroxy ester compounds, in particular, optimisation of the reaction temperature was done. The yield for both diamine and dihydroxy ester synthesis were ~ 80 % and 85% respectively. Few of the synthesised ester compounds were yellow colour oil and few of them were white solid in appearance. The synthesised compounds were fully characterised by nuclear magnetic resonance, infrared resonance spectroscopy and mass spectroscopy. The success of the reaction and the purity of the synthesised compounds were confirmed by these techniques. The intended purpose of the synthesis of these novel amino acid based ester compounds is to use them as dihydroxy and diamine chain extender for polyurethane and polyurethane urea synthesis.

A series of polyurethane and polyurethane urea based on amino acid based chain extender (mentioned above) was synthesised. Polycaprolactone (Mw1000) act as polyol and 4,4-methylenediphenyl diisocyanate act as diisocyanate component for polyurethane synthesis. The control polyurethane synthesised was based on 1,4-butanediol as chain extender with polycaprolactone as polyol and 4,4-methylenediphenyl diisocyanate as diisocyanate component. Synthesised polymers were fully characterised for structural, thermal, surface and mechanical properties. Obtained properties of the amino acid based polyurethane/polyurethane urea were compared with the control polyurethane to evaluate the effect of chain extender type and its structure onto the physiochemical properties of the synthesised polymer. Synthesised amino acid based polyurethane/polyurethane urea showed moderately high molecular weight with narrow polydispersity. The percent yield of the polyurethane/polyurethane urea synthesis was ~ 70%. The amino acid based polymers ranged from completely amorphous to semicrystalline polymer. The hard segment was amorphous in all cases of the amino acid based polyurethane/polyurethane urea. Phase mixed morphology was shown by amino acid based
polyurethane/polyurethane urea as compared to phase segregated morphology observed for control polyurethane. Mechanical properties were tested by obtaining stress strain curve. The amino acid based polymers were weak elastomeric material with low tensile strength and high extensibility as compared to control polyurethane. Non-linear structure of chain extenders and weak hydrogen bonding might be one of the main reasons for low mechanical properties. The amino acid based polyurethane/polyurethane urea were stable up to high temperature. The polymer surface hydrophobicity was increased with the incorporation of amino acid based chain extender.

The benzyloxy carbonyl protecting group of serine amino acid based polyurethane was removed by the use of Hydrogen bromide/Acetic acid solution method. Reaction time was optimised for the deprotection reaction. Nuclear magnetic resonance spectroscopic analysis indicated that deprotection resulted in 62 mol % reduction in benzyloxy carbonyl group content with minimal affecting polyurethane backbone.

Structure property relationship of the polymer was studied by using high molecular weight (Mw 2,000) of polycaprolactone as soft segment. A series of polyurethane and polyurethane urea were synthesised and characterised with polycaprolactone (Mw 2,000 Dalton) as soft segment and obtained properties were compared to polyurethane/polyurethane urea series made with polycaprolactone (Mw 1,000 Dalton). It was observed that as soft segment molecular weight increases, phase segregation, percent crystallinity increases and glass transition of soft segment decreases. Increase in phase segregation morphology has also helped to obtain improved mechanical properties of the amino acid based polyurethane/polyurethane urea. Surface hydrophobicity increases as soft segment molecular weight increases. However, thermal stability was decreased due to phase segregated morphology.

At the end, the in vitro cytotoxicity response of the amino acid based polyurethane/polyurethane urea was evaluated by LIVE/DEAD assay kit. Mouse fibroblast cells were seeded on to the polyurethane/polyurethane urea films. The polyurethane/polyurethane urea did not show any cytotoxic response and healthy cell growth, attachment and proliferation was observed on polymer films which indicates that these polyurethane/polyurethane urea may be useful for biomedical applications.
Acknowledgements

On this great occasion of my thesis submission, first of all I am grateful to the almighty God without whose blessings I would not have been able to complete this challenging journey.

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Last but not the least I would like to thank my husband Rahul Bhardwaj for showing great faith in me. Thanks for being such a wonderful husband and always there to make me feel stronger mentally and providing all love and support to me in fulfilling my motherhood duties during thesis writing.
Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma, in any University, college or any other educational institute. To the best of my knowledge, this thesis contains no material previously published or written by another person except where due reference is made in the text of the thesis.

Signature of Candidate: ..............................................................................................

Date: .............................................................................................................

iv
List of Conference Presentations

Following is a list of conference presentations from the work contained in this thesis.

Conference Presentations


- Sharma S., Harding I., Mayadunne R. M., (February, 2010). Novel complex amino acid based diols as chain extenders in polyurethane synthesis. 20th Annual society for biomaterials and tissue engineering (ASBTE) Queensland, Brisbane, Australia.

- Sharma S., Harding I., Mayadunne R. M., (October, 2010). Novel complex amino acid based diols as chain extenders in polyurethane synthesis. Symposium on biomaterials, Rutgers University, New Jersey, USA.

# Table of Contents

Abstract .......................................................................................................................................... i
Acknowledgements ...................................................................................................................... iii
Declaration ................................................................................................................................... iv
List of Conference Presentations .................................................................................................. v
Table of Contents ......................................................................................................................... vi
Index of Figures .......................................................................................................................... xii
Index of Schemes ........................................................................................................................ xv
Index of Tables .......................................................................................................................... xvi
Abbreviations ........................................................................................................................... xviii

Chapter 1: Introduction ................................................................................................................ 1
1.1 Introduction ............................................................................................................................. 2
1.2 Research Hypothesis ............................................................................................................... 3
1.3 Layout of thesis ....................................................................................................................... 5

Chapter 2: Literature Review ........................................................................................................ 6
2.1 Introduction ............................................................................................................................. 7
  2.1.1 Structure of polyurethane ............................................................................................. 7
  2.1.2 Advantage of using polyurethane ................................................................................ 9
  2.1.3 Role of polyurethane .................................................................................................... 9
2.2 Polyurethane history and development ............................................................................... 10
2.3 Components of polyurethane ............................................................................................. 12
  2.3.1 Diisocyanate ............................................................................................................... 13
  2.3.2 Types of diisocyanate .................................................................................................. 13
  2.3.3 Polyols ........................................................................................................................ 15
  2.3.4 Chain extender ........................................................................................................... 16
  2.3.5 Primary reaction of isocyanate group ........................................................................... 19
    2.3.5.1. Reaction with polyol/hydroxyl group ................................................................. 20
    2.3.5.2. Reaction with amines ...................................................................................... 20
    2.3.5.3. Reaction with water ......................................................................................... 20
    2.3.5.4. Secondary reaction of isocyanate group ........................................................ 21
2.4 Synthesis of segmented polyurethane ............................................................................... 21
2.5 Morphology in polyurethanes ............................................................................................. 23
2.6 Structure-property relationship of polyurethane ................................................................. 26
  2.6.1 Soft segment ............................................................................................................. 26
3.5.2 Synthesis of L-Z-amino acids based dihydroxy ester compounds

3.5.2.1 Reaction with caesium salt

3.5.2.2 Octane-1, 8-diyl bis (2-amino-4-methylpentanoate) – 318

3.5.2.3 Optimisation of reaction conditions

3.5.2.4 Characterisation

3.5.2.4.1 Butane-1,4-diylbis(2-(benzyloxycarbonylamino)-3hydroxypropanoate)-318

3.5.2.4.1.1 $^1$H-Nuclear Magnetic Resonance spectroscopy

3.5.2.4.1.2 Fourier Transform Infrared spectroscopy

3.5.2.4.1.3 Mass Spectroscopy

3.5.2.4.2 Butane-1, 4-diylbis (2- (benzyloxycarbonylamino) 3(4hydroxyphenyl)

3.5.2.4.2.1 $^1$H-Nuclear Magnetic Resonance Spectroscopy

3.5.2.4.2.2 Fourier Transform Infrared Spectroscopy

3.5.2.4.2.3 Mass Spectroscopy

3.6 Conclusion

4.1 Introduction

4.2 Materials

4.3 Experimental Procedures

4.3.1 Synthesis of polyurethane

4.3.2 Synthesis of control polyurethane

4.3.3 Deprotection of Z-group

4.3.4 Synthesis of polyurethane urea

4.4 Characterization of polymers

4.4.1 Gel permeation chromatography

4.4.2 Solubility

4.4.3 Nuclear magnetic resonance spectroscopy

4.4.4 Fourier transform infrared spectroscopy

4.4.5 Thermal Characterization

4.4.6 Water contact angle measurement

4.4.7 Compression moulding

4.4.8 Mechanical properties

4.5 Results and discussion

4.5.1 Polyurethane synthesis reaction

4.5.2 Characterization of polyurethane

4.5.2.1 Gel Permeation Chromatography
Index of Figures

Figure 1: Chemical structure of urethane within a polymer ............................................ 7
Figure 2: Resonance structure of isocyanate group, R can be aliphatic or aromatic group ....................................................................................................................... 13
Figure 3: Chemical structure of common diisocyanate employed in polyurethane synthesis .................................................................................................................. 14
Figure 4: Chemical structure of common polyols employed in polyurethane synthesis 16
Figure 5: Chemical structure of common chain extenders employed in polyurethane synthesis.................................................................................................................. 17
Figure 6: Primary and secondary reactions of isocyanate group ................................... 19
Figure 7: One step and two step (prepolymer) method of polymerization .................... 21
Figure 8: Role of chain extender in polymer synthesis .................................................. 44
Figure 9: Reaction of hydroxyl and amine group with the diisocyanate group .......... 45
Figure 10: Chemical structure of L-amino acids used in the current research work .... 48
Figure 11: Structures of synthesised L-amino acids based diamine ester compounds .. 54
Figure 12: General reaction mechanism of Fischer esterification reaction .......... 55
Figure 13: 1H-NMR spectrum of 310 (spectrum C), 311 (spectrum B) is shown in comparison with compound 309 (spectrum A) ....................................................... 58
Figure 14: Zwitter ion form of L-leucine amino acid (301) ................................. 60
Figure 15: FTIR spectrum of 301 (spectrum A) and 311 (spectrum B) .............. 60
Figure 16: 1H-NMR spectrum of compound 314 ....................................................... 65
Figure 17: 1H-NMR spectrum of model compound 314-M ................................. 67
Figure 18: 2D-COSY- NMR of compound 314 ....................................................... 70
Figure 19: FTIR spectrum of compound 314 ....................................................... 71
Figure 20: Chemical structures of L-Z-amino acids based dihydroxy ester compounds ................................................................. 76
Figure 21: 1H-NMR spectrum of compound 318 (spectrum C) and compared to 1H-NMR spectrum of starting material 306 (spectrum B) and 317(spectrum A) ....... 80
Figure 22: FTIR spectrum of compound 318.......................................................... 82
Figure 23: 1H-NMR spectrum of compound 320 ....................................................... 83
Figure 24: FTIR spectrum of compound 320 ....................................................... 85
Figure 25: Structure of polycaprolactone (PCL), 4,4- methylenediphenyl diisocyanate (MDI) and 1,4-Butanediol (BDO) ................................................................. 91
Figure 26: Chemical structure of Series 1 PU .............................................................. 104
Figure 27: GPC traces of Series 1 PU ......................................................................... 106
Figure 28: 1H- NMR spectra of PCL-1-Z-Ser-PU ....................................................... 108
Figure 29: FTIR spectra of PCL-1-Z-Ser-PU ................................................................. 110
Figure 30: DSC thermograms for 1st and 2nd heating run of PCL-1-Z-Ser-PU .......... 113
Figure 31: DSC thermograms for 1st and 2nd heating run of PCL-1-Z-Thr-PU .......... 114
Figure 32: DSC thermograms for 1st and 2nd heating run of PCL-1-BDO-PU ......... 117
Figure 33: TGA analysis of Series 1 PU ....................................................................... 119
Figure 34: Tensile stress – strain curve of Series 1 PU ................................................ 122
Figure 35: Tensile stress- strain curve of PCL-1-BDO-PU ........................................... 123
Figure 36: 1H-NMR spectra of the reaction mixture during deprotection of Z-group from polyurethane: Spectra A for protected polymer, spectra B for deprotected polymer after 10 minutes of reaction, Spectra C for deprotected polymer after 30 minutes of reaction .......................................................................................... 130
Figure 37: GPC traces of protected and deprotected PCL-1-Z-Ser-PU ...................... 132
Figure 38: FTIR absorption spectra of (A) Protected PCL-1-Z-Ser-PU (B) Deprotected PCL-1-Ser-PU ........................................... 134
Figure 39: The chemical structure of PCL-1-Leu-PUU showing urea and urethane linkages ........................................................................................................... 135
Figure 40: Chemical structure of Series 1 PUU ........................................................... 137
Figure 41: GPC traces of Series 1 PUU ........................................................................ 138
Figure 42: 1H-NMR spectra (Spectrum A) PCL-1-Leu-PUU, (Spectrum B) MDI and (Spectrum C) PCL ...................................................................................................... 140
Figure 43: FTIR spectra of PCL-1-Leu-PUU ................................................................. 142
Figure 44: DSC thermograms for 1st and 2nd heating run of PCL-1-Leu-PUU .......... 144
Figure 45: DSC thermograms for 1st and 2nd heating run of PCL-1-Tyr-PUU ......... 146
Figure 46: TGA analysis of Series 1 PUU ................................................................. 148
Figure 47: The Stress strain curves for Series 1 PUU .................................................. 151
Figure 48: DSC thermograms for the 1st and 2nd heating run of PCL-2-Z-Ser-PU ... 165
Figure 49: DSC thermograms for the 1st and 2nd heating run of PCL-2-Z-Thr-PU ... 165
Figure 50: FTIR absorption spectrum of polyurethane (A) PCL-1-Z-Ser-PU (B) PCL-2-Z-Ser-PU
Figure 51: FTIR absorption spectra of carbonyl and N-H region of polyurethane (A) PCL-1-Z-Ser-PU and (B) PCL-2-Z-Ser-PU
Figure 52: Different types of phase morphology present in polyurethane structure
Figure 53: TGA analysis of Series 1 PUs and Series 2 PUs
Figure 54: Tensile stress – strain curve for PCL-2-Z-Ser-PU
Figure 55: Tensile stress – strain curve for PCL-2-Z-Thr-PU
Figure 56: DSC thermograms for the 1st and 2nd heating run of (A) PCL-2-Leu-PUU and (B) PCL-2-Ileu-PUU
Figure 57: DSC thermograms for the 1st and 2nd heating run of (C) PCL-2-Val-PUU and (D) PCL-2-Tyr-PUU
Figure 58: FTIR absorption spectra of polyurethane (A) PCL-1-Leu-PUU and (B) PCL-2-Leu-PUU
Figure 59: FTIR absorption spectra of carbonyl and N-H region of polyurethane (A) PCL-1-Leu-PUU and (B) PCL-2-Leu-PUU
Figure 60: Hydrogen bonding interactions in polyurethane urea
Figure 61: TGA analysis of Series 2 PUUs
Figure 62: TGA analysis of Series 1 PUUs
Figure 63: The Stress strain curves for Series 2 PUU
Figure 64: The Stress strain curves for PCL-2-Tyr-PUU
Figure 65: Fluorescence images of mouse fibroblast cells on Series 1 polymers surfaces (A) 1,4- Butanediol (Control), (B) & (C) Series 1 PU, (D) TCPS control, (E) and (F) Series 1 PUU (magnification x 200). Circle showing dead cells with red fluorescence
Figure 66: Fluorescence images of mouse fibroblast cells on Series 2 polymers surfaces (A) PCL-1-BDO-PU (Control), (B) & (C) Series 2 PU, (D) TCPS control, (E) and (F) Series 2 PUU (magnification x 200). Circle showing dead cells with red fluorescence
Index of Schemes

Scheme 1 : Reaction scheme for the preparation of polyurethane and polyurethane urea 8
Scheme 2 : Schematic representation of the synthesis of diamine ester compound 311 53
Scheme 3: Schematic representation of the synthesis of dihydroxy ester compound 318
........................................................................................................................................ 75
Scheme 4 : Schematic representation of the synthesis of PCL-1-Z-Ser- PU ............... 102
Scheme 5 : Deprotection reaction to remove Z group from PCL-1-Z-Ser-PU .......... 128
Index of Tables

Table 1 : Biodegradable Polyurethane Elastomers ................................................................. 10
Table 2 : Peak integration data obtained for 1H-NMR spectrum of 311 ............................... 59
Table 3 : Selected FTIR frequencies (cm⁻¹) of 301 and its ester derivative 311 .................. 62
Table 4 : Peak integration data obtained for 1H-NMR of compound 314 ........................... 66
Table 5 : Chemical shift (ppm) position of 1H-NMR spectrum of 314 and 314-M ............... 68
Table 6 : Optimisation of reaction temperature for alkali metal salt based reaction .......... 78
Table 7 : Peak integration data obtained for 1H-NMR spectrum of compound 318 .......... 81
Table 8 : Peak integration data obtained for 1H-NMR spectrum of compound 320 .......... 84
Table 9 : Abbreviations used for Series 1 PU .................................................................... 103
Table 10 : Composition of Series 1 PUs ........................................................................... 105
Table 11 : Molecular weights of Series 1 PU ................................................................. 105
Table 12 : FTIR peak assignment for PCL-1-Z-Ser-PU ..................................................... 111
Table 13 : Thermal properties of Series 1 PUs ................................................................. 112
Table 14 : Water contact angle values for Series 1 PU .................................................... 121
Table 15 : Mechanical properties of Series 1 PU (mean ± SD, n = 6) ............................... 123
Table 16 : Composition of PCL-1-Z-Ser-PU for deprotection reaction ......................... 127
Table 17 : Molecular weight of PCL-1-Z-Ser-PU before and after deprotection reaction ... 133
Table 18 : Nomenclature for Series 1 PUU .................................................................... 136
Table 19 : Composition of Series 1 PUU ........................................................................... 137
Table 20 : Molecular weight of Series 1 PUU ................................................................. 139
Table 21 : FTIR peak assignment for PCL-1-Leu-PUU ...................................................... 143
Table 22 : Thermal properties of Series 1 PUU ................................................................. 144
Table 23 : Contact angle values for Series 1 PUU ............................................................. 150
Table 24 : Mechanical properties of Series 1 PUU (mean ± SD, n = 6) ......................... 152
Table 25 : Polyurethanes abbreviations used for Series 2 PUs ........................................ 163
Table 26 : Comparison of molecular weight of Series 1 and 2 PUs ................................. 164
Table 27 : Thermal properties of Series 2 and 1 PUs ....................................................... 166
Table 28 : Assignment of the major absorption bands in the FTIR spectra of the PCL-MDI based PU (Mattia and Painter, 2007), (Yilgor and Yilgor, 2007) .................. 169
Table 29 : Contact angle values for Series 1 and 2 PU .................................................... 175
Table 30: Mechanical Properties of Series 1 and 2 PUs (mean ±SD, n=6) .................178
Table 31: Abbreviations used for PCL 2,000 based Series-2 PUUs .........................180
Table 32: Molecular weight of Series 2 and Series 1 PUU ......................................181
Table 33: Thermal properties of Series 2 PUUs and its comparison with Series 1 PUUs ......................................................................................................................................................184
Table 34: Assignment of the major absorption bands in the FTIR spectrum of the MDI- PCL based Polyurethane .........................................................................................................................189
Table 35: Characteristic temperature on TGA curves for Series 1 and 2 PUUs .............194
Table 36: Contact angle values for Series 1 and 2 PUUs (mean ± SD, n = 5) ..............196
Table 37: Mechanical properties of Series 1 and 2 PUUs (mean ± SD, n = 6) ..........198
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU</td>
<td>Polyurethane</td>
</tr>
<tr>
<td>PUU</td>
<td>Polyurethane urea</td>
</tr>
<tr>
<td>LDI</td>
<td>Lysine diisocyanate</td>
</tr>
<tr>
<td>HDI</td>
<td>Hexane diisocyanate</td>
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<td>PCL</td>
<td>Polycaprolactone</td>
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<td>PEG</td>
<td>Polyethylene glycol</td>
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<td>Methylene-diphenyl diisocyanate</td>
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<td>Butanediol</td>
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<td>Isocyanate group</td>
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<tr>
<td>BDI</td>
<td>Butane diisocyanate</td>
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<tr>
<td>HMDI</td>
<td>Hexamethylene diisocyanate</td>
</tr>
<tr>
<td>SPUU</td>
<td>Segmented polyurethane urea</td>
</tr>
<tr>
<td>PTMO</td>
<td>Polytetramethylene oxide</td>
</tr>
<tr>
<td>CL</td>
<td>Caprolactone</td>
</tr>
<tr>
<td>DBTL</td>
<td>Dibutyltin dilaurate</td>
</tr>
<tr>
<td>SS</td>
<td>Soft segment</td>
</tr>
<tr>
<td>HS</td>
<td>Hard segment</td>
</tr>
<tr>
<td>DMA</td>
<td>Dynamic mechanical analysis</td>
</tr>
<tr>
<td>SAXS</td>
<td>Small angle X-ray scattering</td>
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<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>HSC</td>
<td>Hard segment concentration</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
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<td>PEO</td>
<td>Polyethylene oxide</td>
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<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>WAXD</td>
<td>Wide angle X-ray diffraction</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
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<tr>
<td>----------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>PTMG</td>
<td>Polytetramethylene glycol</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Human umbilical vein endothelial cells</td>
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<tr>
<td>Gly-Leu</td>
<td>Glycine-Leucine</td>
</tr>
<tr>
<td>Z</td>
<td>Benzyloxy carbonyl</td>
</tr>
<tr>
<td>p-TsOH</td>
<td>para-toluene sulphonic acid</td>
</tr>
<tr>
<td>MHz</td>
<td>Mega hertz</td>
</tr>
<tr>
<td>¹H-NMR</td>
<td>Proton nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>¹³C-NMR</td>
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</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
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<td>d₆-DMSO</td>
<td>Deuterated Dimethyl sulfoxide</td>
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<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>EI</td>
<td>Electron impact</td>
</tr>
<tr>
<td>PFK</td>
<td>Perfluorokerosene</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionisation</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>UV</td>
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<tr>
<td>IUPAC</td>
<td>International union of pure and applied chemistry</td>
</tr>
<tr>
<td>Na₂CO₃</td>
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</tr>
<tr>
<td>CE</td>
<td>Chain Extender</td>
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<td>MS (ESI)</td>
<td>Electrospray ionization mass spectroscopy</td>
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<td>DMF</td>
<td>Dimethyl sulfoxide</td>
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<td>Caesium hydroxide</td>
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<td>COOH</td>
<td>Carboxylic acid</td>
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<td>NMP</td>
<td>N-Methylpyrrolidone</td>
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<tr>
<td>Mw</td>
<td>Weight average molecular weight</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Mn</td>
<td>Number average molecular weight</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation Chromatography</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>LiBr</td>
<td>Lithium Bromide</td>
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<tr>
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<td>Differential scanning calorimetry</td>
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<td>Tg</td>
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<td>Melting temperature</td>
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<tr>
<td>DCM</td>
<td>Dichloromethane</td>
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<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<tr>
<td>Xc</td>
<td>Crystallinity</td>
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<tr>
<td>MPa</td>
<td>Megapascal</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>HBr/HAc</td>
<td>Hydrogen Bromide/Acetic acid</td>
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<tr>
<td>MDA</td>
<td>Methylene dianiline</td>
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<tr>
<td>TCPS</td>
<td>Tissue culture polystyrene</td>
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<tr>
<td>EthD</td>
<td>Ethidium homodimer</td>
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<tr>
<td>AM</td>
<td>Acetoxymethyl</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>PBS</td>
<td>Phosphate Buffer Solution</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>ECS</td>
<td>Fetal calf serum</td>
</tr>
<tr>
<td>MEM</td>
<td>Minimum essential medium</td>
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</table>
Chapter 1: Introduction
1.1 Introduction

In the last few decades, there has been significant interest and demand for the development of biodegradable and biocompatible polymers for biomedical applications such as tissue engineering (Ozdil and Aydin, 2014, Guo and Ma, 2014, Sobczak, 2015), drug delivery (Wang and Wang, 2012), scaffold formation (Qizhi et al., 2013), and biomedical devices (Lamba et al. (1998), Guelcher, 2008, Krishnan et al., 2014). There are several important physiochemical properties required by the polymer to be used in such areas, including biodegradability and biocompatibility as mentioned above, but also good mechanical properties and easy polymer processing (Lanza et al., 1997, Medine et al., 2012).

The class of polymer, “polyurethane”, refers to a surprisingly wide range of polymeric materials available for use in the biomedical field. There are particular features of this material which offer substantial advantages over other available polymers (Davis and Mitchell, 2008, Shelke et al., 2014). The key advantage is the composition of the polyurethane system, which allows materials to take on a range of different material properties and degradation kinetics to suit various biomedical applications including permanent implants and temporary biodegradable scaffolds (Gunatillake and Adhikari, 2003).

Segmented polyurethanes are synthesised from various polyols, diisocyanates and chain extenders. These monomers can be structurally manipulated to achieve a wide range of bio-material properties (Martina and Hutmacher, 2007). The use of polyurethanes as biomaterials has been explored for various implants such as pacemakers and as vascular grafts (Oertel, 1994). The main criterion for the selection of monomers depends on the biocompatibility and non-toxicity of the degradation product (Marcos-Fernández et al., 2006). Amino acid based polymers are developed and studied as biomaterials due to their high biocompatibility and biodegradability. Several amino acid based polymers are used for biomaterial applications (Bourke and Kohn, 2003). The use of amino acid as one of the component for polyurethane synthesis will enhance the biocompatibility of the polymer (Sarkar, 2007). Polyurethanes based on amino acids offer several advantages
including biocompatibility, biodegradability and a range of material properties which can be tailored by changing the structure of the polymer (Skarja, 2001).

1.2 Research Hypothesis

The main research hypothesis tested was the synthesis of segmented polyurethanes by the incorporation of novel amino acid based diester chain extenders with tuneable physiochemical properties for potential use in biomedical applications, i.e. can these segmented polyurethanes be successfully synthesised, are they biocompatible, and what are their mechanical properties A further aim is to test those properties as a function of the polymer structure, in particular the molecular weight of the polymer soft segment monomer.

A number of steps will be taken to fulfil these goals, as follows:

1. The novel amino acid based diester chain extenders were synthesised and characterised. The presence of amino acid as one of the components of the polyurethane will increase its biocompatibility and its biodegradation, as amino acids are naturally occurring biomolecules and these are already present in the human body. The chances of obtaining toxic cell responses during in vitro studies should then be decreased. The introduction of hydrolysable ester linkage through chain extender will enhance the degradation property of the polyurethane (Guelcher, 2008). Moreover, the incorporation of amino acid will also enhance the enzyme mediated degradation of the polyurethane (Skarja and Woodhouse, 2001). Two series of L-amino acid chain extended polyurethanes and polyurethane urea’s were to be synthesised. The diol used to link amino acid groups and form chain extender is to be a simple, linear dihydroxy compound and is selected based on its low toxicity (Kartvelishvili et al., 1997).

2. A series of polyurethanes and polyurethane urea’s were synthesised based on Methylene diisocyanate, Polycaprolactone and amino acid based chain extenders. They will then be fully characterised for their resultant physiochemical properties and compared to a control polyurethane based on 1,4-butanediol as the
chain extender, polycaprolactone is to be used as the soft segment as this polyol is one of the biologically acceptable polyols reported in the literature (Chasin and Langer, 1990), (Gunatillake and Adhikari, 2011). The aromatic diisocyanate 4,4-methylenediphenyl diisocyanate (MDI) is used as the diisocyanate. MDI was selected because of its high reactivity and better resultant mechanical properties, largely due to its aromatic structure (Vermette, 2001). Toxic degradation products from aromatic polyurethanes are reported in the literature (Szycher and Siciliano, 1991), providing a downside to the use of MDI. However whether the concentration of these toxic products can reach physiologically significant levels in vivo is still inconclusive and has not been resolved yet (Coury, 2004), (Guelcher, 2008), (Blais, 1990). No evidence of toxicity of the resultant by product (4,4-methylenedianiline (MDA) of MDI is reported (Liljensten et al., 2002, Gisselfält et al., 2002). Moreover several MDI based polyurethanes are currently used as biomedical implants (Gunatillake and Adhikari, 2011, Gisselfält and Helgee, 2003).

3. Third, the effect of soft segment chemistry will be observed by using two different molecular weights of the soft segment (a high value, 2000 Mw and a low value, 1000 Mw). The structure property relationship will then be observed via thermal, surface and physical testing of the two polymer series formed. This will be applied to both PU and PUUs.

4. Initial in vitro cell cytotoxicity study was performed on the synthesised polymers. Mouse fibroblast cells will be used. Cells viability will be evaluated using a standard LIVE/DEAD® assay kit. Tissue culture polystyrene (TCPS) will be used as the positive control.

The final objective of this dissertation can be specified as follows:

1. Synthesis and characterisation of novel L-amino acid based diester chain extenders.
2. Synthesis of polyurethanes and polyurethane ureas based on novel L-amino acid based chain extenders. Structural, thermal, mechanical and surface properties of the synthesised polymers compared to control polyurethane.
3. Study of the effect of the soft segment molecular weight on the structure property relationship of amino acid based polyurethanes and polyurethane ureas.

1.3 Layout of thesis

The rest of the thesis is divided into six chapters. Chapter 2 describes the existing literature on polyurethane and its applications. It includes polyurethane history, chemical reaction of diisocyanate group, structural morphology of segmented polyurethane and the role of amino acid in polyurethane synthesis. In Chapter 3, the synthesis and characterisation of novel amino acid based diester dihydroxy and diester diamine compounds (which later acts as chain extender for polyurethane and polyurethane urea synthesis) are described. A detailed characterisation of these chain extenders will show their successful synthesis. The successful incorporation of amino acid based diester dihydroxy and diester diamine compounds as chain extender into polyurethane and polyurethane urea will be described in Chapter 4. This chapter will also include a detailed structural, thermal and mechanical characterisation of the polymers. Chapter 5 includes detailed analysis of the structure property relationship of the polyurethane and polyurethane urea based on different molecular weights of the soft segment. A library of polymers is developed and the effect of structural variation is examined in terms of different physiochemical properties to understand the underlying principal of structure property correlation. Chapter 6 includes a brief preliminary cytotoxicity screening (LIVE/DEAD assay) of the synthesised polymers to determine any substantial general cytotoxic response and Chapter 7 summarizes the research and discusses direction for future work.
Chapter 2: Literature Review
2.1 Introduction

A vast number of biodegradable polymers have been synthesised in recent times and their structure property relationship has been studied in detail (Vroman and Lan, 2009, Dumitriu, 2002). Biodegradable polymers are now in great demand with increasing applications in biomedical engineering such as drug delivery, medical devices, wound dressing, and as scaffold for tissue engineering. To fulfil these new demands, biodegradable polymers have been found to be encouraging candidates by characteristic ability to manipulate their physio mechanical properties. This can be typically achieved by regulating the ratio and nature of the starting material used for polymer synthesis. There are several polymer classes available which are showing potential to be used as biodegradable biomaterials for tissue engineering applications, but perhaps the most promising are biodegradable polyurethanes (Kumar et al., 2001).

Polyurethane structures contains urethane linkages with in the polymer chains. The urethane linkage is equivalent to carbamate linkage in organic chemistry (Hepburn, 1982). The structure of the urethane link is shown in Figure 1.

![Chemical structure of urethane within a polymer](image)

**Figure 1**: Chemical structure of urethane within a polymer

2.1.1 Structure of polyurethane

Polyurethane elastomers are produced through the reaction of diisocyanate and polyl and low molecular weight diols or diamines as chain extender. The chemical reaction between an isocyanate group with hydroxyl group generate urethane linkage while, reaction between isocyanate and diamine group produce characterstic urea linkage. Polyurethane synthesis reaction has been employed to synthesise a range of thermoplastic and thermoset polyurethanes in literature (Sobczak, 2015, Shi, 2004). Thermoplastic polyurethanes are prepared by reacting three classic compounds: a diisocyanate, a
difunctional polyol and a dihydroxy or diamine chain extender. These monomers react to form a linear, segmented copolymer consisting of alternating soft and hard segment blocks, which are the characteristic feature of thermoplastic polyurethanes. The general chemical structure of thermoplastic polyurethane produced using the prepolymer linking method is shown in Scheme 1 “adapted from Gunatillake and Adhikari” (2011).

The soft and hard segments are mostly thermodynamic incompatible, which leads to the two phase morphology of the polymer. The soft segment domains are primarily composed of polyols and are generally amorphous in nature, leading to the characteristic elastic properties of the polymer. The hard segments are primarily composed of diisocyanate chains linked with the chain extender and are generally glassy or semicrystalline in nature, contribute to the characteristic mechanical properties of the polymer.
2.1.2 Advantage of using polyurethane

Polyurethane is a versatile and commercially important material, already in large production. The urethane link is the main bond usually present within the polymer backbone, but it is the flexibility of polyurethane structure to incorporate other functional groups into the polymer network makes it more versatile as compared to other available biomaterials. A range of different functional groups can be incorporated into the polyurethane network such as ester, ether groups which can contribute to different range of properties acquired by polyurethanes. Thus the polyurethane can form rigid hard thermosetting materials as well as much softer elastomers (Lamba et al., 1998), (Wirpsza, 1993). Hence polyurethanes can be design to have specific properties of hardness, abrasion, chemical resistance, mechanical and elastic properties and also other specific tissue engineering properties such as blood and tissue compatibility (Biesman, 2002). It has been reported in the literature (Gunatillake and Adhikari, 2011) that biocompatibility and biodegradability are not the only characteristic required by material to act as an ideal candidate for tissue engineering applications. Along with these properties, the material needs to show optimum surface characteristic properties to encourage cells growth and proliferation. It is expected that an ideal degradable biomaterial will possess good mechanical and biological properties compliant with suitable degradation mechanism and easy fabricating ability. Among the currently available synthetic polymers, polyurethane offers various advantages in designing materials to fulfil these requirements. The availability of a huge range of starting materials with an easy to follow two step prepolymer synthetic method and opportunity to formulate the desired polymer with targeted properties are the several benefits available with the use of polyurethanes (Petrovic and Ferguson, 1991). Overall, the flexibility in polyurethane synthesis, along with its processing and bio-friendly properties have made it a preferable choice over other available synthetic polymers for biomedical applications.

2.1.3 Role of polyurethane

Polyurethane materials have a huge role in everyday materials, for example in the construction industry, as coatings, adhesives and textiles, household furnishing, medical devices (Saunders, 1964). These days, there is a high demand for polyurethane material in the bioengineering field. But its role as biomaterial in medical field started in early
1960, when polyurethane was used for in situ bone fixation and polyurethane coatings were applied to cardiovascular implants (Santerre et al., 2005, Soletti et al., 2011, Boretos and Pierce, 1967). Bio-stable polyurethanes have been extensively investigated as long term medical implants in the form of cardiac pacemakers and vascular grafts (Oledzka et al., 2007, Zoltowska et al., 2014, Liu et al., 2012a, Thottappillil and Nair, 2015). Table 1 “adapted from Sobczak et.al.” (2015) shows some of the biomedical grade polyurethanes that are commercially available and in use currently.

<table>
<thead>
<tr>
<th>Table 1 : Biodegradable Polyurethane Elastomers</th>
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<tr>
<td><strong>Product Name</strong></td>
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<td>SynBioSys</td>
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2.2 Polyurethane history and development

Until the 1920s and 1930s polymeric materials were largely based on natural or modified natural materials. In 1937, Otto Bayer, of the Farben Industrie, Leverkusen, Germany,
performed and patented the pioneering work on synthetic polyurethane (Bayer, 1937). Otto Bayer and colleagues took advantage of the known poly-addition reaction of isocyanate and alcohol functional groups to develop a linear polymer possessing a large number of urethane bonds and trademarked it as “Perlon U” and this proved as a huge commercial success.

In early 1950’s, attempts to synthesise polyurethane elastomers was based on the use of naphthalene-1, 5-diisocyanate (NDI) and resultant elastomer produces was very tacky and gummy material. The reason for such appearance might be the use of bulky diisocyanate (NDI) and lack of polymer melt stability. However the properties of these elastomers were improved tremendously when short chain extenders were introduced into the polymer network and also NDI was replaced by MDI (Oertel, 1994).

In the 1950’s, aromatic diisocyanate plays a huge part in the development of polyurethane chemistry. Toluene diisocyanate (TDI) and methylenediphenyl diisocyanate (MDI) were the two main aromatic diisocyanate used to develop polyurethane. In 1954, DuPont chemists successfully synthesise the TDI based polyurethane fibre called “spandex” followed by the synthesis of MDI based polyether urea with trade name of “Lycra” (Dieterich et al., 1994).

In 1957, a new family of polyurethane was made available by Schollenberger et.al. (1958). He introduced a new “cross linked” thermoplastic polyurethane elastomer. This was based on MDI, adipic acid and 1,4-butanediol (BDO). The polymer showed useful properties such as high elasticity, extensibility and solubility. Nevertheless, the structure property relationship of the synthesised polyurethanes was still not well understood. Cooper et.al. (1966) was arguably the first to gain a good understanding in this area. They reported that the excellent elastomeric properties of a thermoplastic polyurethane is the result of phase separated hard and soft segment morphology. During the 1960s, MDI become the raw material of choice for the rapidly evolving applications of polyurethane (Joshi, 2009).
In addition to elastomers, polyurethanes can also be produced as foams (rigid and flexible), adhesives, binders, coatings, and paints. Because of their unique properties, polyurethanes have found a wide variety of applications in commercial areas such as the automotive, furniture, construction, seating, exterior panels, structural foam, furniture, house-hold electrical materials, and refrigerator insulation (Saunders, 1964), (Wright and Cumming, 1969). These days polyurethanes especially biodegradable version have made their way to biomedical applications. Polyurethanes are now synthesised in custom made manner to fulfil specific medical applications.

2.3 Components of polyurethane

The three main components used for polyurethane synthesis are: a diisocyanate, a polyol and a dihydroxy or diamine chain extender (Ortel, 1994). These three monomers react to form a linear, segmented polyurethane made up of alternating hard and soft segments. The chemical reaction between the isocyanate group and the hydroxyl or amine group produces urethane and urea groups respectively. Polyurethane morphology is highly affected by the choice and structural composition and ratio of the reactants, synthesis method and processing conditions. A variety of materials are available to produce polyurethanes of desired physiochemical properties. In general, the resultant properties of the final polyurethane can be projected based on the choice of specific components (Lamba et al., 1998), (Pinchuk, 1995), (Hepburn, 1991). For example, aliphatic reactants have more molecular flexibility than cycloaliphatic groups, followed by aromatic groups. As a result, polyurethanes having aromatic group in their backbone shows enhanced mechanical properties, while aliphatic polyurethanes are generally produce softer and weaker materials. Similar to the role of molecular flexibility in the polyurethane, presence of asymmetric or symmetric reactants along with bulky side groups also prevent the alignment of polymer segments which in turn leads to reduced tensile strength (Caracciolo et al., 2009). Additionally, hydrogen-bond forming groups in polyurethane structure promotes phase segregation and hence improved mechanical properties. Overall, it shows that a clear understanding of the reactant chemistry help in designing the polyurethane with property required for specific application (Parrag, 2010).
2.3.1 Diisocyanate

The diisocyanate is the central component in polyurethane synthesis since the diisocyanate/hydroxyl reaction produces the characteristic urethane group that defines the polymer. The isocyanate group (NCO) is highly reactive, especially with nucleophile agents. The reactivity of the NCO group is determined by the electrophilic characteristic of the central carbon atom of the cumulated bond. The electron structure of the isocyanate group can be described using its resonance structure, as shown in Figure 2.

\[
\begin{align*}
R-N=O & \quad R-N=O & \quad R-N=O & \quad R-N=O \\
1 & 2 & 3 & 4
\end{align*}
\]

**Figure 2**: Resonance structure of isocyanate group, \( R \) can be aliphatic or aromatic group

The fourth structure in Figure 2 forms only in the case of aromatic isocyanates, where the aromatic ring can stabilize the negative charge of the nitrogen atom. The formation of this resonance structure explains the higher reactivity of aromatic isocyanates compared to aliphatic isocyanates (Bagdi, 2010).

The resonance structure illustrates that the NCO group can react with either electron donors or electron acceptors. Most reactions of isocyanate involve addition at the N=C double bond. Aromatic isocyanates are generally more reactive than aliphatic, as the electron withdrawing nature of the benzene ring makes the isocyanate carbon more susceptible to nucleophilic attack. The presence of bulky side groups in the ortho position on aromatic isocyanate, or branched or bulky substituent on aliphatic molecules will sterically hinder the approach of electron donors and reduce the rate of the reaction.

2.3.2 Types of diisocyanate

Aromatic and aliphatic diisocyanate are utilised to attain special properties desired in the final product. For example, aliphatic diisocyanate based polyurethane produce light stable polymer and aromatic diisocyanate based polyurethane will undergo photo-degradation,
if exposed for long enough, (Wang, 1998). The incorporation of aromatic diisocyanate in the polymer network produces a stiffer polymer chain with a higher melting point as compared to aliphatic isocyanates. In general, aliphatic diisocyanate based polyurethane shows weaker mechanical properties as compared to polyurethanes based on aromatic diisocyanate (Skarja, 2001). The commonly used and commercially available aromatic and aliphatic diisocyanates for synthesizing polyurethanes are shown in Figure 3.

**Figure 3**: Chemical structure of common diisocyanate employed in polyurethane synthesis

The molecular rigidity of the aromatic structure of the aromatic diisocyanate might be a reason for better hard segment polymer chain packaging which in turn strengthen the hard segment interaction through pi-electrons (Pinchuk, 1995), (Skarja, 2001). The two most commonly used aromatic diisocyanates in polyurethane synthesis are toluene diisocyanate (TDI) and methylenediphenyl diisocyanate (MDI). Out of two, MDI has
superior reactivity and provides better mechanical properties to the synthesised polymer. (Zia et al., 2009, Szycher, 1999). Other aromatic diisocyanates available are p-phenylene diisocyanate (PPDI) and 1,5-naphtalene diisocyanate (NDI) (Randall and Lee, 2002).

For the development of biodegradable polyurethane, aliphatic diisocyanates are recommended over aromatic diisocyanates. Since the use of aromatic diisocyanate can lead to the release of toxic and carcinogenic by-products upon degradation (Kavlock et al., 2007, Szycher and Siciliano, 1991). The first commercially produced aliphatic diisocyanate was 1,6-hexamethylene diisocyanate (HDI). Several others aliphatic diisocyanates are now in commercial use, including methylene bis(p-cyclohexyl isocyanate) (H_{12}MDI), cyclohexyl diisocyanate (CHDI), and 1,4-butanediisocyanate (BDI) (Lutz and Börner, 2008). Recently, a lysine amino acid based diisocyanate (LDI) i.e. 2,6-DiisocyaantoEthylCaproate, has been used to develop biodegradable polyurethanes. It degrades into less toxic (and possibly non-toxic) by-products and hence is the preferred choices for use in the development of biodegradable and biocompatible polyurethanes (Zhang et al., 2000).

### 2.3.3 Polyols

Polyols generally result in the soft segment of biodegradable polyurethanes. Polyols are generally low molecular weight hydroxyl terminated polymers. Different types of polyols has been used to synthesise polyurethane such as polyesters, polyethers, hydrocarbon polymers and/or polydimethylsiloxanes. Figure 4 shows the chemical structure of some common soft segments polyols used for biodegradable polyurethanes synthesis. Esterification reaction between acid and diol is carried out to produce polyesters. One of the most commonly used polyester polyol used for biodegradable polyurethane synthesis is polycaprolactone (PCL). It has been widely used to make biocompatible polyurethane due to its high biodegradability property (Heijkants et al., 2005) along with a low melting point of approximately 60°C and a glass transition temperature of -60°C (Liu et al., 2006).
Other polyol such as polyether diols are synthesised either by ring opening polymerization of tetrahydrofuran or alkylene oxides addition to polyols (Hinrichsen, 1994).

Polyols contributes to the flexibility and elongation property of the polyurethanes and, they approximately constitute 50 – 70 % of the total polymer composition. The choice of soft segment is often one of the critical factor in determining the properties of the overall PU. For example, elastomeric biodegradable PUs, can be achieved by the use of polycaprolactone (PCL) as soft segment and are particularly attractive for soft tissue applications (Gorna and Gogolewski, 2002, Gorna et al., 2002), (Kylmä and Seppälä, 1997), (Storey et al., 1994), (Parrag and Woodhouse, 2010).

**2.3.4 Chain extender**

Chain extenders typically have molecular weight ranging from 40 – 300 Dalton and can be classified as hydroxyl terminated or amine terminated. Difunctional compounds are considered as chain extender, whereas if compounds with higher functionality are used, they would be considered as cross linkers in polyurethane synthesis. The incorporation of chain extender into the hard segment in polymer network plays a very important role in
polyurethane structure and hence to its properties. For an example, polyurethane based on chain extender permits hard segment segregation and hence improves the physical properties of the resultant polymer along with an increase in the hard-segment glass transition temperature ($T_g$) (Wang, 1998). However, in case of no use of chain extender, polyurethane is formed by directly reacting diisocyanate and polyol. Generally it has very poor physical properties (low tensile strength) and often does not exhibit micro-phase separation. This shows the importance of using chain extender and how it influences the overall property of the synthesised polyurethane.

A number of di and poly-functional active hydrogen reactants are used as chain extenders, branching agents or cross linkers in the synthesis of polyurethanes. Zhang et.al. (2006) define the significance of chain extender as “The role of the chain extender is to produce an ‘extended’ sequence in the copolymer consisting of alternating chain extender and diisocyanate. These extended sequences, generally hard segments, and act both as fillers and as physical crosslink sites increasing mechanical strength in the polymer”

The most important chain extenders for polyurethane synthesis are aliphatic and aromatic diols and diamines (Hollinger, 2012). In general, polyurethanes produced with aromatic chain extenders and diamine based chain extenders have better physical properties as compare to polyurethane based on aliphatic and diol based chain extender. This is due to the formation of better crystalline hard segment domain in polymer structure which further enhances the mechanical properties of the polyurethane. The chemical structures of commonly used chain extenders are shown in Figure 5.

![Figure 5: Chemical structure of common chain extenders employed in polyurethane synthesis.](image-url)
Typical examples of diols reported in the literature and used as chain extender are 1,2-ethanediol, dipropylene diols. Conventional chain extenders such as 1,4-butanediol (BDO) and 1,6-hexanediol are used in preparing most biodegradable polyurethane formulation (Lamba et al., 1998), (Gunatillake and Adhikari, 2011). Aliphatic diamines used as chain extender include 1,2-ethanediameine, propylene diamine, hexylene diamine. 1,4-butanediameine (putrescine) is a naturally occurring compound and is used as chain extender in biodegradable polyurethane due to its low reactivity (Guan et al., 2002). Cycloaliphatic diamines include isophorone diamine, 1,4-cyclohexyl diamine along with aromatic diamines are often used for the synthesis of polyurethanes (Frisch and Kordomenos, 1985). Overall, it can be concluded that the major influence of chain extender structure on polyurethane properties is attributed to its effect on hard segment ordering, which, in turn, affects the polyurethane crystallinity and mechanical properties. Generally short chain, compact and symmetrical molecules favours better ordering of the hard segment.

Several research groups (Guan et al., 2004, Guan and Wagner, 2005, Guan et al., 2002), (Skarja and Woodhouse, 2000, Skarja, 1998, Elliott et al., 2002), (Marcos-Fernández et al., 2006) have developed naturally occurring amino acid based novel chain extenders for the synthesis of biodegradable polyurethane synthesis. The introduction of these amino acid based chain extender also contribute towards a method of introducing degradable chemical moieties into the hard segment of polyurethanes. Amino acids and short peptides can easily be incorporated as chain extenders in the form of dihydroxy and diamine compounds (Lipatova et al., 1983), (Guan et al., 2005), (Marcos-Fernández et al., 2006), (Sarkar et al., 2009), (Skarja, 2001), (Parrag and Woodhouse, 2010). Using this approach allows the choice of hard segment for achieving specific physical and mechanical properties while still promoting overall polymer degradation. As a result, PUs can be developed that are susceptible to enzyme degradation and also promote cell attachment and proliferation. The incorporation of these amino acid based chain extender into the polyurethane provides an opportunity to design and develop synthetic biomimetic PUs with modified physical, chemical, mechanical and degradation properties (Parrag and Woodhouse, 2010).
2.3.5 Primary reaction of isocyanate group

Isocyanate (NCO) group is highly reactive in nature and can promote reactions with other functional groups which further enhances the diversity of polyurethane copolymers. Examples of the primary and secondary reactions of isocyanate group with different functional groups are shown in Figure 6.

![Reaction Diagram]

**Figure 6**: Primary and secondary reactions of isocyanate group

All of these reactions can occur during the synthesis of polyurethane (Hepburn, 1991), (Hood, 2012).
2.3.5.1. Reaction with polyol/hydroxyl group
The reaction of isocyanate with a polyol results in urethane bond formation. This is one of the primary reaction occur during polyurethane synthesis. This is an exothermic reaction and highly influenced by the structure of both reactants. Especially, aliphatic polyols with primary hydroxyl end groups are the most reactive followed by secondary alcohol or polyol and least reactive are phenols.

2.3.5.2. Reaction with amines
Reaction of isocyanate with primary amines, at room temperature and in the absence of catalyst, is about 100 to 1000 times faster than the reaction with primary alcohol. The reactivity of amines increases with the basicity of the amine, and aliphatic amine reacts much faster than aromatic amines. In the case of aromatic amines, steric hindrance and electron withdrawing substituent reduces the reactivity toward isocyanate.

2.3.5.3. Reaction with water
One of the most common side reactions of the NCO group is with any water in the reaction mixture. Water is hard to completely remove from the reaction as it involves hygroscopic materials such as dimethylformamide (DMF) or dimethylacetamide (DMAc) as solvent. Even trace amounts of water present in the synthetic reaction can significantly affect the stoichiometric value of the NCO group and hence affect the final polyurethane composition and properties. The main product of the reaction with water is a substituted carbamic acid, which breaks down into an amine and carbon dioxide. The amine then reacts with further isocyanate to yield the substituted urea. In order to prevent NCO reaction with water, care must be taken to dry the reactant and solvents. Catalyst choice is also critical in preventing NCO reaction with water.

There are two main type of catalyst are used in polyurethane synthesis: tertiary amines and organometallic species. The most common organometallic catalyst is tin based, e.g. dibutyltin dilaurate (DBTL) or stannous octoate (Joshi, 2009). These catalysts are widely used in polyurethane synthesis as they are readily soluble in reaction solvent, are not very volatile and have a low odour. Tertiary amines, unlike organometallic catalysts, increase the reaction rate of NCO with amines, hydroxyl group and water. To minimise the effect
of water, therefore, an organometallic catalyst has been suggested. The organometallic species will increase the reaction rate of NCO with amine and hydroxyl functional group, but not with water (Lamba et al., 1998), (Hepburn, 1991), (Hood, 2012).

2.3.5.4. Secondary reaction of isocyanate group

Isocyanates, under certain conditions, may react with the active hydrogen atoms of the urethane and urea linkages to form allophanate and biuret linkages respectively. The reaction of isocyanate with urea groups is significantly faster than with urethane groups. However, these linkages are thermally labile and will dissociate at elevated temperature.

2.4 Synthesis of segmented polyurethane

Polyurethanes are produced in a one-step or a two-step (involving a prepolymer) process, by either bulk or solution polymerization, as shown in Figure 7 (Joshi, 2009).

![Diagram](image)

**Figure 7**: One step and two step (prepolymer) method of polymerization

Segmented polyurethanes consist of linear segmented polymer composed of individual hard and soft segments. The reaction temperature of polyaddition reaction between diisocyanate and diamine or dihydroxy group is generally in the range 80 – 100°C. Molar
ratio of isocyanate and polyl or hydroxyl group is very critical in obtaining high molecular weight polyurethane. It is suggested that while formulating a polyurethane structure, this ratio should maintain at one and even a slight deviation in the stoichiometry of the reacting groups can limit the final polymer molecular weight (Król, 2007), Hepburn, (1991).

In the one-shot method of polyurethane synthesis which is normally used for bulk polymer synthesis, all the reaction components (such as polyl, isocyanate and chain extender) are simultaneously mixed vigorously at the same time, and allowed to polymerise. The resulting polymer has a random distribution of monomer units along the polymer chain. The reaction is highly exothermic and is very quick.

Two step or prepolymer method produce alternating or block co-polyurethanes (Scheme 1, Section 2.1.1). First step includes the reaction of diisocyanate with polyl to form prepolymer via urethane linkage. In the second step, a low molecular weight chain extender is added to the reaction mixture and it would link the prepolymer segments, yielding high molecular weight polymer. If terminal isocyanate groups from the prepolymer react with terminal amine groups from the diamine chain extender, a urea linkage is formed, and the end product is a polyurethane urea. Alternatively, if a diol chain extender is used, additional urethane functional groups are formed leading to polyurethane urea. The two step solution polymerization pathway has been reported by several researchers (Gunatillake and Adhikari, 2011), (Lamba et al., 1998), (Randall and Lee, 2002, Liu et al., 2012b)

Solvent is commonly used as reaction medium in two step polyurethane synthesis method. The role of the solvent is to provide a non-reactive medium in which the all the starting materials and resulting polymer are all soluble. Polar aprotic solvents such as N, N-dimethylacetamide (DMAc), dimethylformamide (DMF), dimethylsulphoxide (DMSO), and N-methyl pyrrolidine (NMP) are commonly used for solution polymerization reactions. Usually polyurethanes are prepared with the use of catalysts such as dibutyl tin dilaurate (DBTL) and stannous octoate (Engels et al., 2013). Temperature of polymerisation reaction ranges from 80°C – 100°C and anhydrous
conditions are recommended to minimise the side reactions during polymerisation reaction (Blackwell and Gardner, 1979).

The method used for synthesis of polyurethane has a marked influence on morphology as well as the physical properties of the resulting polymer. One step method of polymer synthesis produces a polyurethane network with random distribution of monomer units along the polymer chains. While two step method generate a more regular structured polymer. It seems that in two step method, prepolymer formed in first step are well connected with each other via chain extender providing a regular and repeated hard and soft segment sequence in polyurethane structure. Hence the overall size distribution of polymer is narrow which may impart better mechanical properties to the polyurethane (Joshi, 2009).

2.5 Morphology in polyurethanes

Segmented polyurethanes are composed of alternating hard and soft blocks and depend on the incompatibility of these two segments, polymer phase morphology arise. In specific circumstances, soft and hard segment of polyurethane can segregate into phase separated morphology or these two segment can mix with each other to form phase mixed morphology. Hence the compatibility and incompatibility between these two segments define the elastomeric polymer properties. Generally the soft segments present in the polymer structure are in rubbery state and have their glass transition temperature \( T_g \) below the temperature of use. Hard segment melting temperatures \( T_m \) are above normal usage temperatures and hence the hard segments are rigid, glassy and/or crystalline (Marcos-Fernández et al., 2006). Overall the factors which might affect phase morphology in polyurethanes includes hard and soft segment polarity, length and structure of each segment, their tendency to crystallize, hydrogen bonding interactions between each segment and overall polymer composition (Hood, 2012).

For segmented polyurethanes, increased phase segregation generally results in improved mechanical properties. Hard segments act as physical crosslink sites and soft segments
adding some flexibility to the polymer (Foks et al., 1990). The elastic nature of segmented polyurethanes is a consequence of the thermodynamic incompatibility that serves as the driving force for phase separation (Cooper and Tobolsky, 1966). Hence, the design of elastic polyurethanes therefore requires appropriate chemistry to promote phase segregation (Wang and Hsieh, 1997), (Smith, 1977), (Parrag, 2010).

A lot of research has been done in the past to understand the morphology of segmented polyurethane (Camberlin and Pascault, 1984). Morphology of polymer can be studied at three different levels, based on the size of the structure present in the polymer network. The smallest structural level is the molecular structure followed by domain structure which has a dimension of ~ 50-1000 Å and act as second level of polymer morphology in polyurethane structure. The third level of morphology is characterised by the spherical semicrystalline spherulites where the size is generally in the micron range (Petrovic and Ferguson, 1991). A lot of information can be collected from each structural level by investigating them with different characterisation techniques such as nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FTIR), small angle X-ray scattering (SAXS), electron microscopy and polarized light microscopy. A collective study of different morphology levels can deliver detail information about the polymer block sequence, its distribution, phase volume fraction, size, shape and orientation of each segment. Each level of morphology provides detail information about the structure of polyurethane. For example, molecular structure provides detail information about the polymer block sequence or its distribution. A second level of polymer morphology determines phase volume fraction, size, shape, orientation as a function of segment content and sample history. Characterisation of all structural levels can be complemented by thermal analysis methods such as differential scanning calorimetric (DSC) and dynamic mechanical analysis (DMA) to render additional, if somewhat less direct, information on the domain structure (Wang, 1998).

Bonart et al. (1969) and Clough et al. (1968) provided the first evidence for the formation of two phase structure in segmented polyurethane. But due to lack of sufficient data from small angle X-ray scattering (SAXS) techniques, it was not further confirmed the presence of two phase morphology in polyurethane. However, the first direct observation
of the presence of two phase morphology in segmented polyurethane was obtained by Koutsky et al. (1970). Koutsky and co-workers used electron microscopy technique to study the commercially available polyether and polyester urethane with 38% hard segment and showed that the sizes of the hard domains ranged from 30 to 100 Å.

Later on, extensive work was carried by couple of research group such as Thomas et al. (1987), Chen-Tsai et al. (1986), and Serrano et al. (1987) to define the morphology of TDI/BDO based polyurethanes with varying hard segment concentration. They have used transmission electron microscopy (TEM) and SAXS techniques to demonstrate that this polymer exhibit two phase morphology. It could not, however, be assumed that complete phase separation occurred. In fact, there was evidence that appreciable hydrogen bonding existed between the hard and soft segments, which implied incomplete phase separation.

Following this, Li and co-workers (1988) validate that at low hard segment concentration, the hard segment phase was dispersed in a matrix of soft segment, in the form of cylinders or spheroids and at very high hard segment content, isolated soft segments were embedded in a hard segment matrix. They used polybutadiene based polyurethane (MDI/BDO) and SEM and SAXS data to study the morphology of segmented polyurethane. Similarly, Estes et al. (1971) also proposed a two phase model for polyurethane morphology with semicontinuous and interpenetrating domains.

Wilkes et al. (1974) has noticed the appearance of large supermolecular structures in the form of spherulite in segmented polyurethane. Their observation was further confirmed by Chang and Thomas (1979), who also proposed spherulite morphology when studying PCL/MDI/BDO polyurethanes with varying soft segment concentration. They report that by increasing the hard segment concentration, the size of spherulite also increases.

Crystallization is also one of the important property shown by segmented polyurethane. Percent crystallinity present in the hard and soft segment can provide additional information on the morphology of polyurethanes. The crystalline form of the hard segments depends on their structure, as well as on the crystallization conditions (Petrovic and Ferguson, 1991). More recent models for the obtained crystallization properties of
segmented polyurethane have been proposed by several researchers including Hernandez et al. (2003, 2008). However, in the past, a simple model for the packing arrangement of MDI/BDO hard segments was proposed by Bonart et al. (1969, 1974). Following the trend, Blackwell and co-workers (1979, 1981, 1982, 1984) also proposed a more complex model for the chain conformation and packing of MDI/BDO hard segment of polyurethane. They proposed a planar zigzag conformation for 1,4-butanediol (BDO) and V-shape for MDI units with hydrogen bonding present within the urethane groups. All of these studies were based on different characterisation techniques such as SAXS, FTIR, DSC and atomic force spectroscopy (AFM). Despite these efforts, the exact nature of micro-phase structure within polyurethanes has yet to be elucidated.

Overall it was observed that morphology of segmented polyurethane influence the final properties of polymer. Hence a control over the morphology is essential in order to obtain the desired product properties. A profound knowledge of morphology is thus vital to understanding structure-property relationships (Noshay and McGrath, 1977).

2.6 Structure-property relationship of polyurethane

Polyurethane properties are strongly affected by the structure of the chain segments. Several excellent papers have been published dealing with structure-property relationship (Schollenberger and Dinbergs, 1973), (Schneider et al., 1975), (Saunders, 1964, Seefried et al., 1975), (Heikens et al., 1968), (Morbitzer and Hespe, 1972), (Wang, 1998). The property differences caused by structure variations such as the type of diisocyanate, polyol and the nature of chain extender can be classified as “soft segment effective” and “hard segment effective”. The role of these hard and soft segment in the structure property relationship for polyurethanes is described here in detail.

2.6.1 Soft segment

Typically, soft segments (SS) with average molecular weights of 1,000 – 5,000 Dalton are used for synthesis of thermoplastic polyurethanes. Different types of polyol have been used as the soft segment. The most frequently used soft segment for polyurethane
synthesis are polyether and polyester polyols. Polyester based polyurethane generally show superior tensile strength as compared to polyether based polymers (Yen and Cheng, 1994). This is often attribute to the strong hydrogen bonding between N-H and C=O of ester group as compare to the weak urethane N-H– ether oxygen bond. Another reason might be the high tendency of polyester to crystalize. However, polyether based polyurethanes show a high degree of phase segregation than polyester due to greater incompatibility between polyether soft segment and polar hard segment (Prisacariu, 2011), (Paik Sung and Schneider, 1978), (Buckley et al., 2010). Polyether urethane shows high degree of hydrolytic susceptibility than that of ester linkage (Dieterich et al., 1994). Among the commonly used polyether’s, polytetramethyleneoxide (PTMO) gives elastomers the best physical properties. This result is believed to be caused by the regularity of the PTMO structure, which permits crystallisation upon extension. However, for polyesters, polycaprolactone (PCL) is the preferred soft segment for biomedical grade polyurethane, since it is considered non-toxic and has been approved for use in biodegradable medical devices (Engelberg and Kohn, 1991).

In general, increasing SS length creates the potential for increased strain-at-break. Polyester-based PUs show higher tensile strengths then their polyether-based PUs. Strain induced crystallization of soft segment leads to a self-reinforcing effect, which enhances the modulus of the polymer (Wilkes and Wildnauer, 1975), (Morbitzer and Hespe, 1972). Yeh et.al.(2003) studied soft segment structure development during deformation of a segmented polyurethane-urea elastomer. Stress induced crystallization of PTMO as a soft segment was observed with increasing strain up to 300 – 500%.

Korley et.al.(2006) studied the effect of crystallinity in soft segment on overall polymer properties. The research group has investigated the morphology and mechanical behaviour of polyurethanes based on polyethylene oxide (PEO) and polyethyleneoxide-polypropyleneoxide-polyethylenoxide (PEO-PPO-PEO) as soft segments with varying hard segment content. The study demonstrate that soft segment imparts flexibility into the polymer structure along with ordered soft segment also acts a reinforce filler during deformation process, improving the overall toughness of the material.
Difference in molecular weight of the soft segment also affects the properties of polyurethane. A study about the effect of increasing molecular weight on polymer morphology has been reported in literature (Velankar and Cooper, 1998). For this study, polyester based soft segment with different molecular weights (Mw- 830, 1250, 2000 and 3000 Dalton) but with constant composition were used to synthesise a series of polyurethane. Characterisation was done by using techniques such as differential scanning calorimetry (DSC), small angle x-ray scattering (SAXS) and rheology. The research group demonstrated that polyurethane based on low molecular weight soft segment were amorphous in nature while polymer based on high molecular weight soft segment showed phase segregation morphology. Similar studies of the effect of PCL molecular weight on polyurethane properties was reported by Hejikants et.al.(2005). The PCL molecular weights were in the range of 750 to 2800 Da and polyurethanes were synthesised using two step methods without the use of catalyst. The tensile strength usually increased from 38.7 MPa for PCL750 to 55 MPa for PCL1900, while the elongation at break increased from 870% to 1173%. Polyurethanes based on PCL molecular weight 1600 Da and lower exhibited crystalline urethane and amorphous PCL phase with some dispersed in hard segment. In polyurethane with PCL molecular weight higher than 1600 Da, an additional SS crystalline phase was observed. It was observed that as molecular weight of PCL increases, tensile strength and elongation at break for polyurethane increases. These studies show that materials with improved mechanical properties can be designed by the appropriate choice of softs segment molecular weight. 

Bogart et.al. (1983) have studied the structure property relationship of MDI-PCL-BDO based PUs. Soft segment molecular weights of 830 and 2,000 Dalton were used. They reported an increased tendency for the soft segments to crystallise as the molecular weight of those soft segments increased. Kloss et.al. (2002) reported a similar observation for PCL based polyurethanes. Wang et.al. (2003) has also described the effect of PCL molecular weight on the degree of crystallinity. WAXD studies showed that PCL 530 based polyurethane were amorphous in nature and high molecular weight PCL based polymer were semicrystalline in nature.
Overall, based on the reports stated in literature it can be concluded that the softs segment highly influences the overall properties of polyurethane which includes mechanical, hydrolytic, phase segregation and crystalline behaviour of polymer.

### 2.6.2 Hard segment

The hard segment (HS) is composed of chain extender and diisocyanate. Both components contribute to the structure-property relationship of the polyurethane. The effect of the chain extender on material properties depends on the type and structure of chain extender used. This is particularly relevant to this thesis, since the synthesis and incorporation of novel amino acid based chain extenders into polyurethane is one of the main feature of this thesis. It is therefore very beneficial to discuss the effect of chain extenders on the structure-property relationship of polyurethanes.

#### 2.6.2.1 Chain extender

Several reports have been described by various authors about the effect of chain extender (CE) on morphology and overall properties of polyurethane (Hong et al., 1992), (Blackwell et al., 1982), (Pandya et al., 1988). Chain extender length, structure and its functionality can influence hard segment (HS) packing and crystallinity (Vlajic et al., 1990, Petrović et al., 1998, Liaw, 1997).

When diamine is used as the chain extender, (urea bond is formed) better physical properties are obtained than if a diol based chain extender (urethane bond is formed) is used. Due to the greater basicity of amine, diamine reacts more rapidly with a given diisocyanate than diol. Therefore diamines may be preferred, particularly in systems employing low reactivity diisocyanates (e.g. aliphatic examples). The urea group generated by the NH₂/NCO reaction enables the development of a highly cohesive hydrogen bonding network within the hard segment. This is due to the greater hydrogen bonding potential for the urea group in comparison to urethane. This network acts to increase phase separation as well as increasing hard domain cohesion, both of which lead to improved physical properties (Gogolewski, 1989), (Takahara et al., 1985).
Yen et al. (2003) observed a high mechanical properties for diamine chain extended PUs in comparison to BDO chain extended PUs, probably because of greater hydrogen bonding exist between the hard segment and hence improves the mechanical properties of the synthesised polyurethane. Other studies reported in literature (Paik Sung et al., 1980a, Adhikari et al., 2000, Wang and Cooper, 1983, Ahn et al., 1994) also demonstrated the influence on polymer properties from the choice of urea over urethane groups and reports the similar findings of enhanced mechanical properties of PU. Structure of chain extender also influence the end properties of the PUs. Polyurethanes based on aromatic chain extenders are usually stiffer (higher modulus) than those PUs based on aliphatic chain extender. It might be due to reduced hard segment chain mobility and increased inter-chain interaction (Blackwell and Gardner, 1979).

It has been reported in the literature (Bonart et al., 1974) that the number of carbon atoms present in the backbone of chain extender can affect the polymer properties. Gisselfalt et al. (2003) has reported that use of aliphatic chain extender with an even number of carbon atom present in chain extender has improved the mechanical properties of polyurethane as compared to when odd number of carbon atom based chain extender was used. This is believed to result from the formation of a hydrogen bonded network in the minimum energy molecular conformation, which promotes a high level of hard segment interaction and phase separation (Blackwell et al., 1982).

The structure of chain extender has a pronounced effect on polyurethane properties. The non-linear chain extender tends to reduce the tensile properties of the synthesised polyurethane as compare to the when corresponding aliphatic linear chain extender based PUs were tested. The low mechanical properties might be due to poor hard segment packing (weak hydrogen bonding) due to bulky pendant group present as irregular structure in the backbone of synthesised polyurethane. The steric hindrance due to irregular arrangement of hard segment chains prevents hard segment domain formation. This would lead to incomplete phase separation which in turn effect the mechanical properties of the polyurethane (Hepburn 1993). Bae et al. (1999) studied the effect of chain extender structure on the mechanical behaviour of polyurethanes. They synthesised two series of MDI-PTMG based polyurethane containing linear and non-linear chain
extenders. The Dynamic mechanical analysis (DMA) of both the series of polymers were studied and compared to observe the effect of the chemical structure of chain extender on PUs. Linear chain extenders such as 1,3-propanediol, 1,4-butanediol (BDO), 1,5-pentanediol; non-linear chain extenders such as 1,4-pentanediol, 2,5-hexanediol; and cyclic chain extenders such as 1,4-cyclohexanediol, and 1,4-cyclohexanediol. and 1,4-cyclohexanediolmethanol were used. 1,4-butanediol (BDO) has been used as the control aliphatic linear chain extender. Polyurethane’s (PUs) based on non-linear chain extenders showed higher soft segment glass transition temperatures ($T_g$) and lower young’s modulus as compared to control BDO based PUs (Asefnejad et al., 2011) and melting temperature of hard segment increases from 10°C to 170°C with the use of linear chain extender. BDO based PU showed higher young’s modulus value which corresponds to the presence of well-ordered hard segment. Similar trends were also observed for soft segment $T_g$ when cyclic chain extenders were used.

Vlajic et.al. (1990) studied the effect of chain extender structure and hard segment content on the properties of the polymer. Elastomers from symmetrical and rigid difunctional chain extenders were shown to have better mechanical and physical properties than cross linked polymers. The influence of chain extender length on copolymer properties was investigated by Pandya et.al.(1988). The $T_g$ of polyurethane based on the homologous series of saturated diol as chain extenders decreases from lower to higher members i.e. $T_g$ of hexanediol (used as chain extender) is lower than that of ethane diol. $T_g$ is found to increases from butanediol to butynediol extended polymer. i.e. unsaturation in the chain extender increases $T_g$ (Jayashree, 1999), (Nair et al., 2010). Chain extenders based on amino acids have also been developed to enhance enzyme mediated degradation of polyurethane (Skarja, 2001). The role of these chain extenders in rendering synthetic polyurethanes to become more bio-friendly will be discussed in Section 2.8.

In general, it was observed that polyurethanes prepared from non-linear chain extenders have non-crystalline hard segment domains with a low degree of order which result in poor mechanical properties (low young’s modulus).
2.6.2.2 Diisocyanate

Diisocyanate is the major component of the hard segments (HS) in polyurethanes. The hard domains generally contain diisocyanate and chain extender units (McBane et al., 2007), (Gogolewski, 1989). Cyclic and aliphatic diisocyanates impart different characteristics to the polyurethane. HS structure and its distribution within the polymer network strongly influence polymer morphology and thermal behaviour (Paul et al., 1999, Pandya et al., 1986), (Schneider et al., 1975).

As discussed earlier, Cooper et.al. (1966) reported that “the versatile properties of PU are attributable to the micro-phase separated structure that these materials present”. The effect of the chemical structure of diisocyanate has a strong influence on the micro-phase property of polyurethane. PUs based on aromatic MDI and 1,4-butanediol, form a zigzag structure in which the benzene rings of MDI are arranged at right angles to each other (Blackwell et al., 1984). Schneider and co-workers (1975) reported that 2,6-toluene diisocyanate (2,6-TDI) based polyurethanes are more hydrogen bonded and hence more phase separated than 2,4-toluene diisocyanate (2,4-TDI) based PU. Differential scanning calorimetric (DSC) and Fourier transform infrared spectroscopy (FTIR) techniques were used in this study. Li and co-workers (1993) has also evaluated the effect of use of aliphatic (HDI) and aromatic diisocyanate (MDI) on polymer overall properties. They used SAXS and DSC techniques to conclude that PU based on HDI had higher mobility as compared to MDI based PU. The morphological difference between aromatic diisocyanate (MDI) and aliphatic diisocyanate (HDI) was also investigated by Fernandez Borja et.al. (2008). DSC and FTIR results concluded that MDI based PUs are less phase segregated as compared to HDI based PUs.

In another study, Song et.al. (1996) synthesized PUs based on aliphatic polyester and three aromatic diisocyanates MDI, XDI and TDI. Their aim was to study the effect of isocyanate geometry on the crystallinity and physical properties. They found from DSC and SAXS data that the PUs crystalline behaviour depends on the degree of the efficient packaging of the molecules in the diisocyanate structure. It means, an ordered packaging of the molecules in diisocyanate would lead to high crystalline structure of the hard segment of the PUs. Hence, they have reported the decreasing order of crystallinity of the
hard segment based on the different diisocyanate present in the polyurethane MDI>XDI>TDI. The results revealed that the higher order of hard segment (HS), higher will be the crystallinity and higher will be the thermal stability of the soft segment. The crystalline behaviour of HS leads to the higher degradation temperature. Similar observations about the relationship of crystallinity to type of diisocyanate to polymer thermal and mechanical properties was also reported by Zia et.al. (2009).

Different characterisation techniques were used by Bogart et.al. (1983) to study the structure property relation of PCL-BDO based PU. Two different types of diisocyanate i.e. MDI and CHDI were used for the study. High level of hard segment crystallinity was observed in MDI based PU as compared to CHDI based PU which was amorphous in nature. DSC results showed that as hard segment content increases, the hard segment T<sub>m</sub> increases and longer the hard segment better will be the phase separation. Mechanical properties were also observed by DMA and stress strain curve. They have reported that as hard segment percentage increases, young modulus increases and ultimate elongation decreases in both the cases. Overall it can be concluded that, increase in hard segment content increases the hard segment crystallinity, increases hard segment melting temperature and also increased phase segregation morphology. A similar observation for the effect of domain size on tensile properties has been suggested by Smith et.al.(1977).

Abouzahr et.al. (1982,) also studied the effect of hard segment (HS) content on MDI based PUs. Different percentage of HS was used to synthesise PUs and based on the properties observed related to percent hard segment, PUs were divided into four category. It was observed that at very low HS percentages, polymer was poorly phase separated and weak elastomeric properties were obtained. At very high hard segment content, soft segment was dispersed within the hard segment and polymer behaved as a brittle plastic. However, at moderate level, polymer was phase separated with higher modulus and extensibility. Similar observations were made by Prisacariu et.al. (2010), on PUs based on crystallisable HS.

Yilgor et.al.(2007) studied the influence of hydrogen bonding and diisocyanate symmetry on micro-phase morphology on non-chain extended, polyether urethanes and polyether
ureas. Polyurethanes based on symmetrical diisocyanates, such as 1,4-phenylene diisocyanate (PPDI), 1,6-hexamethylene diisocyanate (HDI) and 1,4-cyclohexyl diisocyanate (CHDI), all showed phase segregated morphology, and PUs based on unsymmetrical diisocyanates such as bis(4-isocyanatocyclohexyl)methane (HMDI), 2,6- and 2,4-toluene diisocyanate (TDI), and 1,3-phenylene diisocyanate (MPDI) did not show any micro-phase morphology (at room temperature). Their study shows the important role of hard segment symmetry on the morphology of polyurethanes (Joshi, 2009).

In general, the selection of diisocyanate and chain extender is made on a complementary basis to generate maximum hard segment interaction and cohesion. Therefore, if an aliphatic or unsymmetrical diisocyanate is employed, the use of diamine chain extender can serve to improve the ultimate material properties in comparison to dihydroxy (diol) based chain extenders. Conversely, if a symmetric aromatic diisocyanate is used, a dihydroxy or a branched diamine chain extender may be employed while retaining adequate mechanical properties.

2.7 Hydrogen bonding

Polyurethanes are extensively hydrogen bonded. A variety of hydrogen bonds are possible. Degree of phase separation can provide an indication of the level of hydrogen bonding present in the polyurethane (Miller et al., 1990), (Lee et al., 1987), (Seymour and Cooper, 1973), (Srichatrapimuk and Cooper, 1978). Phase separation favours inter-urethane, inter-urea or urethane urea hydrogen bonding while phase mixing shows less hydrogen bonding. Fourier transform infrared (FTIR), is a commonly used spectral technique to study the hydrogen-bonding properties of polyurethane (Born and Hespe, 1985), (Koberstein et al., 1986), (Coleman et al., 1986), (Coleman et al., 1988), (Harthcock, 1989), (Paik Sung et al., 1980a), (Bonart et al., 1974). Polyether urethane has fewer hydrogen bonds (40%) as compared to polyester urethane of the same hard segment content. Increasing the hard segment content will increase hydrogen bonding in polyurethane. At room temperature, approximately 90% of the N-H groups in the hard segment of a typical polyurethane are hydrogen bonded (Seymour and Cooper, 1973). However, the balance between hard and soft segment hydrogen bonding will also depend
on the particular hard and soft segment chemistry used, and the accompanying phase separation.

The primary force for micro-phase separation is believed to be the strong intermolecular interaction (hydrogen bonding) between urethane units. Thus, qualitative information of phase separation can be extracted from hydrogen bonding between the two phases. If hydrogen bonds exist only within the hard segment domains, phase separation occurs to a greater extent. By contrast, if they can be formed between the soft and hard segments, the interphase hydrogen bonding enhances the degree of phase mixing (Joshi, 2009), (Wang, 1998).

The extent of hydrogen bonding can be affected by structure and composition of the polyurethane as well as temperature. It has been reported in the literature (Lamba et al., 1998) that hydrogen bonding will start to dissociate as the temperature increases and this process can be accelerated by the glass transition of the hard segments. Thermal annealing of polyurethane can lead to the rearrangement of hydrogen bonds (Ishihara et al., 1974). Thermal FTIR studies by McKiernan et al. (2002) showed the existence and high concentrations (~75%) of hydrogen bonding present in aliphatic polyurethane even in the melt. They also reported that hydrogen bonding controls the crystallization, packing, and morphology of polyurethanes. FTIR has been used as a sensitive tool for the characterization of hydrogen bonding (Lee et al., 1987), (Senich and MacKnight, 1980), (Sung and Hu, 1981), (Tang et al., 1995), (Teo et al., 1997), (Painter et al., 1991).

Valuable information can be obtained from the N-H and C=O stretching vibrations of an FTIR spectrum of polyurethane which appear ~3200-3400 and 1600-1700cm\(^{-1}\) respectively (Seymour and Cooper, 1973), (Sung and Schneider, 1975), (Nakayama et al., 1969), (Luo et al., 1996), (Brunette et al., 1981), (Sung and Schneider, 1977). In summary, it can be concluded that the extent of inter segmental as well as interphase hydrogen bonding play an important role to modify the physical and mechanical properties of the polyurethane. (Fernández d'Arlas et al., 2008).
2.8 Polyurethanases as biomaterials

Segmented polyurethanes represent an important class of synthetic polymers for potential tissue engineering and other biomedicine applications. Using non-toxic soft segment polyols, hard segment chain extenders allows the development of a whole new family of biodegradable polymers which may exhibit diverse properties and thus may be suited to a wide array of applications, but most importantly are biocompatible and often biodegradable. Technically, it may be impossible to develop truly biocompatible polymers (Williams, 2014) however cell compatibility has been widely used in the literature to be synonymous with “biocompatible” and will be continue to be the convention used here, knowing that the distinction may need to be made in future.

A significant advantage of working with PUs, in comparison to other biomaterials, is that flexibility in the chemistry used in the synthesis process allows the development of polymers with diverse physical, chemical, mechanical, and degradation properties. *A priori* knowledge of the particular properties needed for site specific applications enables researchers to tailor the PU properties to meet those requirements. Moreover, the ability to incorporate specific amino acid and peptide sequences into the backbone structure of the polymer confers unique biological functionality to these synthetic materials. The formation of tailor made synthetic polymers, with the ability to adjust their material properties whilst maintaining biocompatibility, could have important implications in, for example, the development of ideal biomaterial scaffolds for tissue engineering.

Degradable polyurethanes can be synthesised by introducing hydrolysable linkages (most commonly esters) into the polymer backbone in a variety of ways. The most common approach has been the use of degradable soft segment polyester diols such as polylactic acid or polycaprolactone. This approach has the advantage of employing well characterised and accepted polyesters. Thus the chances of producing toxic degradation products upon polymer degradation can be reduced or omitted (Vermette, 2001).

Alternatively, to further enhance the degradation process, hydrolysable linkages may be inserted via chain extenders, leading to degradable hard segments, which otherwise are
the slowest segments to degrade in polyurethane. This approach is less common, however naturally occurring amino acid or peptide based chain extenders with hydrolysable ester linkages have been synthesised and incorporated into polyurethanes. The introduction of biodegradable chain extenders into the hard segment is considered as one of the most convincing method to design polyurethane with easily degradable hard segment (Gunatillake and Adhikari, 2011), (Pulapura and Kohn, 1992). The idea of incorporating amino acid as one of the monomeric units in the form of a chain extender has three attractive features (Skarja and Woodhouse, 1998):

1. Non-toxic products would be released upon polymer degradation (although the study of polymer degradation and characterisation of the degradation products was outside the scope of this work).

2. Enzyme mediated degradation can be tailored into the polymer based on the known amino acid based enzyme profile at the site of application (although, again, the study of enzyme mediated degradation was outside the scope of this work).

3. The side chain functional group (hydroxyl and amine groups) of different amino acids can be used to generate a pendant group on the polymer backbone. Such pendant groups may then be used as reactive sites, for example as a drug carrier.

As mentioned above, modification of the chain extender is one of the strategies used by researchers to enhance the hard segment degradation rate. The incorporation of chain extenders based on amino acids has been explored by several research groups (Rockwood et al., 2007), (Liu et al., 2012b), (Elliott et al., 2002, Rechichi et al., 2008, Parrag and Woodhouse, 2010) to develop PUs for soft tissue engineering. For example, Gu et.al. (2015) has reported the synthesis of biodegradable polyurethane urea (PUU). Lysine amino acid based Poly-lysine-PCL oligomer was used as the soft segment. Overall polymer degradability was improved by introducing poly-lysine oligomer into the main chain of the PUUs. The results of cells viability tests indicated that the PUUs showed good biocompatibility with endothelial cells and the thermostability and hydrophilicity of PUUs increased with increased poly-lysine oligomer content.
In other studies, Wang et al. (2014) has reported the synthesis of polyurethane urea based on a novel tripeptide as chain extender. Phenylalanine-lysine ethyl ester-phenylalanine (PLP) was used as chain extender and provide site for trypsin cleavage. PEG, HDI was used as polyol and diisocyanate. In a similar way, a series of L-amino acid based polyurethane urea was developed by Chan-Chan et al. (2013). L-amino acid such as L-arginine, L-glycine and L-aspartic acid was used as the chain extender, PCL 2000 (Mw) was used as polyol and $H_{12}MDI$ was used as the diisocyanate. The polymers showed good mechanical properties. Lu et al. (2012) has illustrated the synthesis of polyurethane urea based on cysteine amino acid. Cell compatibility results indicated that the synthesised polymer performs better than controls. The evolving aim of these amino acid based chain extender synthesis is to produce biodegradable, and more importantly, biocompatible, polyurethanes predominantly for use in biomedical applications.

Rechichi et al. (2008) synthesised phenylalanine amino acid based chain extender and prepared a series of PUU. PCL or PCL-PEG-PCL act as soft segment and MLDI act as diisocyanate component. Cell studies indicated that polyurethanes were non-toxic and encourage cell adhesion and proliferation. In another study, Fromstein et al. (2002) investigated the effect of blending the amino acid based PUUs on polymer properties such as degradation rate to assess their suitability for biomedical engineering. The dipeptide Gly-Leu has also been introduced as part of the chain extender by reacting with cyclohexanediol (Parrag and Woodhouse, 2010). The incorporation of growth factors to improve cell growth has also been explored with biodegradable polyurethanes. Based on the studies reported in literature, it seems promising that PUs/PUUs based on amino acids, amino acid derivatives and peptides can act as potential biomaterials for soft tissue engineering applications.

The flexibility available in polyurethane chemistry to tailor physio chemical, mechanical and biological properties have made this class of synthetic polymer to act as potential biomaterial for biomedical applications. Currently, there is a need to develop biodegradable polyurethanes which can fulfil the requirement of an ideal biomaterial with improved biocompatibility and controlled biodegradation to address the materials needed in tissue engineered products and therapies. Hence a good understanding of the relationship of the molecular structure of polyurethanes with mechanical,
biocompatibility and degradation properties plays a vital role in designing biodegradable polyurethane for soft tissue applications. A number of research studies are reported in literature (Thilak Gunatillake, 2011), where either amino acid or peptides have been used to synthesise custom made chain extenders which have later been used for PU/PUU synthesis. The effect of these newly developed custom made amino acid based chain extender on PUs/PUU physiological properties is very well documented in literature. For example, Skarja et.al.(1998) have prepared polyurethanes based on two different soft segments (PCL, PEO) with phenylalanine diester chain extender. Enzyme mediated erosion of L-phenylalanine based PUs was also demonstrated by Ciardelli et.al.(2004).

In another study, Kavlock et.al.(2007, 2013) have developed a family of biodegradable PUUs based on BDI, PCL, and tyramine-1,4-diisocyanatobutane-tyramine or its tyrosine analogue as chain extender. The phenyl group in these chain extenders are expected to impart rigidity similar to PU with MDI based hard segments. The new PUUs supported in-vitro attachment and proliferation of viable MG-63 human osteoblast like cells. Sarkar et.al.(2009) has illustrated the synthesis of L–tyrosine based chain extender and evaluated its effect on structure property relationship of PUs when this amino acid based chain extender was incorporated in PU. Similarly, Fernandez Borja et.al.(2008) investigated the synthesis of a series of biodegradable non-toxic polyurethane urea based on L-lysine or L-ornithine ethyl esters as chain extender. Mori et.al.(1985) and Sosa et.al.(1985) have also reported the synthesis of L-serine amino acid based chain extenders and later used them for segmented biodegradable polyurethane synthesis. As a part of evaluating the significance of use of amino acid based chain extender in PU/PUUs synthesis, Guan et.al. (2005) prepared a family of polyurethane urea based on either PCL or triblock copolymer PCL-PEG-PCL as the soft segment. BDI was used as the hard segment and the peptide Ala-Ala-Lys, act as the chain extender. Synthesised polymers were flexible. Endothelial cells adhesion was >140% of tissue culture polystyrene on PU surfaces. Following this trend, Parrag et.al.(2010) has exemplified the synthesis of biodegradable PUU based on chain extenders containing di-peptide Gly-Leu for fibroblast compatibility. Mouse embryonic fibroblasts cells were successfully cultured up to 28 days. Many studies (Harris et al., 2006) report on fibroblast compatibility with biodegradable PUs having different chemical structure.
Whilst these reports provide excellent progress towards the final goal of tailor made, biodegradable and biocompatible polyurethanes and polyurethane urea, there remains much to do before a systematic understanding of the structure function of biomedical grade polymers is fully known. A major goal of this thesis, then, is to systematically synthesise a series of L-amino acid based chain extenders, incorporate them into polyurethane and polyurethane urea, and thereby formulate a systematic series of potentially unique polymers which can be tested for structure function relationships.

2.9 Technical Approach

As discussed in Section 2.8, different amino acids have been used as chain extenders in the synthesis of biocompatible and biodegradable polymers, however relatively little work has been reported on amino acids (as chain extender) for polyurethane synthesis. There is thus a need to continue generating a library of polyurethanes based on different L-amino acid based chain extenders. This includes the synthesis and characterisation of different L-amino acids which can be the basis of chain extender, and then incorporation of these into polyurethanes. Characterisation of the polymer to evaluate the effect of incorporated amino acid on the structure property relationship of polyurethane then follows. Keeping this project goal in mind, the following technical approach will be employed:

The approach followed is to develop segmented polyurethanes based on L-amino acids. L-amino acid based dihydroxy and diamine diester compounds are synthesised and later used as chain extenders for segmented polyurethane, and polyurethane urea synthesis. PCL will be used as the soft segment and MDI will be used as the diisocyanate. MDI was used because of its high reactivity. It is recognised that MDI is often not ideal for obtaining a genuinely biodegradable polyurethane because of reported toxic degradation products (Marcos-Fernández et al., 2006), (Vermette, 2001). However, as these chain extenders are synthesised for the first time, the highly reactive diisocyanate was chosen in order to achieve a high molecular weight of the synthesised polyurethane. The resultant polymers will be screened for obvious signs of bio-incompatibility with the philosophy that if they are incompatible, then the knowledge of structure-property gained through synthesis and testing of novel polymers will be of benefit in the general scientific sense,
and if they are compatible then future testing can be used to determine their ultimate development as biomedical polymers.

Following synthesis, the polymers are to be characterised in detail, using different techniques such as nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and tensile testing. The effect of soft segment molecular weight will be investigated and an evaluation (albeit a preliminary one) of cell cytotoxicity of the synthesised material will be reported.
Chapter 3: Synthesis and characterization of L-amino acids based ester compounds
3.1 Introduction

Polyurethanes represent a large family of polymeric materials with an enormous diversity of chemical composition and properties. These properties have contributed to their widespread applications in many field and their use in a range of commodity products (Saunders, 1964, Vermette, 2001) particularly in biomedical application areas such as cardiovascular (Gogolewski et al., 1987, Gogolewski and Pennings, 1983), artificial organs (Zdrahala and Zdrahala, 1999), tissue replacement (Lelah and Cooper, 1986) and augmentation (Lamba et al., 1998, Gorna and Gogolewski, 2006).

3.1.1 Polyurethane Structure

Compared to many industrially prepared polymers, polyurethanes possess complex chemical structures, typically arising from three components - a diisocyanate, a polyol, and a chain extender. These three parts in the polyurethane structure enables one to create a virtually infinite number of materials with various physicochemical and mechanical characteristics. However, although there are three components, polyurethane polymer structures are frequently referred to as two-phase structures in which the soft segment and hard segment domains are present in polymer structure. The hard segment is composed of the diisocyanate and the chain extender, while the soft segment is made up of the polyol moieties used. For this reason, polyurethanes are often referred to as segmented block copolymers. This particular molecular architecture, as well as the collective intrinsic properties of each ingredient, contributes to the unique characteristics of the polyurethane when compared to other homo polymers (Changhong, 2006, Hepburn, 1982).

As reported in the literature, the most common formulation for biodegradable polyurethane synthesis is based on the use of hexane diisocyanate (HDI) (Woo et al., 2000), or lysine diisocyanate (LDI) (Han et al., 2011, Hassan et al., 2006, Storey et al., 1994) as the diisocyanate, and a combination of polyethylene glycol (PEG) (Sreenivasan, 1991, Yeganeh et al., 2005), and/or polycaprolactone (PCL) (Bruin et al., 1990, Storey et al., 1994, Gorna et al., 2002, Wang et al., 2003) as the polyl segment. The popularity of these components is probably due to their world-wide acceptance as biocompatible and biodegradable for polyurethane synthesis. There is, however, a lot of variation observed
in literature about the choice and use of novel chemicals/compounds to act as monomers/starting material or chain extenders for biodegradable polyurethane synthesis. Specifically, in case of chain extenders, the choice could be synthetic or naturally occurring compounds and the choice of starting material for the chain extender depends on the properties and application of the resultant polyurethane (Jayakumar et al., 2006), (Tatai et al., 2007).

### 3.1.2 Chain Extenders

The chain extender generally forms a crucial part of the hard segment of polyurethane and by incorporating different types of chain extenders into polyurethane, one can easily manipulate the structural and mechanical properties of that polyurethane to better tailor the material to its future applications (Hepburn, 1991, Nair and Laurencin, 2007, Zhang et al., 2006). Chain extenders are low molecular weight difunctional species that react with diisocyanate to form a linear extended structure in the polymer. As the name suggests, chain extenders are normally used to extend polymeric chains by joining two existing polymer chains (Gunatillake et al., 2003). The role of chain extenders in polyurethane synthesis is shown in Figure 8. The polymer chains linked in this way need not be of the same molecular weight giving rise to diverse and complex final polymer structure.

**Figure 8**: Role of chain extender in polymer synthesis

The functional groups that are typically used in chain extenders for polyurethane synthesis are hydroxyl and amine. The popularity of these functional groups is mainly due to their excellent reactivity with diisocyanate functionality, with each functional group reacting to produce two structurally distinct species. Figure 9 shows the reaction
of the two different functional group (OH/NH\textsubscript{2}) with diisocyanate group. The hydroxyl group reacts with diisocyanate to form a urethane linkage and the amine group reacts to form a urea link. Based on the type of bond present, the resultant polymer is often referred as polyurethane (PU) or polyurethane urea (PUU) (Perrin et al., 1997).

![Figure 9: Reaction of hydroxyl and amine group with the diisocyanate group](image)

There are several commercially available aliphatic dihydroxy and diamine compounds such as 1,4-butanediol (Hong et al., 2007), 1,4-butanediamine (Guan et al., 2004) that have been used as chain extenders for PU/PUU synthesis. Chain extenders can contribute to the overall physiochemical properties of polymer, so are also often custom made to suit the final polymer properties and the applications.

3.1.3 The Role of amino acid based chain extender in polyurethane biocompatibility properties

As mentioned earlier in section 3.1.2, chain extenders can be synthesised from naturally occurring molecules or can be chemically designed to fulfil the end-polymer requirements. One such requirement which has been attracting considerable recent interest is the design and development of novel biocompatible and biodegradable polymers for tissue engineering applications (Gunatillake and Adhikari, 2003, Jagur-Grodzinski, 2006). Amino acid based diisocyanates such as LDI and other biocompatible monomers such as HDI, PEG, and PCL have been used as starting materials to produce such biodegradable polyurethanes (Lakshmi and Cato, 2006). To further enhance the biocompatibility of the resultant polyurethane, there is an increase in the use of biocompatible chain extenders such as those synthesised from naturally occurring
compounds, e.g. L-amino acids and peptides. This approach will also help to improve the biodegradability of the polymer. Thus a bio-compatibility related shift has been observed from using the conventional (1,4-butanediol) compounds to naturally occurring and synthetically modified molecules as chain extenders or as a part of polyurethane composition. For example, L-amino acids (Skarja, 1998, Sarkar et al., 2009, Marcos-Fernández et al., 2006), peptides (Parrag and Woodhouse, 2010, Silva et al., 2003), monosaccharide or oligosaccharide such as glucose (Zetterlund et al., 1997), sucrose (Jhurry and Deffieux, 1997), polysaccharide chitin (Silva et al., 2003, Atef El-Sayed et al., 2010), cellulose (Hanada et al., 2001), starch (Kim et al., 2007, Kendaganna Swamy and Siddaramaiah, 2003, Alfani et al., 1998), alginate (Yun et al., 2007) and amino diol (Li et al., 2002, Biemond et al., 2011) amongst others, have been used for polyurethane synthesis.

L-amino acid based chain extenders synthesis will be the focus of this thesis and have been reported previously in the literature (Chapter 2, section 2.8). For example, Kastrava et al. (1999) has reported on the use of straight chain aliphatic diols such as 1, 4-butanediol, 1, 3-propanediol and 1, 6-hexanediol and esterified with L-amino acids such as L-valine, L-leucine, L-isoleucine, norleucine, L-phenylalanine and L-methionine amino acid to synthesize amino acid based diamine ester compounds and later use them as chain extenders in the preparation of polyester amides and polyurethanes (Kartvelishvili et al., 1997, Katsarava et al., 1999, Kartvelishvili et al., 1996), Skarja et al. (2000) have recently illustrated the synthesis and the use of amino acid based diamine diester as chain extenders for the preparation of biodegradable polyurethanes. Guelcher et al. (2005) has reported the synthesis of tyrosine and its derivative tyramine based polyurethane synthesis. Amino alcohol (2-amino-1-ethanol) based chain extenders have been used to formulate biodegradable polyurethane (Caracciolo et al., 2008). Sarkar et al. (2008) ; and others (Marcos-Fernández et al., 2006) have reported the synthesis of L-tyrosine, L-lysine and L-ornithine amino acid based chain extenders respectively and later used them for segmented biodegradable polyurethane synthesis. More recently, Parrag et al. (2010) has exemplified the synthesis of a peptide based chain extender and its incorporation into polyurethane.
Following on from this literature trend (as discussed in Chapter 2, section 2.8), this chapter details the synthesis and characterisation of L-amino acid based diamine and L-Z-amino acid based dihydroxy ester compounds, some of which are novel. These compounds will then be used as chain extenders for polyurethane synthesis and this will be discussed in the following chapter 4.

3.1.4 Strategy to be used for the synthesis of amino acid based chain extenders

In this chapter, the reaction scheme devised by Huang et.al. (1979) and Skarja et.al. (2000) was followed to synthesise amino acid based diamine ester compounds which were later used as chain extender for polyurethane urea synthesis. The reaction scheme is originally based on the Fischer esterification reaction which includes the reaction of an acid with alcohol to form ester bond and water is released as by product. The reaction scheme is based on the use of L-amino acid as acid and an aliphatic dihydroxy compound as alcohol to form the ester compound. A Lewis acid is used as a catalyst for the reaction. In this chapter, a series of ester derivatives are synthesised based on different L-amino acids. For example, L-leucine, L-valine, L-isoleucine, L-tyrosine L-phenylalanine amino acids are used during esterification reaction and reacted with an aliphatic dihydroxy compound (1,8-octanediol) to synthesise different amino acid based diamine ester series.

L-Z-amino acids were used to synthesis dihydroxy ester compounds which were later used as chain extenders for polyurethane synthesis. The amine (NH₂) group of L-amino acid was protected by a benzyloxy carbonyl group (Z). The reaction was carried out by reacting the caesium salt of L-Z-amino acid with an alkyl halide to synthesise dihydroxy ester compounds. This method has been used in the past for solid state peptide synthesis (Gisin, 1973). L-Z-serine, L-Z-threonine and L-Z-tyrosine amino acids were used, in this chapter, to synthesis a novel series of dihydroxy ester based compounds for later use as chain extenders.

The main objective of the work discussed in this chapter is to describe the methods of synthesis and characterization of L-amino acid based diamine/dihydroxy ester compounds. In developing these compounds and then incorporating them as chain extenders into PU and PUU, ester functionality and naturally occurring amino acids will
be incorporated into the polymer (PU/PUU) backbone, making them more susceptible to both hydrolytic and amino acid specific enzymatic degradation (Shah et al., 2009) for future work.

However this thesis is mainly focused on the synthesis of amino acid based ester compounds (with diamine and dihydroxyl functional groups) and to incorporate them as chain extender in polyurethane synthesis. Later on, their effect on the physio mechanical properties of the synthesised polymer will be studied. The chemical structures of the L-amino acids and L-Z-amino acids to be used for the synthesis of the L-amino acid based ester compounds described in this chapter, are shown in Figure 10.

![Chemical structure of L-amino acids used in the current research work](image)

**Figure 10**: Chemical structure of L-amino acids used in the current research work

The uniqueness of this part of research work is the innovation of the L-amino acid based ester compounds. These L-amino acid based diamine esters and dihydroxy ester compounds are synthesised for the first time and later used (for the first time), as chain extenders, in polyurethane and polyurethane urea synthesis (see chapter 4). L-amino acids such as phenylalanine (Skarja and Woodhouse, 2002) tyrosine (Sarkar et al., 2008), ornithine (Guelcher et al., 2005), and serine (Sosa et al., 1985, Sarkar et al., 2009) have been used to synthesise chain extenders in the past and used for polyurethane synthesis. However, to the best of this author’s knowledge, the novel synthesised amino acid based
ester compounds reported in this chapter have not been reported before. The novel dihydroxy ester compounds synthesised using the above mentioned L-Z-amino acids are also reported here for the first time.

3.2 Materials

Chemicals such as L-valine, L-leucine, L-isoleucine, L-phenylalanine, L-tyrosine, Benzyloxy carbonyl (Z)-L-serine, Z-L-threonine, Z-L-tyrosine and 1,8-octanediol, 1,4-diodobutane, hydrogen bromide/acetic acid solution, were obtained from sigma Aldrich, USA and used as received. Monohydrate p-toluene sulphonic acid (p-TsOH) and caesium carbonate were obtained from Merck, Sydney. Benzene as reaction solvent was obtained from sigma Aldrich, USA. All other solvents such as ethanol, chloroform and chemicals used for purification methods were of analytical grade and were obtained from sigma Aldrich, USA. Deionised water was used for all purposes. Unless stated otherwise, all reactions were carried out in oven dried glassware and under nitrogen atmosphere. Chemical reaction temperatures were controlled using an IKA brand magnetic temperature modulator.

3.3 Experimental Procedure

The following section describes in detail of the experimental procedure followed and characterization techniques used for the synthesis of L-amino acid based diamine and dihydroxy ester compounds.

3.3.1 Synthesis of diamine ester compounds

3.3.1.1 Octane-1, 8-diyl bis (2-amino-4-methylpentanoate) (311)

1, 8-octanediol (309) (4.02 g, 0.0275 mol) was dissolved in 50 mL of benzene. L-leucine (301) (9.53 g, 0.057 mol) was added to the reaction mixture following the addition of p-toluene sulphonic acid (13.06 g, 0.0687 mol). After the addition, the reaction mixture was allowed to stir under reflux for 24 hr at 110°C. The collection of water as side product using a Dean Stark tube favoured the forward reaction. The mixture was cooled to room temperature and the solvent removed under reduced pressure. Crude product in the form
of tosylate salt was obtained and dried under vacuum for 24 hours. The crude product was purified by washing three times with 60°C distilled water and anhydrous ethanol. The white tosylate salt of L-leucine amino acid (310) was dried under vacuum at 60°C for 48 hours. The tosylate salt of L-leucine (310) was converted to free diamine ester following the purification scheme reported by Skarja et al. (2000). The tosylate salt was added to the distilled water at 90°C with continuous stirring following the slow addition of molar excess of sodium bicarbonate to the solution. The diamine diester compound (311) (which is not soluble in water) appeared as an oil and floated to the top of the aqueous layer. The oil was extracted with chloroform (5x100 mL) using a separating funnel. The organic layer (oil dissolved in chloroform) was washed with brine and dried over sodium sulphate, filtered and solvent removed under reduced pressure. A light yellow colour oil was obtained as product (311) with 80% yield.

Based on the synthetic method describe above for compound 311, a series of different L-amino acids (302, 303, 304, 305, Figure 10) were converted to their diamine ester derivatives and referred as compound 312, 313, 314 and 315 respectively with 80% yield in all cases. The chemical structure of the synthesised compounds is shown in Figure 11. The detail characterization results for compound 312, 313, 314 and 315 are reported in Appendix A at the end of the thesis.

3.3.2 Synthesis of dihydroxy ester compounds

3.3.2.1 Butane-1,4-diylbis(2-(benzyloxycarbonylamino)-3-hydroxypropanoate)

(318)

In the first step, an aqueous solution of L-Z-serine amino acid (306) (1.0 g, 4.18 x 10⁻³ mol) in 12 mL of absolute ethanol and 6 mL of distilled water was titrated to neutral pH 7.0 by drop wise addition of 15 mL of 20% (w/v) caesium carbonate solution. Stirring was continued throughout the whole procedure. After the titration, the solvent (ethanol and water) was evaporated under reduced pressure and compound caesium salt of L-Z-serine amino acid (316) was obtained as a white solid material.

In the second step, to a solution of 316 (12.0 g, 0.032 mol) in 20 mL DMF under inert atmosphere at 50°C, 1,4-iodobutane (317) (2.5 g, 8.066 x 10⁻⁶ mol) was added using a
syringe into the reaction mixture. After the addition, the solution was allowed to stir for 3 hours. The reaction mixture was cooled to room temperature. The crude product was obtained by adding 10 mL of water to the reaction mixture and the aqueous layer extracted with dichloromethane (3 x 10 mL). The combined organic solution was washed with 100 mL water, dried with sodium sulphate, filtered and the solvent removed under reduced pressure. The residue was chromatographed (hexane: acetone, 6:4 ratio) to obtain the product **318** as colourless solid with 85% yield.

Based on the synthetic method described above for compound **318**, two other L-amino acids (**307, 308**) are converted to their corresponding dihydroxy ester derivatives and referred as compound **319, 320** with 85% yield in all cases. The chemical structure of the synthesised compounds is shown in Figure 20. The detail characterization results for compounds **319** and **320** are reported in Appendix A at the end of the thesis.

### 3.4 Characterization

#### 3.4.1 Nuclear magnetic resonance spectroscopy

Unless otherwise specified, all $^1$H and $^{13}$C Nuclear magnetic resonance spectroscopy (NMR) spectra were recorded at room temperature (25°C) using a Bruker Av400 spectrometer at 400 MHz and 100.6 MHz respectively, or a Bruker Av200 spectrometer at 200 MHz and 50 MHz respectively. Chemical shifts (δ) are measured in parts per million (ppm) using known solvent (deuterated dimethylsulphoxide, d$_6$-DMSO) chemical shifts as an internal reference standard (2.49ppm for $^1$H-NMR and 39.5ppm for $^{13}$C-NMR for the (DMSO-d$_6$)). $^1$H-NMR spectroscopic data are recorded as follows: chemical shift (δH) (relative integral, multiplicity) whereby multiplicity is defined as: s for singlet; d for doublet; t for triplet; q for quartet or quintet; m for multiplet, or combinations thereof.

#### 3.4.2 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra were obtained using a ThermoNicolet 6700 spectrometer using a SmartATR (attenuated total reflectance) attachment fitted with a diamond window. The sample was analysed (32 scans) at 25°C in the transmission mode over 4000-700 cm$^{-1}$.
3.4.3 Mass Spectroscopy
Electrospray mass spectra were recorded on a Thermo scientific Q-Exactive FTMS instrument using electrospray ionization (ESI) employing 35 eV cone voltage, employing lock spray and sodium iodide as a reference sample.

3.4.4 Thin layer chromatography
Thin layer chromatography (TLC) was performed using 0.25 mm thick plates precoated with Merck Kieselgel 60 F254 silica gel, and visualized under ultra violet (UV) light (254nm and 365 nm). All organic compounds were named according to the IUPAC guidelines.

3.5 Results and Discussion

3.5.1 Synthesis of L-amino acids based diamine ester compounds
The Fischer esterification reaction was used to synthesise diamine ester compounds. The Fischer (sometimes called Fischer-Speier) esterification is a condensation reaction first described by Emil Fischer and Arthur Speier in 1895 (Lamba et al., 1998). It is an example of a nucleophillic acyl substitution reaction based on the electrophilicity of the carbonyl carbon atom and nucleophilicity of the alcohol. Basically, 2 molecules of L-amino acid was reacted with 1 molecule of linear dihydroxy compound in the presence of excess p-Toluene sulphonic acid to form diamine diester compound. p-Toluene sulphonic acid (p-TsOH) was used to catalyse the esterification reaction. Scheme 2 depicts the synthetic pathway of diamine ester compound 311 based on compound 301 and 309. Benzene was used as solvent and the reaction was carried out under reflux conditions at 110°C for 24 hours.
Scheme 2: Schematic representation of the synthesis of diamine ester compound 311

The acid catalyst plays two important roles in this reaction, as described below, and to fulfil its role, a small excess of acid catalyst monohydrate \( p\)-toluene sulphonic acid (\( p\)-TsOH) was used.

Firstly, it acts as a catalyst for the esterification reaction by increasing the electrophilicity of the carbonyl oxygen of the amino acid and thus enhancing nucleophilic attack by the alcohol.

Secondly, it protonates the amine group of the amino acid to form a tosylate salt which prevents that group from participating in side reactions which could lead to unwanted by-products and decreased yields.

Water is released as a side product of the reaction and thus the reaction was driven to completion by trapping the released water in a Dean - Stark tube. Crude product in the form of tosylate salt 310 was obtained by evaporating benzene under reduced pressure and purified by following the purification scheme developed by Skarja et.al. (2000).

In brief, the crude product 310 was dried under vacuum and dissolved in distilled water at 90°C. Neutralisation of the acid was performed in order to obtain the tosylate free diamine diester compound 311. Sodium bicarbonate (\( \text{Na}_2\text{CO}_3\)) was used as the base to
neutralise the crude product 310. Release of gas, presumably carbon dioxide, and a change in pH of the solution was observed during titration.

Sodium bicarbonate breaks the ionic interactions between p-toluene sulphonic acid and the amine group of the amino acid. This results in the formation of a water soluble sodium salt of p-toluene sulphonic acid. The water insoluble diester diamine appears as oil on the surface of aqueous layer. The purified diamine ester product 311 was obtained through organic solvent extraction as described in the experimental procedure section 3.3.

Based on the synthetic pathway described in scheme 2, L-amino acids 302, 303, 304, and 305 were esterified using compound 309 as linking diol to synthesise a series of diamine ester compounds, specifically 312, 313, 314 and 315 respectively. The chemical structure of the resultant diamine ester compounds is shown in Figure 11. The pure compounds 311, 312, and 313 were a light yellow oil in appearance whilst compounds 314 and 315 were colourless solids. In all cases, the yield was ~80% and the products were stored in a desiccator until further use.

**Figure 11**: Structures of synthesised L-amino acids based diamine ester compounds
The detailed reaction mechanism of the Fischer esterification is shown in Figure 12 (Lamba, 1998).

The reaction mechanism involves several steps:
1. Proton transfer from the acid catalyst ($p$-TsOH) to the carbonyl oxygen of the amino acid increasing the electrophilicity of carbonyl carbon atom.
2. The carbonyl carbon is attacked by the nucleophillic oxygen atom of the alcohol.
3. Proton transfer from the oxonium ion to a second molecule of the alcohol gives an activated complex.
4. Protonation of one of the hydroxyl group of activated complex gives a new oxonium ion.
5. Loss of water from this oxonium ion and subsequent deprotonation gives the ester.

![Figure 12: General reaction mechanism of Fischer esterification reaction](image)

Figure 12: General reaction mechanism of Fischer esterification reaction
As mentioned in section 3.1.4, synthesis of diamine diester compounds using naturally occurring L-amino acids has been previously reported. For example, Huang et al. (1979) used ethylene glycol as the linking diol and L-phenylalanine as amino acid. Skarja et al. (1998) used 1,4-cyclohexane dimethanol as the linking diol and phenylalanine as the amino acid, and also used the combination of 1,6-hexanediol with Z-lysine amino acid to synthesise diamine ester compounds. They reported the use of these compounds as chain extenders for polyurethane synthesis (Skarja and Woodhouse, 2000). However, the diamine ester compounds reported in the literature as of today differ in chemical structure to those reported in this thesis based on the choice of the linking diol (1,8-octane diol, 309) and its combination with L-amino acid for the esterification reaction.

3.5.2 Characterization

To authenticate the structure of the synthesised diamine ester compounds, $^1$H and $^{13}$C-nuclear magnetic resonance (NMR) spectra were obtained. Fourier transform infrared Spectroscopy (FTIR) and Mass spectroscopy (MS) were another techniques used to confirm the synthesis of targeted compounds. NMR peak assignments were based on the spectra of the constituent starting materials, in comparison to reference spectrum of similar molecules and evaluation of integrated peak area. Peak assignments from $^1$H-NMR and $^{13}$C-NMR were used to show the successful synthesis of targeted compounds (or otherwise). Unless otherwise stated, deuterated dimethylsulphoxide (d$_6$-DMSO) was used as solvent for NMR spectroscopy. Conclusive proof of successful synthesis is, however, not always clear due to the following:

- The presence of similar chemical environments for different protons and carbon atoms within the structures, resulting in considerable overlap of peaks making assignment a difficult task.
- Due to the difference in the solubilities of the synthesised ester compounds with respect to their corresponding L-amino acids, the $^1$H-NMR of L-amino acids could not be obtained in d$_6$-DMSO (solvent for NMR spectroscopy) and hence L-amino acids $^1$H-NMR could not be used to analysed final product.

Nevertheless, Fourier Transform Infrared Spectroscopy (FTIR) and Mass Spectroscopy (MS) were performed and were adequate to prove the structure of all synthesised
compounds (i.e. those for which $^1$H-NMR was challenging). Furthermore, $^1$H-NMR spectra for the starting material (309) and crude tosylate form of the final compound (soluble in d$_6$-DMSO) were able to be performed and were consistent with the predicted structure. The use of a model compound and its $^1$H-NMR spectrum to further provide the evidence for the synthesis of targeted ester derivatives is also reported. Model Compound of known composition was used as a control to confirm the structure of the amino acid based ester compounds via $^1$H-NMR spectroscopy.

To avoid the repetition of text, only one aliphatic diamine ester compound 311 and one aromatic diamine ester compound 314 are described here in detail and only $^1$H-NMR and FTIR and MS characterization results are discussed here in detail and $^{13}$C-NMR data for 311 and 314 are given in Appendix A at the end of thesis. Compounds 311 and 314 were chosen at random for full description here. The characterization results ($^1$H-NMR, $^{13}$C-NMR, FTIR, MS) for the rest of the synthesised final compounds (312, 313 and 315) are also reported in Appendix A.

3.5.2.1 Octane-1, 8-diyl bis (2-amino-4-methylpentanoate) (311)

3.5.2.1.1 $^1$H-Nuclear Magnetic Resonance Analysis

In Figure 13, the $^1$H-NMR spectrum of the purified compound 311 (spectrum B) was compared with the spectrum of the starting material 309 (spectrum A) and to the tosylate form 310 (spectrum C).

The multiplet centred at 4.0ppm in spectrum B, (shown in the inset) is attributed to the terminal methylene protons (a) of 309 which are adjacent to ester functionality. Since these protons are close to the reactive site, they may be used to evaluate the success of the esterification reaction. It was observed that these terminal methylene protons yield a multiplet centred at 3.3ppm in spectrum A (309) when these protons are present next to the reactive hydroxyl group. As expected, the esterification reaction results in the shifting of the peak for the terminal methylene protons of 309 from 3.3ppm (spectrum A) to 4.0ppm (spectrum B) due to the presence of the ester group which has a greater deshielding effect in comparison to the hydroxyl group in 309. Spectrum B in Figure 13
clearly shows the presence of multiplet (shown in inset) at 4.0ppm and this shift has clearly indicated the synthesis of ester linkage.

\[
\delta/\text{ppm (d}_6\text{ DMSO)}
\]

**Figure 13 :** $^1$H-NMR spectrum of 310 (spectrum C), 311 (spectrum B) is shown in comparison with compound 309 (spectrum A)

A similar observation was reported by Skarja *et al.* (1998) during the synthesis of 305 based diamine diester compound. The six terminal methyl protons (i, h) appear as a quartet at 0.9ppm in spectrum B, 311. Due to multiple overlap, the protons (b, c, d, f, g) of 311 (spectrum B) appear between 1.4ppm to 1.7ppm, the overall peak integration for these 9 protons (b, c, d, f, g) was performed and compared to the peak integration of the other assigned protons. The complete removal of acid catalyst $p$-toluene sulphonic acid ($p$-TsOH) from 311 (spectrum B) was confirmed by the disappearance of $p$-TsOH proton signals in the NMR spectrum of 311. For example, the four aromatic protons (k,j) and
three methyl protons (I) of \( p \)-TsOH present in spectrum C \((310)\) at 7.2ppm, 7.5ppm and at 2.3ppm respectively were absent in the spectrum of pure \( 311 \) (spectrum B). Peak integration data for \(^1\text{H}-\text{NMR}\) spectrum of \( 311 \) is shown in Table 2 and reference to NMR tables confirmed the assignment of protons for \( 311 \).

**Table 2 :** Peak integration data obtained for \(^1\text{H}-\text{NMR}\) spectrum of \( 311 \)

<table>
<thead>
<tr>
<th>Proton</th>
<th>Chemical Shift (ppm)</th>
<th>Peak area</th>
<th>No of Protons (Stoichiometric Prediction)</th>
<th>Area/Proton</th>
</tr>
</thead>
<tbody>
<tr>
<td>i and h</td>
<td>0.8-0.9</td>
<td>6.00</td>
<td>6</td>
<td>3.0</td>
</tr>
<tr>
<td>b, f and g</td>
<td>1.5-1.8</td>
<td>4.81</td>
<td>5</td>
<td>0.962</td>
</tr>
<tr>
<td>e</td>
<td>3.2</td>
<td>1.06</td>
<td>1</td>
<td>1.06</td>
</tr>
<tr>
<td>a</td>
<td>4.0</td>
<td>1.99</td>
<td>2</td>
<td>0.995</td>
</tr>
<tr>
<td>c and d</td>
<td>1.3-1.4</td>
<td>5.06</td>
<td>4</td>
<td>1.26</td>
</tr>
</tbody>
</table>

The \(^1\text{H}-\text{NMR}\) spectrum of \( 311 \) displays all the expected structural features of the targeted compound and this demonstrates successful formation of ester linkage and hence expected diamine ester compound \( 311 \). However NMR is not necessarily used to quantify purity, however the lack of peaks for \( 301 \) would suggest that any starting material present must be less than 1% of the final product \( 311 \). Similarly, there are no other peaks unaccounted for which would indicate significant side-product formation. The final product \( 311 \) is therefore estimated at better than 99% pure.

### 3.5.2.1.2 Fourier transform Infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) analysis was performed to determine the presence of specific functional groups such as ester (C=O) and free amine (NH\(_2\)) in compound \( 311 \). The starting material, L-leucine amino acid \((301)\) also contains similar groups and hence to distinguish the carboxyl (C=O) peak present from either ester \((314)\) or acid \((301)\) group, the FTIR of both the compounds were compared and discussed in detail. This analysis further supports the absence of starting material, \( 301 \) in pure synthesised \( 311 \). As mentioned earlier, it was not possible to demonstrate this in regards to the corresponding amino acid \((301)\) through \(^1\text{H}-\text{NMR}\) spectroscopy, due to NMR solvent solubility issues.
It is generally known that L-amino acid (301 in this case) exists in its zwitter ion form in the solid state. The zwitter ion form of 301 is shown in Figure 14.

![Zwitter ion form of L-leucine amino acid (301)](image)

**Figure 14**: Zwitter ion form of L-leucine amino acid (301)

The carboxyl group exists as the carboxylate anion, -CO$_2^-$ and the amine group exists as the ammonium ion -NH$_3^+$. It is anticipated that the peaks associated with these groups will appear at different positions as compared to acid (COOH) and amine (NH$_2$) group present in the compound 311. The IR spectra of 301 and 311 are collectively shown in Figure 15.

![FTIR spectrum of 301 (spectrum A) and 311 (spectrum B)](image)

**Figure 15**: FTIR spectrum of 301 (spectrum A) and 311 (spectrum B)

The discussion mainly includes the carboxyl and amine group peak position in 301 and 311. The FTIR spectrum A shown in Figure 15 is for L-Leucine amino acid (301)
and spectrum B is for 311. The selected FTIR peak positions for both 301 and 311 are recorded in Table 3. It has been reported in the literature (Wade, 1987) that when ionization occurs in the carboxyl group (COO\(^-\)) of amino acids, resonance is possible between the two C-O bonds. In consequence, the characteristic carbonyl (C=O) absorption (1730 – 1755 cm\(^{-1}\) region) vanishes and is replaced by two bands between 1600 - 1550 cm\(^{-1}\) and between 1400-1300 cm\(^{-1}\), which corresponds to the antisymmetrical and symmetrical vibrations of the COO\(^-\) structure. Similar observations were recorded when the 301 and 311 IR spectra are compared. Compound 301 shows characteristic peak for COO\(^-\) structure in the region of 1600 cm\(^{-1}\). The peak observed at 1511, 1576 and 1605 cm\(^{-1}\) corresponds to the asymmetric stretching of COO\(^-\), \(\nu_{\text{As}}(\text{CO}_2^-)\) and 1405 cm\(^{-1}\) corresponds to symmetric stretching of carboxylate anion \(\nu_{\text{s}}(\text{CO}_2^-)\).

The lack of signals in the region 1730 – 1755 cm\(^{-1}\) (characteristic of C=O stretching vibrations) demonstrate that 301 has lost the acidic carbonyl group in favour of a carboxylate ion COO\(^-\) group. In the case of pure 301 (FTIR spectrum A), we can see the characteristic NH\(_3^+\) stretching vibrations (broad band) at 3042 cm\(^{-1}\) \(\nu(\text{NH}_3^+)\); the asymmetric/symmetric N-H bending of NH\(_3^+\) group appears at 1605 cm\(^{-1}\) \(\delta_{\text{AS}}(\text{N-H})\), and at 1511 cm\(^{-1}\), \(\delta_{\text{S}}(\text{N-H})\) respectively. Out of plane vibrations for COO\(^-\) group \(\gamma(\text{COO}^-)\) appears at 838, 848 cm\(^{-1}\). However, a weak peak at 2617 and 2569 cm\(^{-1}\) appears in the FTIR of 301 and this corresponds to the acid, COOH, group of the amino acid. Sometimes a very small percentage of carboxylate anion, (COO\(^-\)) groups are also present as acid groups (COOH). The conversion of COO\(^-\) to COOH is very fast and thus although the peak for COOH appears in the IR, the main species present is the carboxylate anion COO\(^-\). Similar observations for FTIR data of 301 have been reported in the literature (Grosan et al., 2012), (Façonha Filho et al., 2008).

In the case of 311, it can be seen that the vibrational frequencies of both N-H and COO\(^-\) group have changed. Specifically, the symmetrical and asymmetrical N-H bending (1605-1511 cm\(^{-1}\)) from the zwitter ion structure of L-Leucine (301) have disappeared. Instead, a strong band appears at 1600 cm\(^{-1}\), which is due to \(\delta(\text{N-H})\)
vibration from the amine NH₂ moiety. Additional bands at 3376, 3305cm⁻¹ are characteristic of the primary amine functional group in 311.

A frequency shift is also seen case of the vs(CO₂⁻) band and the vas(CO₂⁻) band. A very distinctive sharp peak at 1730cm⁻¹ for C=O of an ester group and stretching of C-O of an ester at 1170cm⁻¹ was observed in 311. These changes clearly confirm that the L-Leucine amino acid (301) COO⁻ group has been converted into ester group in 311. The absence of COOH/COO⁻ peaks in the FTIR of 311 also demonstrate the purity of the synthesised compound 311.

**Table 3**: Selected FTIR frequencies (cm⁻¹) of 301 and its ester derivative 311

<table>
<thead>
<tr>
<th>302</th>
<th>Assignment</th>
<th>311</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1405</td>
<td>Symmetrical COO⁻ stretch: (\nu_s(COO^-))</td>
<td>1385, 1170</td>
<td>Symmetrical COO⁻ stretch: (\nu_s(COO^-))</td>
</tr>
<tr>
<td>1511</td>
<td>Symmetrical NH₃⁺ bending: (\delta_s(N-H))</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1511, 1576, 1605</td>
<td>Asymmetrical COO⁻ stretch: (\nu_{as}(CO₂^-))</td>
<td>1730</td>
<td>Asymmetrical COO⁻ stretch: (\nu_{as}(CO₂^-))</td>
</tr>
<tr>
<td>1605</td>
<td>Asymmetrical NH₃⁺ bending: (\delta_{as}(N-H))</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1600, 3376, 3305</td>
<td>NH₂ bending: (\delta) (N-H)</td>
</tr>
<tr>
<td>3042</td>
<td>NH₃⁺ stretching: (\nu) (NH₃⁺)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2958, 2865, 2979, 1384, 1405, 1385, 1469, 1453</td>
<td>Aliphatic C-H group</td>
<td>2857, 2930, 2953, 1467, 1403</td>
<td>Aliphatic C-H group</td>
</tr>
<tr>
<td>923</td>
<td>C-C group</td>
<td>963</td>
<td>C-C group</td>
</tr>
<tr>
<td>1082, 1033</td>
<td>C-N group</td>
<td>1082, 1033</td>
<td>C-N group</td>
</tr>
<tr>
<td>838, 848</td>
<td>(\gamma(COO^-))</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Vibration mode: \(\nu\) = stretching, \(\delta\) = bending, \(\omega\) = wagging, \(s\) = symmetrical, \(AS\) = asymmetrical
Overall it was observed that both the carboxyl and amine groups are present but in slightly different positions for compounds 301 and 311. It can thus be concluded that the synthesised compound (311) is pure and not contaminated with the residual starting material (301). The different form of carboxyl group relating to each compound was well established by FTIR spectra. The conversion of acid into ester group was well demonstrated. In conclusion, we argue that 301 has been fully converted into the ester derivative 311.

3.5.2.1.3 Mass spectroscopy
Additional evidence for the success of the synthetic reaction and purified product was provided by mass spectroscopy (MS (ESI)) data. The calculated molecular weight of the compound 311 (C_{20}H_{40}N_{2}O_{4}) was 372.5432 and obtained MS based relative molecular weight of compound 311 was \textit{m/z} 373.5431 \textit{[M+H]^+} with molecular ion. The accurate isotopic mass of the molecular ion (\textit{^1H}) present is 1 and hence, the subtraction of molecular weight of molecular ion from the relative molecular weight of expected compound, provides an indication that the accurate molecular weight (372.5432) of the targeted product (311) was achieved. Hence, it proves that the synthesised product was pure.

In conclusion, based on \textit{^1H} NMR, FTIR and MS data, compound 311 was synthesised successfully. The yield of the reaction was 80% and the compound was pure (> 99%).

3.5.2.2 Octane-1, 8-diyl bis (2-amino-3(4-hydroxyphenyl) propanoate) (314)
Compound 304 has been used extensively for polymer synthesis. The phenol ring present in the side chain of 304 can provide better mechanical properties to the polymer when used as chain extender as compared to the use of aliphatic chain extender for polymer synthesis. For example, Sarkar \textit{et.al} (2009) and Gupta \textit{et.al} (2004) have reported the synthesis of a tyrosine based desaminotyrosyl tyrosine hexyl ester (DTH) compound and have used it as a chain extender for polyurethane synthesis. A tyrosine based polymer called pseudo (polyaminoacid), has also been studied and described, in detail, by Bourke and Kohn \textit{et.al} (2003). Following a similar trend, in this thesis, L-tyrosine amino acid
was used to make a diamine ester derivative and later used as chain extender for polyurethane synthesis. The reaction scheme is followed to synthesise and the novel chemical structure of is shown in Figure 11.

3.5.2.2.1 $^1$H-Nuclear Magnetic Resonance spectroscopy

The $^1$H-NMR spectrum of the diamine ester was obtained in d$_6$-DMSO (NMR solvent) and is shown in Figure 16. The pre-cursor (L-tyrosine) was insoluble in d$_6$-DMSO and hence $^1$H-NMR spectrum cannot be used for comparison with the $^1$H-NMR spectrum of the diamine ester compound to further confirm the synthesis of . As mentioned earlier (section 3.5.2), L-tyrosine amino acid (304) is only soluble in D$_2$O and the synthesised diamine ester compound (314) is sparingly soluble in D$_2$O. Hence a model compound, based on aromatic amino acid (L-phenyl alanine), was therefore used to obtain $^1$H-NMR in d$_6$-DMSO spectra and used to compare with the $^1$H-NMR (d$_6$-DMSO) spectra of the targeted 314. The commercially available compound used was L-phenylalanine ethyl ester HCl salt (314-M), an ethyl ester derivative of L-phenyl alanine amino acid. Although compound 314 is based on L-tyrosine amino acid (304), hence an L-tyrosine ethyl ester would have been ideal for the comparison purpose but it was unavailable at the time of synthesis. L-phenylalanine (305) is very similar in chemical structure to L-tyrosine amino acids (304) except the former has a benzene group in its side chain and 304 has a phenyl group in its side chain. This can be easily accounted for when comparing the two structures. 314-M was easily available and was also soluble in d$_6$-DMSO, hence was used as the model compound for 314 to examine the appearance of amino acid protons peaks in 314-M $^1$H-NMR spectrum with respect to amino acid based proton peaks appeared in the synthesised compound 314 spectrum. d$_6$-DMSO was used as solvent for 314 and 314-M for $^1$H-NMR spectra. The $^1$H-NMR spectrum of the diamine ester 314 is shown in Figure 16 and $^1$H-NMR spectrum of the 314-M is shown in Figure 17 and describe later in this section. The terminal methylene protons (a), next to the hydroxyl group, of 309 appear at position 3.3ppm (spectrum A of Figure 13). This signal for these protons shifts to 3.9ppm in the $^1$H-NMR spectrum of 314 (Figure 16). The change in shift is due to the deshielding effect of ester bond formation.
Furthermore, there is no peak observed for the presence of terminal methylene proton of 1,8-octanediol (309) at 3.3ppm in the $^1$H-NMR spectrum of compound 314, demonstrating the absence of the starting material 309. The proton (e) present on the chiral carbon atom and next to the amine group of amino acid appears at position 3.4ppm in Figure 16.

![Figure 16: $^1$H-NMR spectrum of compound 314](image)

As observed in the $^1$H-NMR spectrum of 309 (shown in Figure 13), the methylene protons (b, c, d) of the aliphatic dihydroxy appear at 1.4ppm and 1.2ppm and these protons appear at the same position in the $^1$H-NMR spectrum of 314. The presence of two doublet signals for the four aromatic 304 protons (g, h) at 6.6ppm, 6.9ppm and a quartet signal for the two benzyl methylene protons (f) at 2.7ppm are further evidence to confirm the synthesis of the 314. The ratio of the area of the peak for terminal methylene protons (a) of 309 to the area of peak for the chiral carbon proton (e) of 304 was 2:1 respectively, as expected.
Peak integration data for $^1$H-NMR spectrum of 314 is shown in Table 4 and reference to NMR tables confirmed the assignment of protons for 314. This supports the hypothesis that the synthesised targeted diamine ester product 314 was pure and without traces of the starting material (304, 309). Due to multiple overlaps between c and d, the overall peak integration for these 4 protons were performed and compared to the integration values for the other assigned protons.

**Table 4**: Peak integration data obtained for $^1$H-NMR of compound 314

<table>
<thead>
<tr>
<th>Proton</th>
<th>Chemical Shift (ppm)</th>
<th>Peak area</th>
<th>No of Protons (Stoichiometric Prediction)</th>
<th>Area/Proton</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>3.9</td>
<td>1.95</td>
<td>2</td>
<td>0.97</td>
</tr>
<tr>
<td>b</td>
<td>1.6</td>
<td>1.98</td>
<td>2</td>
<td>0.99</td>
</tr>
<tr>
<td>c and d</td>
<td>1.2</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>e</td>
<td>3.4</td>
<td>0.97</td>
<td>1</td>
<td>0.97</td>
</tr>
<tr>
<td>f</td>
<td>2.7</td>
<td>1.95</td>
<td>2</td>
<td>0.97</td>
</tr>
<tr>
<td>g</td>
<td>6.6</td>
<td>1.93</td>
<td>2</td>
<td>0.96</td>
</tr>
<tr>
<td>h</td>
<td>6.9</td>
<td>1.94</td>
<td>2</td>
<td>0.97</td>
</tr>
</tbody>
</table>

The signals observed in the $^1$H-NMR spectrum of 314 are in full agreement with the predicted structure of 314. Thus the successful synthesis of 314 was concluded.

**Model compound 314 – M**

Figure 17 shows the $^1$H-NMR spectrum of 314-M. The $^1$H-NMR spectrum of 314-M was obtained in d$_6$-DMSO which was the same solvent used to obtain the 314 $^1$H-NMR spectrum.
Both compounds are very similar in structure and are present in the same environment (d<sub>6</sub>-DMSO), however it would still expect some chemical shift of the analogous protons a, e, f because of the salt form of 314-M and the absence of phenol group in 314-M as compared to the diamine structure and phenol group present in 314. It is therefore more focussed on the position of methylene protons (a) related to ethyl ester in 314-M and to 1, 8-octanediol (309) in 314. The methylene protons (a) are present next to an ester group in the synthesised compound 314 and 314-M and present next to a hydroxyl group in the starting compound 309 and hence provide a key assignment to follow the confirmation of 314 synthesis.

It was expected that these protons (a) should appear at the same position in both the targeted (314) and model compound (314-M) due to similar neighbour environment, but in a different position for the starting material 309. Another proton to follow in 314-M spectrum is the proton present on the chiral carbon atom (e). This proton is present next to a carboxyl group in the stating material (304) and next to an ester group in the final compound 314 as well as the model compound 314-M.
A comparison of the $^1$H-NMR spectrum of commercially available 314-M with the synthesised 314 shows, for example, that the methylene protons (a) in both the compounds are present very close to each other (3.9ppm for 314 and 4.0ppm for 314-M) consistent with successful synthesis of 314. The small change in position of the methylene proton (a) in 314-M is probably due to the presence of the methyl group next to this proton in 314-M. Another important proton to look at is the methine proton (e) present on a chiral carbon atom.

As mention above due to the different environment present next to this proton in 314 and 314-M, this proton appeared at 4.2ppm in 314-M and at 3.4ppm in 314 spectra. In general, by comparing the $^1$H-NMR spectra of 314-M to 314, all expected protons at a position quite close to that expected, were observed. Other examples include the quartet signal was observed for two benzyl methylene protons (f) in 314-M at 3.0 – 3.2ppm compared to 2.7ppm in 314, a slight shift in frequency, but consistent with the slight differences in structure between the two compounds and consistent with the same number of such protons in both compounds.

In a similar way, benzyl methylene protons (g, h) appear as two doublet signals at 6.6 – 6.9ppm for the four aromatic protons in 314 and the same protons (g) appear at 7.3ppm in the 314-M spectrum. The slight change in chemical frequency is probably due to the presence of the phenol group in 314 as compared to benzene group present in 314-M. The Table 5 shows the comparison of the peak position of protons appeared in 314 and 314-M $^1$H-NMR spectra.

<table>
<thead>
<tr>
<th>Proton</th>
<th>Compound</th>
<th>a</th>
<th>b</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Shift (ppm)</td>
<td>314</td>
<td>3.9</td>
<td>1.6</td>
<td>3.4</td>
<td>2.7</td>
<td>6.6</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>314-M</td>
<td>4.0</td>
<td>1.1</td>
<td>4.2</td>
<td>3.0 – 3.3</td>
<td>7.3</td>
<td>-</td>
</tr>
</tbody>
</table>

Overall, we can conclude from comparing the $^1$H-NMR spectra of 314 and 314-M, that the $^1$H-NMR spectrum of 314 (Figure 16) displays all the expected structural features of the targeted compound and this confirms the formation of 314. To further support 314...
synthesis, we have also reported the 2-D COSY NMR spectrum of 314 in the following section to show the expected proton coupling in the synthesised compound.

Two dimensional correlation spectroscopy (2-D-COSY)

In the structure of 314 (Figure 18), we expect to observe coupling between the protons on the four equivalent methylene groups (a, b, c, d) of 309 and between the protons at the methine proton (e) and the benzyl methylene protons (f) of 304. Figure 18 shows the 2-D-COSY spectrum of compound 314. The first thing to note about the spectrum is that the 1H-NMR spectrum of the compound being studied is plotted along both the horizontal and vertical axes, and each axis is calibrated according to the chemical shift values (in parts per million, ppm). The COSY spectrum shows distinct spots on a diagonal extending from the upper right corner of the spectrum down to the lower left corner. By extending vertical and horizontal lines from each spot on the diagonal, we can easily see that each spot on the diagonal corresponds with the same peak on each coordinate axis. Lines have been drawn to help identify the correlation. The diagonal peaks serve only as reference points. The important peaks in the spectrum are the off-diagonal peaks.

By extending a horizontal line from the spot at 1.2ppm (which is labelled c, d in Figure 18 and corresponds to the original methylene protons of 309 (Figure 16). This horizontal line eventually encounters an off-diagonal spot (slightly to the left of the same spot in the COSY spectrum) that corresponds to another methylene proton (b) of the original compound 309 at 1.4ppm (b). A vertical line drawn from this off diagonal spot intersects the spot on the diagonal that corresponds to the same methylene proton (b). The presence of this off-diagonal spot, which correlates the methylene proton spot (c, d) and the methylene proton spot (b), confirms that these two protons are coupled to each other as would have expected. A similar result would have been obtained by drawing a vertical line from the 1.4ppm spot (b) and a horizontal line from the 4.0ppm spot (a). The two lines would have intersected at the second off-diagonal spot (at the lower right of the COSY spectrum) showing the correlation between the two protons.
In a similar way, the benzyl methylene protons (f) of the original 304 compound (present at 2.7ppm) are coupled with the methine proton (e) of 304 and the two aromatic protons (g, h) also show coupling on the spectrum at the left side of lower end of spectrum. The vertical and horizontal lines described in this analysis are drawn on the COSY spectrum in Figure 18. The COSY spectrum shows faint other spots, beside the ones discussed previously. These extra spots have much lower intensities than the principal spots on the plot. The COSY method can sometime detect interactions between nuclei that are several atoms apart but still close together spatially to produce off diagonal spots. These minor spots, along with spots due to the solvent (d6-DMSO) and moisture present in the solvent, were ignored for the purpose of compound identification.

From interpretation of the COSY spectrum for 314, it has been demonstrated that all the expected proton coupling was present, further demonstrating the formation of the targeted 314. Hence by analysing the COSY spectrum of 314, the proton couplings observed is in full agreement with the predicted structure of synthesised compound. This was further
supported by $^1$H-NMR spectra of 314 and 314-M compound. Thus the successful synthesis of 314 was concluded.

### 3.5.2.2 Fourier transform infrared spectroscopy

The Fourier transform infrared (FTIR) spectrum of compound 314 is shown in Figure 19. The 2400 – 3600 cm$^{-1}$ region is shown in inset.

![FTIR spectrum of compound 314](image)

**Figure 19**: FTIR spectrum of compound 314

FTIR analysis was performed to determine the presence of amine, ester and phenol groups in the synthesised compound 314. The FTIR spectrum shows a strong peak at 1722 cm$^{-1}$ which is a characteristic feature of carbonyl (C=O) ester linkage. The primary amine (NH$_2$), aliphatic and aromatic C-H group area is shown in the inset of Figure 19. The broad double peak at 3287, 3399 cm$^{-1}$ is attributed to the primary amine (NH$_2$) functional group and may overlap a small hydroxyl peak. The presence of two sharp peaks for the primary amine also indicates the successful generation of free amine from its tosylate salt. It is reported in the literature that tosylate salts yield a broad peak at 2900 cm$^{-1}$ (Wade, 1987, Skarja and Woodhouse, 2000) which was absent in the current FTIR spectrum of 314. The FTIR peaks for the aromatic C-H of the phenol group were present at 2679, 2849 cm$^{-1}$, as shown in the inset in Figure 19. In addition, the aromatic C=C group appears at 1459, 1516, 1595, 1613 cm$^{-1}$ position.
The peak at 2596 cm\(^{-1}\) is due to the aliphatic C-H bond consigned to the aliphatic C-H of compound 314. The C-O stretch for the ester bond was found at 1043, 1185, 1258 cm\(^{-1}\). The distinctive peak for the \(p\)-substituted phenyl ring was observed at 820 cm\(^{-1}\). Overall, it was observed that the spectral characteristic of 314 was found to be in full agreement with the expected FTIR peaks. Thus, the formation of pure 314 was concluded.

**3.5.2.2.3 Mass spectroscopy**

The calculated molecular weight of the compound 314 (C\(_{26}\)H\(_{36}\)N\(_2\)O\(_6\)) was 472.5791 and obtained MS (ESI) based relative molecular weight of compound 314 was \(m/z\) 473.5790 [M+H]\(^{+}\) with molecular ion. The accurate isotopic mass of the molecular ion (H\(^{+}\)) present is 1 and hence, the subtraction of molecular weight of molecular ion from the relative molecular weight of expected compound, provides an indication that the accurate molecular weight (472.5791) of the targeted product 314 was achieved. Hence it proves that the synthesised product was pure.

Overall, it can be conclude that with the supporting evidences obtained from \(^1\)H-NMR, FTIR and MS, the compound 314 was pure > 99% and synthesised successfully with 80% yield.

**3.5.2.2.4 Other Compounds**

In total, 5 different L-amino acid based diamine esters were synthesised using the Fischer esterification reaction (scheme 2). Compounds 311 and 314 were described in detail in section 3.5.1. The remaining synthesised compounds 312, 313, and 315 were the diamine ester derivatives of 302, 303 and 305 amino acid respectively. These products were also analysed by \(^1\)H-NMR and \(^{13}\)C-NMR, FTIR and MS and details are reported in Appendix A. The chemical structures of all the synthesised diamine ester compounds are shown in Figure 11. The synthesised compounds were purified and found to be pure and affording 80% yield in all cases. The aim of the synthesis of these compounds was to use them as chain extender for polyurethane urea synthesis which is described in the next chapter. Here one point needs to be mentioned that out of the above synthesised products, compound 315 was not used further as chain extender for polyurethane urea synthesis. The reason is that this compound is based on L-phenylalanine amino acid and as many
detailed research works has been reported in literature (Skarja, 2001), (Huang et al., 1979) based on the synthesis of L-phenylalanine based chain extender and its incorporation into polyurethane urea as chain extender. Hence it was felt that it is not worth pursuing this compound further for polymer synthesis.

3.5.2 Synthesis of L-Z-amino acids based dihydroxy ester compounds

3.5.2.1 Reaction with caesium salt

The reaction of L-Z-amino acid caesium carboxylate salts with alkyl halide is a very useful reaction for the preparation of L-Z-amino acid based carboxylic esters. Alkali metal carbonates represent one of the most important and widely used classes of weak base in organic synthesis. Often, lithium carbonate (LiCO$_3$), sodium carbonate (Na$_2$CO$_3$), or potassium carbonate (K$_2$CO$_3$) is used, however caesium carbonate (Cs$_2$CO$_3$) has become the first preference as it is less expensive and the reaction is easily carried out under mild conditions and with high yield (Kellogg, 2006).

The first synthetic development using Cs$_2$CO$_3$ were made in the early 1970's by Gisin et. al. (1973) when preparing Merrifield-resins (chloromethylated polystyrene) for solid phase peptide synthesis. Merrifield resin is named after its inventor, Robert Bruce Merrifield (1984 winner of the Nobel Prize in Chemistry), and is used in peptide synthesis via the solid phase peptide synthesis method. The first step in this method is to attach the $N$-amino acid, via a benzyl ester linkage, to the resin and is achieved by the reaction of chloromethylated polystyrene resin with the caesium salt of $N$-Boc (tert-Butyloxycarbonyl) protected amino acid. The caesium salt of Boc-amino acid is more compatible and easier to attach to the polymer resin under mild conditions at room temperature as compared to other sodium, lithium or potassium salts of the amino acid. It is reported in the literature that no racemisation reaction and no deprotection of amino group was observed during esterification reaction. These advantages of using Cs$_2$CO$_3$ has made it a first choice to use for esterification of sensitive compounds such as L-Z-amino acids and peptides. Gisin and Wang et.al.(1977) has reported the use of Cs$_2$CO$_3$ for the esterification of suitably protected amino acid or peptide groups under neutral conditions at room temperature. The reaction was easily carried out and yields were generally very
high (Gisin, 1973). The reported success of Gisin et al. (1977 and 1973), and others (Sharma and Pasha, 1997) with caesium carbonate and alkyl halide based esterification reaction is the basis behind using this method in this thesis to esterify the L-Z-amino acids to synthesise novel dihydroxy ester compounds.

L-Z-serine (306), L-Z-threonine (307), L-Z-tyrosine amino acid (308) were converted to their corresponding dihydroxy ester derivative such as 318, 319, 320 using the above mentioned caesium salt based esterification reaction. The chemical structure of L-Z-amino acid is shown in Figure 10 and the chemical structure of synthesised compounds is shown in Figure 20. The amine (NH$_2$) group of L-amino acid used in the following reaction is protected by a benzyloxy carbonyl group (Z) and the carboxyl group is used to form ester linkage.

### 3.5.2.2 Octane-1, 8-diyl bis (2-amino-4-methylpentanoate) – 318

The synthetic pathway for the synthesis of 318 is shown in Scheme 3. The difunctionalisation reaction contains two steps:

In the first step, L-Z-Serine amino acid (306) was converted to its carboxylate salt 316 using caesium carbonate, Cs$_2$CO$_3$. This was achieved by neutralising the acidic solution (ethanol/water mixture, 2:1) of L-Z-Serine amino acid to neutral pH using an aqueous solution of caesium carbonate (weak base). This step activates the carboxyl group of L-Z-Serine amino acid by converting it into the caesium salt of L-Z-Serine amino acid. After titration, the solvent (ethanol/water) was evaporated under reduced pressure and a white solid material, presumed to be the caesium salt of the amino acid (316), was obtained and stored in a desiccator for the next step. The caesium salt of the amino acid was made in bulk and stored for use in the next step of the reaction.

In the second step, 316 was reacted with 1,4-diiodobutane (317) under an inert atmosphere at the optimised temperature of 50°C for 3 hours. Dimethylformamide (DMF) was used as reaction solvent. The crude product was obtained by solvent extraction with dichloromethane (DCM) from the reaction mixture.
Thin layer chromatography (TLC) of the obtained crude product was done with hexane: acetone (6:4) as mobile phase. The TLC plates were examined under ultra violet (UV) light. Two spots (instead of the expected single spot for the pure compound, 318) were observed for the final crude product mixture obtained in step 2. It was assumed that one spot was for the expected diamine ester compound (318) and the second spot represents an impurity. The two compounds were eventually separated by column chromatography using the same hexane: acetone (6:4) as the mobile phase and pure compound 318 was obtained. The TLC of the pure compound 318 obtained after column chromatography showed a single spot on TLC plates and hence confirmed the purity of synthesised compound. No further purification was undertaken. The percent yield obtained for 318 was 85%.

In the present study, the synthetic route reported in scheme 3 was used for the esterification of compounds 307, and 308 to their corresponding dihydroxy ester compounds 319, 320 respectively. The optimised reaction conditions (temperature and time) were used. The chemical structure of the novel synthesised compound 318, 319 and 320 is shown in Figure 20.
Despite the frequent use of caesium carbonate based methodology in organic synthesis, the actual role of caesium ion in esterification reaction has not, to date, been fully confirmed. It is reported in the ester synthesis literature that a high yield and a faster reaction was observed when caesium carbonate (Cs\(_2\)CO\(_3\)) was used as compared to other available alkali metal salts such as lithium carbonate (Li\(_2\)CO\(_3\)), sodium carbonate (Na\(_2\)CO\(_3\)) or potassium carbonate (K\(_2\)CO\(_3\)) (Strijtveen and Kellogg, 1986), (Dijkstra et al., 1987). Several proposed mechanism regarding the role of caesium have been suggested. For example, Gisin et al. (1973) and Kruizinga et al. (1981) (Kruizinga and Kellogg, 1981) argue that rate of esterification depends on the size of the cation used to generate the carboxylate anion of the amino acid. The larger the size of the cation, the greater the dissociation in polar solvents such as DMF, and thus the greater the nucleophilic activity of the carboxylate ion to displace the halide ion (iodine in the current case) and the greater the reaction rate. Since

**Figure 20**: Chemical structures of L-Z-amino acids based dihydroxy ester compounds

[Chemical structures of L-Z-amino acids based dihydroxy ester compounds]
caesium ion ($\text{Cs}^+$) is the largest ion in size as compare to $\text{Na}^+$, $\text{Li}^+$, $\text{K}^+$ ion and hence practically, it would generate the fastest reaction.

3.5.2.3 Optimisation of reaction conditions

Reaction Temperature and Reaction Time
Room temperature (25°C) was the first choice to carry out caesium carbonate and alkyl halide based esterification reaction as 25°C was the temperature used initially by Gisin and Wang et al. (1977) for solid phase peptide synthesis and amino acid/peptide ester synthesis respectively. The reaction was monitored by TLC with hexane: acetone (6:4) solvent mixture as the mobile phase. It was observed that at room temperature, after 24 hours, the yield of the desired 318 compound was only 50%, with the partially functionalised mono ester product (25%) and unreacted starting material such as 306 and 317 present as significant impurity (25%). The reaction temperature was increased to 30°C, and then in 10°C interval from 30°C to 70°C. It was observed, initially, that as the temperature increased, the desired product 318 appeared as the main product and impurities such as 306, 317 started to disappear. The time for completion of the reaction also gradually decreased as the temperature increased. For example, the time for reaction to complete was 3 hours at 50°C. However, at higher temperatures (60°C and above), degradation products began to appear. As seen on TLC plates, new spots started to appear. No attempt was made to identify these newly emerged impurities; however they were clearly not the desired dihydroxy ester product as seen on TLC plates nor the partially functionalized monohydroxy ester product, or any of the starting materials. The minimum reaction time (3 hour) and max yield (85 %) was obtained at 50°C and hence 50°C was chosen as the optimum temperature and 3 hours as optimum time for this reaction. The result of the reaction temperature and time optimisation is shown in Table 6.

Since the L-Z-amino acids based chain extenders reported in this thesis are novel, there are no literature values of optimization to directly compare against. However, Sharma et al. (1997) has reported the synthesis of Boc protected amino acid esters by the use of caesium carbonate and Merrifield resin and also optimised the reaction conditions such as the temperature, time and solvent. Their research group has reported that at room temperature, esterification took 8 hours to complete whilst it only took 3 hours at 50°C. This result is
consistent with the current observation reported in this thesis in terms of reaction
temperature and time optimisation studies and this was then used as standard optimum
reaction temperature and time for all the L-Z-amino acid based dihydroxy ester compounds
synthesised in this thesis.

**Table 6 : Optimisation of reaction temperature for alkali metal salt based reaction**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (hours)</th>
<th>Dihydroxy Ester (final product) Yield (%)</th>
<th>Monohydroxy Ester product (impurity) Yield (%)</th>
<th>Starting material (impurity) Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>24</td>
<td>50</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>60</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>40</td>
<td>6</td>
<td>70</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>85</td>
<td>15</td>
<td>ND</td>
</tr>
<tr>
<td>60</td>
<td>2</td>
<td>60</td>
<td>unknown impurities started to appear</td>
<td>ND</td>
</tr>
<tr>
<td>70</td>
<td>2</td>
<td>60</td>
<td>unknown impurities started to appear</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND- Not detected

Another parameter of importance when carrying out this reaction is the anhydrous
reaction condition. This reaction is moisture sensitive and small quantities of moisture in
either the reaction solvent (DMF) or in the amino acid based caesium salt can significantly
and detrimentally impact on the reaction yield. The moisture (H⁺ and OH⁻ ions) present
in the second step of the reaction can lead to the conversion of the carboxylate ion (active
form, nucleophile) to the inactive carboxylic acid (COOH) and the caesium ion could be
converted into caesium hydroxide (CsOH). These two species (COOH/CsOH) are then
very difficult to remove from the reaction mixture in step 2 of the esterification reaction
(Scheme 3). As reported in the literature (Sharma and Pasha, 1997), and experienced in
the current work, it is recommended that this reaction should be carried out under a
nitrogen atmosphere in order to avoid moisture contamination in the reaction.

To summarise, unless otherwise stated, the reaction temperature for the synthesis of
compound **318** was 50°C for 3 hours and reaction was carried out under inert nitrogen
atmosphere with 85% yield.
3.5.2.4 Characterisation

$^1$H-NMR, $^{13}$C-NMR, FTIR and mass spectroscopy (MS) data were obtained to confirm the synthesis and purity of final compounds. As shown in Figure 10, the chemical structure of 306 and 307 amino acid are quite similar, hence their respective dihydroxy ester based compounds namely 318 and 319 are also structurally similar. To avoid repetition, therefore, only one aliphatic ester compound 318 and one aromatic ester compound 320 are described in detail, and only $^1$H-NMR, FTIR and MS results of these two compounds, are discussed here in detail. $^{13}$C-NMR data for 318 and 320 can be obtained from Appendix A. Analysis of the compound 319 with full characterisation data is also reported in Appendix A at the end of thesis.

3.5.2.4.1 Butane-1,4-diylbis(2-(benzyloxycarbonylamino)-3hydroxypropanoate)-318

3.5.2.4.1.1 $^1$H-Nuclear Magnetic Resonance spectroscopy

In Figure 21, $^1$H-Nuclear Magnetic Resonance ($^1$H-NMR) spectra of 317 (spectrum A), 306 (spectrum B) and 318 (spectrum C) are shown. $d_6$-DMSO was used as the NMR solvent. The $^1$H NMR spectra shown in Figure 21 can be interpreted using peak position of the terminal methylene proton (a) of 317 and the alpha proton (a) of 306. These protons are in close proximity to the ester bond in the final compound 318, thus the shift in the peak of these protons will be observed in $^1$H-NMR of 318.

The changes in the signals associated with these protons can be used to follow the esterification reaction. The terminal methylene proton (a) next to iodine group appears as triplet at 3.1ppm in the $^1$H-NMR spectrum A of 317. The signals for these protons (a) have moved to 4.0ppm (spectrum C) in final compound 318. A similar observation was detected in the case of the alpha proton (a) of 306 present next to a carboxylic acid.
The alpha proton (a) signal appears as a multiplet at 4.0ppm in the 306 spectrum B. The signal for same alpha proton (c) has moved downfield to 4.2ppm in the spectrum C of 318. The ester bond has a greater deshielding effect than either the iodine group (317) or the carboxylic group (306) and hence these protons have moved down field in the 1H-NMR spectrum 318.

The methylene protons (d) present in the side chain of 318 are seen as a triplet at 3.6ppm in spectrum C. The peak area ratio for the terminal proton of 317 (a) to the alpha proton (c) of 306 and to the protons in the side chain of 306 (d) was as expected as 2:1:2 respectively. The ratio provides evidence in support of the synthesis of 318.
The signals for aromatic protons (g) and methylene protons (f) of the benzyloxy carbonyl group (Z) group of 318 were observed correspondingly as a doublet at 7.3ppm and a singlet at 5.0ppm, also consistent with the synthesis of the targeted compound 318. The methylene proton (b) of 317 appears as a triplet at 1.6ppm in the spectrum C of 318. The N-H proton (e) of carbamate (Z) group of 318 appears as a multiplet at 4.9ppm in spectrum C. Peak integration data obtained from ^1H-NMR spectrum (Figure 21) for compound 318 is shown in Table 7.

### Table 7: Peak integration data obtained for ^1H-NMR spectrum of compound 318

<table>
<thead>
<tr>
<th>Proton</th>
<th>Chemical Shift (ppm)</th>
<th>Peak Area</th>
<th>No of Protons (Stoichiometric Prediction)</th>
<th>Area/Proton</th>
</tr>
</thead>
<tbody>
<tr>
<td>g</td>
<td>7.3</td>
<td>5.02</td>
<td>5</td>
<td>1.04</td>
</tr>
<tr>
<td>f</td>
<td>5.1</td>
<td>2.00</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>e</td>
<td>4.9</td>
<td>0.97</td>
<td>1</td>
<td>0.97</td>
</tr>
<tr>
<td>c</td>
<td>4.1</td>
<td>1.05</td>
<td>1</td>
<td>1.05</td>
</tr>
<tr>
<td>d</td>
<td>3.6</td>
<td>2.00</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>a</td>
<td>4</td>
<td>2.00</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>b</td>
<td>1.6</td>
<td>2.00</td>
<td>2</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Comparison of peak area/proton ratio indicates close agreement with the predicted protons for 318 and thus the formation of 318 was concluded.

#### 3.5.2.4.1.2 Fourier Transform Infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) was used to provide further evidence for the confirmation of the structure of compound 318, and the spectrum region from 630 – 1830cm\(^{-1}\) is shown in Figure 22. The 2700 – 3700cm\(^{-1}\) region of FTIR is shown as an inset in Figure 22. A typical broad peak at position 3492cm\(^{-1}\) (shown in inset) was observed for the primary hydroxyl (O-H) group present in compound 318. N-H stretches for the amine (NH\(_2\)) group are shown at position 3337 – 3356cm\(^{-1}\). Broad peaks in the region of 3059 – 2947cm\(^{-1}\) correspond to aliphatic and aromatic C – H stretches. A characteristic ester (C=O) peak (keto) was detected as a strong band at 1737cm\(^{-1}\). The carbonyl peak (C=O) for the Z-group urethane link of 318 was observed at 1684 and 1669 cm\(^{-1}\).
Figure 22: FTIR spectrum of compound 318

The C-O and C-N stretches for ester and urethane linkages were observed at 1190 and 1220 cm\(^{-1}\) respectively. A very strong distinctive peak for the mono substitution phenyl ring within the Z-group of 318 was present at position 697, and 752 cm\(^{-1}\). All the major functional groups expected in 318 were demonstrated by FTIR spectrum and thus the spectrum is consistent with the formation of compound 318.

3.5.2.4.1.3 Mass Spectroscopy

Additional evidence for the success of the synthetic reaction and purified product was provided by mass spectroscopy (MS(ESI)) data. The calculated molecular weight of the compound 318 (C\(_{26}\)H\(_{32}\)N\(_2\)O\(_{10}\)) was 532.5415 and obtained MS based relative molecular weight of compound 318 was \(m/z\) 571.5413 [M+K] \(^+\)with molecular ion. The accurate isotopic mass of the molecular ion (K\(^+\)) present is 39.0 and hence, the subtraction of
molecular weight of molecular ion from the relative molecular weight of expected compound, provides an indication that the accurate molecular weight (532.5415) of the targeted product 318, was achieved. Hence it supports the observation that the synthesised product is pure.

In conclusion, FTIR data have shown the presence of all major functional groups present in compound 318, complementing the earlier observations from NMR analysis. Additional evidence for the success of synthesised pure > 99% compound 318 was provided by mass spectroscopy. Hence it can conclude that the target compound 318 was synthesised successfully with 85% yield.

3.5.2.4.2 Butane-1, 4-diylbis (2- (benzyloxy carbonylamino) 3(4 hydroxyphenyl) propanoate) – (320)

3.5.2.4.2.1 $^1$H-Nuclear Magnetic Resonance Spectroscopy
The $^1$H-NMR spectrum was obtained for compound 320 and is shown in Figure 23.

![Figure 23: $^1$H-NMR spectrum of compound 320](image-url)
The shift of the alpha proton (c) of 308 and the terminal methylene protons (g) of 317 was the key to the assignment of the structure for the product 320. These protons are present next to the ester linkage in the final compound 320 hence the shift in these protons signal should demonstrate the successful synthesis of 320 (and the absence of any signal at the original position should indicate purity). The $^1$H-NMR spectrum signals for c, g proton are shown in the inset in Figure 23. As shown in Figure 23, the alpha proton (c) of 308 appears as a multiplet at 4.1ppm and the terminal methylene protons (g) of 317 appears as a broad multiplet at 3.9ppm (spectrum A of Figure 21). The downfield shift of the proton signal was due to the deshielding effect of the ester linkage.

The benzyl methylene protons (d) in the side chain of 320 appear as a quartet at 2.8ppm. The four phenol protons (e, f) of the amino acid side chain of 308 appear as two doublet signals at 6.6 and 7.0ppm respectively. The five benzyl aromatic protons (a) of the Z-group of 308 appear as a multiplet at 7.5ppm in $^1$H-NMR of 320. The methylene protons (b) of the Z-group appear as a singlet at 5.0ppm. The methylene protons (h) of 317 appears as triplet at 1.6ppm in 320 $^1$H-NMR spectrum.

Note that the continued presence of the Z-group protons in the $^1$H-NMR spectrum of 320 as shown in Figure 23 demonstrates the stability of Z-group during the esterification reaction. Peak integration data obtained from $^1$H-NMR spectrum for 320 is shown in Table 8.

Table 8: Peak integration data obtained for $^1$H-NMR spectrum of compound 320

<table>
<thead>
<tr>
<th>Proton</th>
<th>Chemical Shift (ppm)</th>
<th>Peak area</th>
<th>No of Protons (Stoichiometric Prediction)</th>
<th>Area/Proton</th>
</tr>
</thead>
<tbody>
<tr>
<td>d</td>
<td>2.8</td>
<td>2.00</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>b</td>
<td>5.0</td>
<td>1.79</td>
<td>2</td>
<td>0.895</td>
</tr>
<tr>
<td>c and g</td>
<td>4.1 and 3.9</td>
<td>2.84</td>
<td>3</td>
<td>0.948</td>
</tr>
<tr>
<td>e</td>
<td>6.6</td>
<td>1.81</td>
<td>2</td>
<td>0.905</td>
</tr>
<tr>
<td>f</td>
<td>7.0</td>
<td>1.82</td>
<td>2</td>
<td>0.91</td>
</tr>
<tr>
<td>h</td>
<td>1.6</td>
<td>1.75</td>
<td>2</td>
<td>0.878</td>
</tr>
<tr>
<td>a</td>
<td>7.5</td>
<td>4.49</td>
<td>5</td>
<td>0.898</td>
</tr>
</tbody>
</table>
Comparison of peak area/proton ratio indicates close agreement with the predicted protons for 320 and hence this confirms the formation of the dihydroxy ester compound 320.

3.5.2.4.2.2 Fourier Transform Infrared Spectroscopy
To further support the structure of synthesised 320, FTIR spectrum was obtained and is shown in Figure 24. The 2500 - 4000 cm\(^{-1}\) region of FTIR spectrum is shown (in inset) in Figure 24.

A typical broad double centred peak arises at 3287 and 3399 cm\(^{-1}\) corresponding to O-H and N-H groups in the structure of 320. Aromatic (C=C) and aliphatic (C-H) carbon atoms from the side chain of 320 and Z-group of 320 appear at 2849, 3004, 30063 cm\(^{-1}\). The characteristic carbonyl peak (C=O) of the ester group appears at 1722 cm\(^{-1}\). Ester bond C-O and urethane bond C-N stretches are shown at 1031, 1056, 1190, 1220 cm\(^{-1}\).

Figure 24 : FTIR spectrum of compound 320
Aliphatic and aromatic C-C, C=C and C-H peaks were observed at 2849, 3004, 3063 cm\(^{-1}\). A sharp peak for mono substituted phenyl ring is shown at 824 cm\(^{-1}\). The peak for amine group as N-H stretch is appeared at 3399 cm\(^{-1}\). The presence of FTIR peaks for specific functional groups such as ester, hydroxyl, and urethane further supports the synthesis of 320. All the major functional groups expected in 320 were demonstrated by FTIR spectrum and thus the spectrum is consistent with the observation of the formation of compound 320.

3.5.2.4.2.3 Mass Spectroscopy

Additional evidence for the success of the synthetic reaction and purified product (320) was provided by mass spectroscopy (MS (ESI)) data. The calculated molecular weight of the compound 320 (C\(_{38}\)H\(_{40}\)N\(_2\)O\(_{10}\)) was 684.9812 and obtained MS based relative molecular weight of compound 320 was m/z 707.8812 [M+ Na]\(^+\) with molecular ion. The accurate isotopic mass of the molecular ion (Na\(^+\)) present is 22.9 and hence, the subtraction of molecular weight of molecular ion from the relative molecular weight of expected compound, provides an indication that the accurate molecular weight (684.9812) of the targeted product 320, was achieved. Hence it supports the observation that the synthesised product is relatively pure.

In conclusion, absence of peaks for the starting materials in both \(^1\)H-NMR and FTIR shows that starting material is not present in the synthesised final compound. Overall, the synthesised compound 320 was found to be relatively pure by NMR, FTIR and MS characterization affording 85% yield.

3.6 Conclusion

This chapter was devoted to the synthesis and characterization of L and L-Z-amino acid based dihydroxy and diamine ester compounds. Two different series of ester based compounds were synthesised. For the first series, Fischer esterification was utilised to synthesise L-amino acid based diamine ester compounds with yields of approximately 80% and good purity in all cases. For this acid catalysed reaction, L-phenylalanine, L-leucine, L-isoleucine, L-valine and L-tyrosine, were converted to their corresponding
diamine ester compounds with 1, 8-octanediol as the linking diol. The novelty of the current research work, however, lies in the use of the straight chain aliphatic linking diol -1, 8-octanediol, producing a diamine ester as reported in the current thesis.

For the second series, alkali metal salt based reaction was used to synthesise novel L-Z-amino acid based dihydroxy ester compounds. The method has been reported in the literature where it was used for solid phase peptide synthesis. Caesium carbonate was used as the alkali metal salt and the N-group protected L-amino acids, L-Z-serine, L-Z-threonine and L-Z-tyrosine, were used to synthesise the novel dihydroxy ester compounds. The L-Z-amino acid based ester compounds reported in this thesis are synthesised for the first time. The reaction conditions, such as temperature and reaction time, were optimised and a purification scheme was developed to obtain the pure product. The progress of the reaction was followed by thin layer chromatography and the yield of the reaction was 85% in all cases.

The synthesised compounds for each series were characterised by \textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR, FTIR and MS spectroscopy. Following purification the synthesised compounds were confirmed to be pure by the absence of starting material peaks in each of the above characterisations.

The synthesised compounds possessed varied functional groups (hydroxyl and amine group), which further provide opportunity to modify them for tailored applications, or to use them as chain extenders for polyurethane synthesis. The formation of difunctional compounds (dihydroxy and diamine) are of great importance when dealing with polymers especially polyurethane. Their use as a chain extender for polyurethane synthesis will be demonstrated in the following chapter 4.
Chapter 4: Synthesis and characterization of amino acid based polyurethane (PU) and polyurethane urea (PUU)
4.1 Introduction

Polyurethanes are an important class of synthetic polymers widely investigated for use in medical implants due to their excellent physio mechanical properties and good biocompatibility. In recent times, amino acid based polyurethanes have been developed to investigate the influence of amino acids to improve cell compatibility as well as biodegradability. Amino acids can be incorporated into the polyurethane structure as the chain extender (Zhang et al., 2000). Various amino acids based chain extenders have been used for polyurethane synthesis and are reported in the literature (Guelcher et al., 2005), (Marcos-Fernández et al., 2006). A detailed literature review on the synthesis and incorporation of amino acid based chain extenders into polyurethane and polyurethane urea was reported in Chapter 2 (section 2.8).

The key objective of incorporating an amino acid component into polyurethane is to first enhance the biocompatibility and biodegradability of the polymer for in vivo applications. Second, this will also provide an opportunity to tailor the physio mechanical properties of the synthesised polymer. L-amino acid based polyurethane (PU) and polyurethane urea (PUU) are synthesised and described in this chapter. The amino acid based ester compounds reported in Chapter 3 were used as chain extenders (CE). A two-step method, involving synthesis of a prepolymer, was used to synthesise the polymers. It is reported in the literature that the two step method gives good control of polymer architecture (Król, 2007).

A series of PUs and PUUs were thus synthesised using 4,4-methylenediphenyl diisocyanate (MDI), Polycaprolactone (PCL) and amino acid based chain extenders (CE). The use of amino acid based CEs were considered advantageous for a number of reasons, as discussed in Chapter 2. In brief, the advantages are:

1. To promote the release of non-toxic degradation product during polyurethane degradation process
2. To facilitate the enzymatic degradation subsequent to in vivo implantation.
3. To introduce the hydrolysable ester linkages into the hard segment of the polyurethane to enhance especially the polymer hard segment degradation process.

Despite of these advantages, the newly synthesised amino acid based dihydroxy and diamine chain extenders are significantly higher in molecular weight (Mw) and the chemical structures are more complex (non-linear) with bulky pendant groups which will have a significant effect on the polyurethane properties and morphology.

To test the above, the properties of amino acid based PU and PUUs are compared to a control PU based on the conventional linear chain extender, 1,4-Butanediol (BDO). BDO is a commercially available aliphatic dihydroxy compound and has been previously used as a chain extender for polyurethane synthesis (Caracciolo et al., 2008), (Miller et al., 1985), (Lee and Tsai, 2000).

It was anticipated that the mechanical properties of the amino acid based PUs and PUUs may be different from conventional BDO based PU. A detailed investigation of their physical and chemical properties is therefore necessary to understand the potential of these materials for use as biodegradable scaffolds and other applications where biodegradable polymers are sought. Previously there has been some work reported in this area where amino acid based chain extenders had been incorporated into PU and PUUs (for detail, see Chapter 2). For example, Kartvelishvili et.al. (1997) has described degradable polyurethane synthesis containing amino acid based diamine diester compounds similar to those reported in Chapter 3 (Figure 11) However, Kartvelishvili et.al. Incorporated these compounds into non-segmented polyurethanes, which usually have significantly different mechanical properties (i.e. stiff and hard), in comparison to segmented polyurethane which is soft and flexible as reported in this thesis. Segmented PUs consists of ’soft’ and ‘hard’ segments derived, respectively, from polyol and diisocyanate/chain extender combinations. Due to the relative incompatibility of these two segments for each other, segmented PUs exhibit a two phase morphology. Skarja et.al. (1998) have also synthesised amino acid based segmented polyurethane with a view to enhance the enzymatic degradation of the hard segment. These chain extenders were
diamine rather than the new dihydroxy variant used in this thesis. Newly synthesised amino acid based diamine chain extenders are also used in this thesis.

In this chapter, a range of synthesised amino acid based diamine diester compounds such as 311, 312, 313, 314 and dihydroxy diester compounds such as 318, 319, 320 have been incorporated as chain extender into PUUs and PUs respectively. The synthesis and characterisation of these compounds is reported in Chapter 3. The polymers (PUs/PUUs) reported in this chapter are synthesised for the first time as novel synthesised amino acid based chain extenders were used for their synthesis.

The chemical structure of PCL, MDI used to make PUs and PUUs reported in this chapter are shown in Figure 25. 1,4-butanediol (BDO) was used as chain extender for control PU synthesis and its structure is shown in Figure 25. The chemical structures of novel amino acid based chain extender used for PUs and PUUs synthesis were shown in Figure 20 and 11 respectively, in Chapter 3.

![Structure of polycaprolactone (PCL), 4,4- methylenediphenyl disocyanate (MDI) and 1,4-Butanediol (BDO)](image)

**Figure 25 :** Structure of polycaprolactone (PCL), 4,4- methylenediphenyl diisocyanate (MDI) and 1,4-Butanediol (BDO)

The main aim of this chapter is to synthesise and fully characterise a series of PUs and PUUs based on the novel amino acid based chain extenders. A library of polymers is developed to study the effect of chain extenders on the physio mechanical properties of the materials and compared with control PU based on BDO as chain extender. This will hopefully be useful for future biomaterial applications for example tissue engineering.
4.2 Materials

Polycaprolactone (PCL) as polyol (Mw 1057) was dried at 60°C for 48 hr under vacuum (0.1 torr) prior to use and 4,4-methylenediphenyl diisocyanate (MDI), was used as received from Sigma Aldrich. Since MDI can react with moisture in the air, pure MDI was distributed into small dry bottles and fresh MDI was used for each reaction. 1,4-butaneol (BDO) was stored under nitrogen atmosphere. BDO was vacuum dried at 60°C each time before use. Anhydrous DMF (<50ppm water) was obtained from sigma Aldrich. The solvents were analytical grade and were used as received. Deionised water was used for all purposes. Stannous octaoate (Alrich) was obtained from Sigma Aldrich and kept moisture free and used as received. All other solvents and chemicals used for purification methods were of analytical grade. Unless stated otherwise, all reactions were carried out in oven dried glassware. An inert atmosphere of nitrogen was used while working with anhydrous solvents. Chemical reaction temperatures were controlled using an IKA brand magnetic temperature modulator.

4.3 Experimental Procedures

4.3.1 Synthesis of polyurethane

Polycaprolactone (PCL, Mw-1057) was used as polyol and 4,4-methylenediphenyl diisocyanate (MDI) was used as diisocyanate for polymer synthesis. The amino acid based dihydroxy diester compounds (318, 319, 320) synthesised and reported in chapter 3 are used as chain extender here. 1,4-Butaneol (BDO) was used as control chain extender for control PU synthesis while PCL (1057) and MDI remain the same as polyol and diisocyanate component respectively for control PU. A two step or prepolymer method was adopted for the synthesis of polyurethane. Stannous octaoate was used as catalyst and N,N-dimethylformamide (DMF) was used as solvent for the polymerization. The series of PUs based on PCL 1057 molecular weight is referred to as Series-1-PU where 1 represent the ~ 1000 Mw of PCL. The polymerisation reaction was carried out under a completely dry environment under inert (dry N₂ was used) atmosphere. PUs were synthesised with equivalent hard and soft segments (50 weight.% each) in all the cases.
The synthesis of polyurethane based on 318 (Z-Serine amino acid based dihydroxy chain extender) as chain extender is described here as an example.

Polymerization was conducted in a 250 mL, 3 neck round bottom flask equipped with magnetic stirrer, nitrogen gas inlet and outlet fitted to the two necks of the flask, and an additional funnel fitted to the third neck. PCL (25.02g / 0.023 mol) was added to the flask. Reaction solvent, anhydrous DMF (50 mL) was added to the flask using a syringe. 0.05 mol% (2-3 drops) of stannous octoate as catalyst was added to the reaction mixture under dry and inert atmosphere with continuous stirring. MDI (12.57g / 0.050 mol) was charged to the reaction flask at room temperature. The temperature was increased to 80°C and the reaction was allowed to proceed for 3 hours at this temperature, and slowly cooled to room temperature (25°C) with continuous stirring. The temperature of the reaction was carefully maintained within the range of ± 3°C. Chain extension step was then carried out by simultaneous addition of Z-serine amino acid based dihydroxy chain extender (318) (13.0g / 0.024 mol) to the prepolymer solution under vigorous stirring. Typically, 318 was dissolved in a minimum amount of anhydrous DMF (10 mL) and was added drop wise to the reaction mixture via a syringe to react with the NCO terminated pre-polymer. The NCO/OH ratio was maintained at 1.05.

The temperature of the reaction was then gradually increased to 80°C and stirred for another 12 hours at the same temperature. The temperature of the reaction was controlled within the range of ± 3°C. After 12 hours, the reaction was quenched by pouring the reaction mixture into a cold concentrated aqueous solution of sodium chloride. At this point, solid polyurethane polymer precipitates out from the reaction mixture. The final polymer was filtered out and washed with distilled water. The washing was repeated at least three times to remove any impurities and unreacted material. The polymer was dried under vacuum at 40°C for 48 hours. The yield of the synthesised polymer was 70%. The synthesised polyurethanes were stored in sealed plastic bags for the purpose of characterisation and future experiments. Synthesised PU will be referred as Series 1 PU which includes PCL-1-Z-Ser-PU which represent PU synthesised on Z-serine based dihydroxy chain extender (318) and 1 represent the ~ 1000 Mw of PCL.
Following the similar prepolymer synthesis protocol, 319 (Z-Threonine amino acid based dihydroxy diester) compound was incorporated into PU as chain extender. Prepolymer method followed is illustrated in Scheme 4. 70% yield was obtained from the polymerisation reaction. PU synthesised in this series will be referred as Series 1 PU, where PCL-1-Z-Thr-PU represent a PU based on Z-threonine based dihydroxy chain extender (319) and 1 indicate to ~1000 Mw of PCL. Composition of Series 1 PU is described in Table 10 and chemical structure of Series 1 PU is shown in Figure 26.

An important point related to the use of compound 320 as chain extender needs to be mentioned. PU obtained based on 320 as chain extender was of very low molecular weight (~ 12,000) polymer as compared to PU based on 318 and 319 as chain extender. Synthesised PU was very tacky to handle and could not be processed further for characterisation. The exact reason for this is not known. But it might be that compound 320 has phenol groups available for chain extension step in polymerisation reaction. The phenol groups might not be as reactive as compared to primary and secondary alcohol present in 318 and 319 respectively. In general, the OH group of phenol should be more reactive but perhaps the static hindrance effect due to the presence of bulky groups has restricted the active participation of phenol in the chain extension step of PU synthesis and hence the polymer did not propagate to produce high molecular weight PU. Due to time limitation, no further work was done in order to obtain high molecular weight polymer based on 320. The obtained polymer was not suitable for further characterisation and it was felt that the synthesis of PU based on 320 needs to be terminated at this stage of research work and can be looked into near future. Based on the situation, it was decided that only 318 and 319 chain extender based PU data should be included and discussed in the thesis.

4.3.2 Synthesis of control polyurethane

The same two step solution polymerization method was used for the preparation of control polyurethane containing equivalent hard and soft segments (approximately 50 weight. % each). The prepolymer method followed is illustrated in Scheme 4. PCL (Mw~1057) act as polyl and MDI act as diisocyanate. 1,4-butanediol (BDO) was used as chain extender for control PU synthesis. Briefly, polymerizations were carried out in 250 mL, three-neck,
round bottom flask equipped with magnetic stirrer. Flask was fitted with a nitrogen gas inlet, outlet and an addition funnel. PCL (25.02g / 0.023 mol) was added to the flask followed by anhydrous DMF (50 mL) as solvent. 0.05 mol% of stannous octaoate was added to the PCL solution as catalyst in the round bottom flask. MDI (20.9g / 0.083 mol) was added to the flask under constant stirring at room temperature. Reaction temperature was increased to 80°C and reaction was allowed to proceed for 3 hours and then slowly cooled to room temperature (25°C) with continuous stirring. The temperature of the reaction was carefully maintained within the range of ± 3°C. Chain extension step was then carried out by simultaneous addition of 1,4-butanediol (5.05g / 0.056 mol), to the prepolymer solution under vigorous stirring. The temperature of the reaction was then gradually increased to 80°C and stirred for another 12 hours at the same temperature. After 12 hours, the reaction was quenched by pouring the reaction mixture into a cold concentrated aqueous solution of sodium chloride. Solid polyurethane polymer precipitates out from the reaction mixture. The final polymer was filtered out and washed with distilled water. The washing was repeated at least three times to remove the impurities and unreacted starting material. The polymer was dried under vacuum at 40°C for 48 hours. The synthesised polyurethanes were stored in sealed plastic bags for the purpose of characterisation and future experiments. Yield of the reaction was 70%. The synthesised polyurethane will be referred as Series 1 PU where PCL-BDO-PU represent the PU based on 1,4-butanediol as chain extender and 1 refers to the molecular weight of PCL~1000 used for PU synthesis. The abbreviations used for Series 1 PU is explained in Table 9. The chemical structure of Series 1 PUs is shown in Figure 26 and the molar ratio of the reactants used for the synthesis of Series 1 PU is shown in Table 10.

4.3.3 Deprotection of Z-group

Preliminary experiment was carried out in an attempt to remove the benzyloxycarbonyl (Z) group from PCL-1-Z-Ser-PU with minimal polymer backbone degradation. The acidolysis method reported in literature (Xie et al., 2007) was followed to remove the Z group from PU backbone. Z group deprotection experiment was carried out only on PCL-1-Z-Ser-PU of Series 1 PU. As mentioned earlier, that it was just an initial work to explore the possibility of removing the Z group from these newly synthesised PU. PCL-1-Z-Ser-PU was choose randomly. For the Z group deprotection experiment, a new batch of PCL-
1-Z-Ser-PU was synthesised based on two step prepolymer method. PCL (Mw1057) act as soft segment and MDI act as diisocyanate. Compound 318 act as chain extender. Reaction conditions remain the same as mentioned in experimental procedure 4.3. Isocyanate index of 1.05 was used with 50 weight percentage hard segment. The quantities of the reactant used for the synthesis of fresh PCL-1-Z-Ser-PU for deprotection of Z group experiment is described in Table 16.

The deprotection reaction was carried out in 250 mL round bottom flask equipped with magnetic stirrer. PCL-1-Z-Ser-PU (2gm) was dissolved in 20 mL of dichloromethane (DCM) and added to the flask. An ice bath was used under the flask to maintain 0°C temperature of the reaction mixture. A 5 mL solution of 25% HBr/HAc was added drop wise with the help of syringe into the flask with continuous stirring. The reaction mixture was stirred vigorously at 0°C for 30 minutes. Reaction time was optimised and reaction progress was monitored by 1H-NMR analysis. The reaction mixture was concentrated under reduced pressure to remove the acid and solvent dichloromethane. Finally, the reaction mixture was poured into a large amount of diethyl ether to precipitate the polymer. The polymer was filtered and dried under vacuum at room temperature. The yield of the reaction was 70%. The obtained deprotected polymer was stored in a sealed plastic bag and referred as PCL-1-Ser-PU.

4.3.4 Synthesis of polyurethane urea

The method of synthesis of polyurethane urea is similar to PU synthesis as described in section 4.3.1. The same two step solution polymerization method was used for the preparation of polyurethane urea containing equivalent hard and soft segments (approximately 50 weight. % each). Stannous octaate was used as catalyst and anhydrous DMF act as reaction solvent. Amino acid based diamine compounds (311, 312, 313, 314) act as chain extenders to synthesise PUU. Detail synthesis of these compound is reported in chapter 3. Polycaprolactone (PCL, Mw-1057) was used as polyl and aromatic diisocyanate, 4,4- methylenediphenyl diisocyanate (MDI) was used as diisocyanate for polymer synthesis. PUUs were synthesised with equivalent hard and soft segments (50 weight.% each) in all the cases The synthesis of polyurethane based on 311
(L-leucine amino acid based diamine chain extender) as chain extender is described here as an example.

Briefly, Polymerization was conducted in a 250 mL, 3 neck round bottom flask equipped with magnetic stirrer, nitrogen gas inlet and outlet fitted to the two necks of the flask, and an additional funnel fitted to the third neck. PCL (25.03g / 0.023 mol) was added to the flask. Reaction solvent, anhydrous DMF (50 mL) was added to the flask using a syringe. 0.05 mol (2-3 drops) of stannous octaoate as catalyst was added to the reaction mixture under dry and inert atmosphere with continuous stirring. MDI (14.21g / 0.056 mol) was charged to the reaction flask at room temperature. The temperature was increased to 80°C and the reaction was allowed to proceed for 3 hours at this temperature, and slowly cooled to room temperature (25°C) with continuous stirring. The temperature of the reaction was carefully maintained within the range of ± 3°C. Chain extension step was then carried out by simultaneous addition of L-leucine amino acid based diamine chain extender (311) (11.42g / 0.030 mol) to the prepolymer solution under vigorous stirring. Typically, 311 was dissolved in a minimum amount of anhydrous DMF (10 mL) and was added drop wise to the reaction mixture via a syringe to react with the NCO terminated pre-polymer. The NCO/OH ratio was maintained at 1.05. The temperature of the reaction was then gradually increased to 80°C and stirred for another 12 hours at the same temperature. The temperature of the reaction was controlled within the range of ± 3°C. After 12 hours, the reaction was quenched by pouring the reaction mixture into a cold concentrated aqueous solution of sodium chloride. At this point, solid polyurethane polymer precipitates out from the reaction mixture. The final polymer was filtered out and washed with distilled water. The washing was repeated at least three times to remove any impurities and unreacted material. The polymer was dried under vacuum at 40°C for 48 hours. The yield of the synthesised polymer was 70%. The synthesised polyurethane urea were stored in sealed plastic bags for the purpose of characterisation and future experiments. Synthesised PUU will be referred as Series 1 PUU which includes PCL-1-Leu-PUU which represent PUU synthesised on L-Leucine amino acid based diamine chain extender (311) and 1 represent the ~ 1000 Mw of PCL. The net yield of the polymerisation reaction was ~70% (in all cases). The chemical composition of the Series 1 PUUs is shown in Table 19 and chemical structure of all the PUUs based on amino acid based diamine compounds are shown in Figure 40.
An important point related to the use of compound 315 as diamine chain extender for Series 1 PUU synthesis needs to discuss here. Compound 315 is based on L-phenyl alanine amino acid. This amino acid has been extensively used in the literature (Skarja and Woodhouse, 1998) in regards to synthesis of diamine chain extender and later incorporate it in PUU synthesis. Hence it was felt that, instead of making PUU based on this diamine compound, we should be focused on the synthesis of PUU based on rest of the diamine chain extenders. Hence, compound 315 was not used for Series 1 PUU synthesis.

4.4 Characterization of polymers

The synthesised polymers were characterized extensively by various techniques to determine the structure and understand the basic properties of the polymers. The characterization studies include structural, thermal, surface and mechanical characterization.

4.4.1 Gel permeation chromatography

The molecular weights of polymers were determined by Gel Permeation Chromatography (GPC). A Waters 515 HPLC pump attached to Waters 590 equipped with a refractive index detector (a Waters 410 differential refractometer) which uses dimethyl formamide (DMF) as solvent (containing Lithium Bromide (LiBr), 0.05 M). The flow rate of the mobile phase was maintained at 1mL/min and the operating temperature was 30°C. Polyurethane sample was dissolved in DMF (approx. 2 mg/mL) and filtered through a 0.5 m (MFS Advantech) syringe filter and injected (50 μl) into an HPLC pump. Calibration of the apparatus was routinely performed with monodisperse polystyrene as standard. Data was analysed using Empower Pro software to determine the average number molecular weight (Mn), weight average molecular weight (Mw) and polydispersity (Mw/Mn) of the polymer. All molecular weights reported here are in terms of polystyrene standard.

4.4.2 Solubility

The solubility of polymers checked in different solvents at 25°C. The concentration of the sample was 0.5 gm/10 mL solvent.
4.4.3 Nuclear magnetic resonance spectroscopy

Unless otherwise specified, all $^1$H NMR spectra were recorded at room temperature (25°C) using a Bruker Av400 spectrometer at 400 MHz or a Bruker Av200 spectrometer at 200 MHz. Chemical shifts ($\delta$) are measured in parts per million (ppm) using known solvent chemical shifts as an internal reference standard (2.49ppm for $^1$H-NMR for the (d$_6$-DMSO-). $^1$H-NMR spectroscopic data are recorded as follows: chemical shift ($\delta$H) (relative integral, multiplicity) whereby multiplicity is defined as: s for singlet; d for doublet; t for triplet; q for quartet or quintet; m for multiplet, or combinations thereof.

4.4.4 Fourier transform infrared spectroscopy

FTIR spectra were obtained using a Thermo Nicolet 6700 spectrometer using a SmartATR (attenuated total reflectance) attachment fitted with a diamond window. The sample was analysed (32 scans) at 25°C in the transmission mode over 4000-700 cm$^{-1}$.

4.4.5 Thermal Characterization

The thermal behaviour of the PUs and PUUs was characterised by Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). The DSC was performed using a DSC30 (Mettler Toledo) instrument. For the DSC measurements, the samples were vacuum dried for 48 hours at room temperature in the presence of phosphorous pentaoxide. A specimen of approximately 5 mg was cut from compression moulded sheet and was encapsulated in an aluminium pan. Samples were scanned in alternating DSC mode from -50°C to 200°C at a rate of 10°C min$^{-1}$ under nitrogen atmosphere. A three stage heating and cooling method was used in which the sample was first heated from -50°C to 200°C and then quenched immediately to -50°C and heated again to 200°C. First heating run provides information about any crystallinity present in the soft segment which might disappear in the second heating run due to the rapid cooling of the polymer sample. DSC thermograms were analysed using Mettler: STAR EV.9.00 software to determine polyurethane glass transition ($T_g$) and melting temperature ($T_m$). Melting point ($T_m$) was taken as the peak temperature of the observed endothermic transition, whereas glass transition temperature ($T_g$) was measured at the half width of the transition after plotting tangents on the curve. The percent crystallinity ($\% X_c$) of the polymer was calculated
according to the melting peak area of the DSC graph by assuming that a perfect PCL crystal has a melting enthalpy of 139.5 J/g [Enthalpy was determined using the values ($\Delta H_m / \Delta H_{m100}$) where $\Delta H_{m100}$ is $\Delta H_m$ is enthalpy of melting temperature of 100% crystalline PCL] (Pitt et al., 1990). Thermo-gravimetric analysis (TGA) was performed with a Mettler Toledo TGA/STDA851 from 0°C to 800°C under nitrogen at a rate of 20°C min$^{-1}$. TGA graphs were analysed using Mettler: STAR EV.9.00 software. The specimens were placed in a ceramic pan and an average of 10 mg of solid sample was used for the experiments.

4.4.6 Water contact angle measurement

Water contact angles were measured using a contact angle meter (KSV CAM200 Instruments Ltd.). Thin films of polyurethane were prepared on thoroughly cleaned and dried glass slides by spin coating of 5 wt% solution of polymer in DMF. The films were dried initially at room temperature for 24 hours followed by vacuum drying at 50°C for another 48 hours to remove the residual solvents. Static water contact angles were measured by depositing a drop of 2 µl of deionised water (Millipore, Elix 5, 15 MΩ) at room temperature (25°C) and then a picture of the surface was taken through a camera (Sony). The contact angle was measured manually on the picture. To verify the contact angle reproducibility, an average of five readings (± standard deviation) from different parts of the each film was taken.

4.4.7 Compression moulding

Compression moulding was performed on a hydraulic press (with a thermostat and water-cooling capability) to obtain polymer films of the synthesised polyurethanes. The polymer was cut into small pieces using clean tin snips and pressed into a 1 mm thick plaque at a temperature above the melting point of the polymer (typically). The temperature was held constant for a period of 5 minutes before cooling in a standard manner under the flow of cold water. A standard mould was used which consisted of a rectangular cavity 100 mm x 60 mm x 1 mm deep cut into a metal plate. Teflon fabric sheet was used on both sides of mould to prevent adhesion of the polymer to the metal. The press used was a model 12-10-1T Wabash hydraulic press. In order to avoid unnecessary degradation of the material, the polyurethanes were not subjected to annealing. Polymer films thickness was
measured with digital callipers. The polymer films were stored in plastic bags in a desiccator prior to characterization.

4.4.8 Mechanical properties
The mechanical properties of the polyurethane films were measured using an Instron model 4468 universal testing machine according to the ASTM D-882 method. The compression moulded plaques were cut into thin strips with a straight section of 40 mm x 5 mm x 0.4 mm. A 100 N load cell was used with a crosshead speed of 100 mm/min at room temperature. At least seven replicate measurements were taken and averaged (± standard deviation). Strong pneumatic grips were used to prevent slippage. Data was analysed using Blue hill v.2.5 software.

4.5 Results and discussion

4.5.1 Polyurethane synthesis reaction
The new amino acid based dihydroxy compounds discussed in Chapter 3 were used as chain extenders to prepare a series of polyurethane. The method used in this thesis for polyurethane synthesis is known as the solution based two-step or “prepolymer method”. The general reaction scheme for the synthesis of Z-Serine amino acid containing dihydroxy (318) chain extender based PU by the two-step method is shown in Scheme 4.

The first step is known as the end-capping step and is associated with the reaction of diisocyanate with polyol to form the prepolymer. The second step is known as the chain extension step, where the chain extender is added to the prepolymer to attain high molecular weight of polymer. The polymerisation reaction follows a step growth polymerisation mechanism. The step growth method allows better control of the reaction as well as copolymer structure than that in the one-step process and was selected as the principal method to synthesise polyurethanes in this study.
Scheme 4: Schematic representation of the synthesis of PCL-1-Z-Ser-PU

Laboratory or experimental synthesis of segmented polyurethanes is generally performed via solution polymerization (Lamba et al., 1998). The solvent provides a non-reactive medium in which the diisocyanate, polyol, chain extender and the resulting polymer are soluble (Gogolewski, 1989). The choice of solvent may affect the effectiveness of the catalyst (Lamba et al., 1998). Anhydrous Dimethylformamide (DMF) was used as solvent and stannous octoate was used as catalyst for both PU and PUU synthesis in this thesis.

The detailed protocol for the synthesis of PU can be obtained from the experimental section (4.3). MDI was used as the diisocyanate. An aromatic diisocyanate (MDI) was chosen because it is widely used in the synthesis of polyurethane due to its high reactivity (Fernández d'Arlas et al., 2008). As mentioned before, novel synthesised amino acid
based chain extenders were used for the first time here. To obtain high molecular weights of polyurethanes based on these novel chain extenders, it was felt that a highly reactive diisocyanate (MDI) was needed. Aliphatic diisocyanates are normally preferred for formulating biodegradable polyurethane, however they are relatively low in reactivity as compared to their aromatic analogues. PCL of molecular weight 1057 was chosen as the polyol because of its known biodegradability (Abedalwafa et al., 2013). The potentially degradable L-amino acid based novel dihydroxy compounds \( (318, 319) \) were used as chain extenders and were incorporated into polyurethane. The polyurethane series based on PCL 1057 is referred to as Series 1 PU.

The polymer abbreviations used in this thesis is based on the type and molecular weight of the chain extender and soft segment used for polymer synthesis. The abbreviations used for Series 1 PUs are shown in Table 9. For example, PCL-1-Z-Ser-PU refers to polyurethane consisting of 1057 molecular weight of PCL, and chain extended with L-Z-Serine based dihydroxy chain extender \( (318, \text{see Chapter 3}) \). Z is the benzyloxycarbonyl group, an amine protecting group present in the chain extender which provides a pendant group in the final PU structure (Scheme 4).

<table>
<thead>
<tr>
<th>Polyol Molecular weight</th>
<th>Diisocyanate</th>
<th>Chain extender (Dihydroxy)</th>
<th>Polyurethane (PU) representative codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycaprolactone (PCL-1) (Mw=1057)</td>
<td>4,4-methyleneedianiline Diisocyanate (MDI)</td>
<td>L-Z-Serine dihydroxy ester ( (318) )</td>
<td>PCL-1-Z-Ser-PU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Z-Threonine dihydroxy ester ( (319) )</td>
<td>PCL-1-Z-Thr-PU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,4-Butanediol (BDO)</td>
<td>PCL-1-BDO-PU (control PU)</td>
</tr>
</tbody>
</table>

An isocyanate index (molar ratio of NCO to OH) of 1.05 was used for the PU synthesis in order to ensure a more complete reaction leading to a high enough molecular weight so that the properties of the material could be evaluated without molecular weight limitations. The amino acid based PUs obtained were yellowish white rubbery solids.
whilst the control BDO based PU was white and opaque in appearance. The polymers were stored in air tight sealed plastic bags. It was observed that amino acid based PUs were very soft and elastic compared with the strong and tough BDO based PU. The percent yield of the polymers synthesised was ~70% in each case. The loss in yield is largely due to loss of low molecular weight polymer during the precipitation process to recover the final polymer from the solvent. These low molecular weight polymers were formed due to the side reactions and are unwanted. The chemical structures of Series 1 PUs are shown in Figure 26.

All PUs contained 0.05% stannous octoate as catalyst, an isocyanate index of 1.05, with 50 weight % hard segment. All PUs described in this and following chapters contained MDI as diisocyanate component. The quantities of starting material used for the synthesis of Series 1 PUs are given in Table 10.
Table 10: Composition of Series 1 PUs

<table>
<thead>
<tr>
<th></th>
<th>PCL-1-Z-Ser-PU</th>
<th>PCL-1-Thr-PU</th>
<th>PCL-1-BDO-PU</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDI (Mw)</td>
<td>250.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDI mass (g)</td>
<td>12.57</td>
<td>12.34</td>
<td>20.9</td>
</tr>
<tr>
<td>MDI (Moles)</td>
<td>0.05</td>
<td>0.049</td>
<td>0.083</td>
</tr>
<tr>
<td>PCL (Mw)</td>
<td>1057</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCL mass (g)</td>
<td>25.02</td>
<td>25.08</td>
<td>25.02</td>
</tr>
<tr>
<td>PCL moles</td>
<td>0.023</td>
<td>0.023</td>
<td>0.023</td>
</tr>
<tr>
<td>CE (Mw)</td>
<td>532.3</td>
<td>560.5</td>
<td>90.12</td>
</tr>
<tr>
<td>CE mass (g)</td>
<td>13</td>
<td>13.16</td>
<td>5.05</td>
</tr>
<tr>
<td>CE moles</td>
<td>0.024</td>
<td>0.023</td>
<td>0.056</td>
</tr>
</tbody>
</table>

4.5.2 Characterization of polyurethane

4.5.2.1 Gel Permeation Chromatography

The molecular weights of Series 1 PUs were measured by gel permeation chromatography. GPC traces for Series 1 PUs are shown in Figure 27 and molecular weights are shown in Table 11.

Table 11: Molecular weights of Series 1 PU

<table>
<thead>
<tr>
<th>Polyurethane (PU)</th>
<th>Number Average Molecular Weight (Mₙ) (Dalton)</th>
<th>Weight Average Molecular Weight (Mₙ) (Dalton)</th>
<th>Polydispersity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Z-Ser-PU</td>
<td>6.67 x 10^4</td>
<td>12.76 x 10^4</td>
<td>1.91</td>
</tr>
<tr>
<td>PCL-1-Z-Thr-PU</td>
<td>5.86 x 10^4</td>
<td>8.70 x 10^4</td>
<td>1.48</td>
</tr>
<tr>
<td>PCL-1-BDO-PU</td>
<td>6.60 x 10^4</td>
<td>16.94 x 10^4</td>
<td>2.56</td>
</tr>
</tbody>
</table>

Series 1 PUs molecular weight are comparable to those reported for amino acid based PUs in the literature (Guelcher et al., 2005), (Sarkar et al., 2009), (Parrag and Woodhouse, 2010). GPC data also indicates a relatively low polydispersity for Series 1 PUs as compared to literature (Bil et al., 2009) values reported for polyurethane synthesis showing the distribution of molecular weight was not broad and was acceptable for PU synthesis.
GPC shows the polymerisation reaction was controlled to form moderately high molecular weight PUs. It is reported in literature, the effect of increasing molecular weight above 25,000 Dalton has little effect on the mechanical properties of PUs (Hepburn, 1991), and all polymers synthesised here were above this value. The GPC data, therefore, indicates that the molecular weights and polydispersity of all Series 1 PUs were sufficient to indicate that good mechanical properties should result.

4.5.2.2 Solubility

The solubility of Series 1 PUs was tested in various organic solvents at room temperature. The results indicate that Series 1 PUs are soluble in polar aprotic solvents such as Dimethylsulphoxide (DMSO), Dimethylacetamide (DMAc), N-Methyl pyrrolidine (NMP), Dichloromethane (DCM) but are insoluble in water and protic solvents such as acetone, ethanol, methanol, ethyl acetate. They were also all partially soluble in Tetrahydrofuran (THF). The insolubility of Series 1 PUs in most of the organic solvents is due to the existence of branched structure in PU which perhaps limit the hydrogen
bonding and hence less solubility. The use of isocyanate excess (1.05) in formulating PUs may also reduce polymer solubility due to the possibility of allophanate linkage formation (Okuto, 1966), (Chao and Tian, 2002).

A similar organic solvent solubility result for tyrosine amino acid based PU has been reported in the literature (Sarkar, 2007) and it was concluded that amino acid based PUs are soluble in most organic solvents but insoluble in protic solvents. The Series 1 PUs solubility results were thus consistent with previous literature findings. The solubility feature indicates that the synthesised PUs are soluble for practical purpose such as polymer processing.

4.5.2.3 1H Nuclear Magnetic Resonance Spectroscopy

Since synthesised segmented PUs are insoluble in chloroform, deuterated dimethylsulphoxide (d6-DMSO) was chosen as the solvent for 1H-NMR spectroscopy. To avoid repetition, only the PCL-1-Z-Ser-PU 1H-NMR spectrum is described here in detail and is shown in Figure 28.

The aromatic protons (11, 13) of MDI appeared at 7.32 and 7.09ppm (m, -C₆H₄-in MDI), respectively. The methylene proton (12) from MDI was assigned to the peak at 3.85ppm. The PCL diol associated protons were also present in the 1H-NMR of PU. For example, the six methyl (CH₃) protons (2, 3) of neopentyl glycol (used for the ring opening of caprolactone to make polycaprolactone (PCL) appeared at 0.875 and 0.195ppm. Methylene protons (CH₂) of PCL (6, 6’, 7,7’, 8,8’) and Z-serine based chain extenders (19) were present at 1.1-1.5ppm. At 2.24ppm terminal methylene (CH₂) protons (5, 5’) present next to the carbonyl (C=O) bond of PCL were observed as a multiplet.
At 3.7 – 4.0ppm, the methylene protons of PCL (9, 9', 1, 4) and of chain extender (14, 15) were observed. The methylene proton (14) next to the hydroxyl group of the chain extender which was involved in the urethane linkage has shifted from position 3.6ppm (spectrum C, Figure 21, Chapter 3) to 4.4ppm due to the change in the environment of protons from hydroxyl to urethane linkage. The $^1$H-NMR spectra of Z-Serine amino acid based dihydroxy chain extender was reported in Figure 21 of Chapter 3. The alpha proton of amino acid (15) appeared at position 4.10ppm in the $^1$H-NMR of the chain extender. This proton has shifted to 4.19ppm in the NMR of PU (Figure 28). The shift of the (14, 15) peaks demonstrate the change in the environment of these chain extender protons from being next to a hydroxyl group in the starting material to being next to a urethane link in the final polyurethane. The benzyl methylene protons (16) of Z-group of amino acid appeared at position 5.02ppm. The aromatic protons (17) of Z-group of amino acid were observed at 7.0 – 7.3ppm as multiplet.
The presence of protons related to the Z-serine based chain extender confirms that the amino acid based chain extender has been successfully incorporated into the polymer. The assignment of chemical shifts in the $^1$H-NMR spectrum of PCL-1-Z-Ser-PU displays all the expected structural features of the synthesised polymer and this verifies the molecular structure and the successful formation of the expected PU. The peak assignments from $^1$H-NMR show that all the three components (PCL, MDI, Chain extender) are present in the polymer. However, due to the presence of similar chemical environments for particular protons, there is considerable overlap of the peaks, making the assignment a difficult task. In general, the presence of characteristic peaks indicates that the polymer is composed of the PCL along with MDI and Z-Serine based chain extender. There is clear evidence of urethane linkages formed during polymerisation.

Some unassigned peaks do appear in the spectra, presumably corresponding to material formed by side reactions and from the unreacted materials/solvents. The intensity of such peaks is considerably lower than the assigned peaks, however, indicating the polymer is of reasonable purity.

Similar $^1$H- NMR spectroscopy assignments for the PCL-1-Z-Thr-PU and PCL-1-BDO-PU were observed and are reported in Appendices B and this further confirms the formation of the Series 1 PUs.

**4.5.2.4 Fourier Transform Infrared Spectroscopy**

FTIR spectra of PCL-1-Z-Ser-PU is shown in Figure 29 and FTIR peaks are reported in Table 12. To avoid repetition, only the PCL-1-Z-Ser-PU FTIR spectrum is described in detail here.

The absorption bands around 3345, 3336cm$^{-1}$ were assigned to urethane N-H bond, peaks observed at 2907, 2867, 2948cm$^{-1}$ represent aliphatic and aromatic C-C/C=C groups in the PU backbone and in the pendant group. The ester bond carbonyl group (C=O) appeared as a strong peak at 1731cm$^{-1}$. Skarja et.al. (1998) has also described the ester carbonyl peak at 1725cm$^{-1}$ for PCL based PU. A band present at 1532cm$^{-1}$ appeared to be for (C=C) in benzene rings of the MDI and Z group.
Sarkar et al. (2009) has reported the appearance of C=\text{C} peak at 1620\,\text{cm}^{-1} due to aromatic benzene ring structure in tyrosine – PCL based PU supporting the current observation of aromatic carbon atom peaks at 1532\,\text{cm}^{-1}. A very strong band at 1220\,\text{cm}^{-1}, 1167\,\text{cm}^{-1} was assigned to $\delta$ (N-H) + $\nu$ (C-N) urethane stretching. Peaks at 1066\,\text{cm}^{-1} and 1083\,\text{cm}^{-1}$ correspond to $\nu$ (C-O-C) ester group of soft segment and $\nu$ (CO-C) of hard segment stretch which confirms the urethane linkage formation.

A weak band at 697\,\text{cm}^{-1}$ indicates the presence of the mono substituted phenyl ring of the Z group on the chain extender. Thus the existence of bands for N-H, C=O of urethane linkage and ester link indicates that expected polymerisation reaction has taken place. Similar observations were recorded by Fernandez Borja et al. (2008) for MDI based PU.
Table 12: FTIR peak assignment for PCL-1-Z-Ser-PU

<table>
<thead>
<tr>
<th>Wave number (cm(^{-1}))</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3345, 3336</td>
<td>(\nu) (N-H) urethane (H-Bonded)</td>
</tr>
<tr>
<td>2907, 2867</td>
<td>(\nu_a) (C-H) in (\text{CH}_2)</td>
</tr>
<tr>
<td>2857, 2948</td>
<td>(\nu_s) (C-H) in (\text{CH}_2)</td>
</tr>
<tr>
<td>1731</td>
<td>(\nu) (C=O) urethane non Bonded</td>
</tr>
<tr>
<td>1699</td>
<td>(\nu) (C=O) urethane H- Bonded</td>
</tr>
<tr>
<td>1532</td>
<td>(\nu) (C-C) aromatic ring</td>
</tr>
<tr>
<td>1220, 1167</td>
<td>(\nu) (C-N) + (\delta) (N-H)</td>
</tr>
<tr>
<td>1412</td>
<td>(\nu) (C-C) aromatic ring</td>
</tr>
<tr>
<td>1083, 1066</td>
<td>(\nu)(C-O-C) in hard segment, (O=C-O-C) in soft segment</td>
</tr>
<tr>
<td>697</td>
<td>Mono substituted phenyl, Z group</td>
</tr>
</tbody>
</table>

\(\nu\)=stretching mode, \(\nu_a\)=asymmetric stretching, \(\nu_s\)=symmetric stretching, \(\delta\)=in-plane bending or scissoring, \(\rho\)=in-plane bending or rocking

In summary, the FTIR spectrum for PCL-1-Z-Ser-PU was found to be in full agreement with the expected polymer structure. Thus the formation of PCL-1-Z-Ser-PU was concluded. Similar FTIR observations for the structural identity of PCL-1-Z-Thr-PU and PCL-1-BDO-PU are reported in Appendix B at the end of thesis. Hence the successful formation of Series 1 PUs was concluded.

4.5.2.5 Differential Scanning Calorimetry

Differential scanning calorimetric (DSC) analysis of the polymers provides important information regarding the morphology of the PU structure. The relative compatibility of soft and hard segments in PUs governs the morphology. A relatively incompatible segment leads to phase separated morphology whereas more compatible systems produce
phase mixing. This behaviour is reflected in the properties of the resulting PUs. The incompatibility arises from chemical structural differences influencing the extent of hydrogen bonding, dipolar interactions, Vander Waals interactions etc. The observed thermal properties for Series 1 PUs are shown in Table 13 and details of the experimental protocol for the DSC experiment are given in the experimental section (4.4.5).

Two heating runs (first and second) for the DSC thermograms were obtained for Series 1 PUs. The first heating run was performed to eliminate any previous thermal history of the PUs sample such that the second heating run was obtained on PUs with the same thermal history. The first heating run provides the information of the crystallinity present in the soft segment which might disappear in the second heating run due to the rapid cooling of the polymer sample. Values of $T_g$ and $T_m$ obtained in both (first and second) heating runs were collectively used as final data for thermal analysis of the polymer sample.

**Table 13 :** Thermal properties of Series 1 PUs

<table>
<thead>
<tr>
<th>Polyurethane</th>
<th>PCL-1-Z-Ser-PU</th>
<th>PCL-1-Z-Thr-PU</th>
<th>PCL-1-BDO-PU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soft Segment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass transition ($T_g$) (°C)</td>
<td>6.1</td>
<td>1.2</td>
<td>-14.8</td>
</tr>
<tr>
<td>Melting Temperature ($T_m$) (°C)</td>
<td>ND</td>
<td>48</td>
<td>ND</td>
</tr>
<tr>
<td>Crystallinity (%)</td>
<td>ND</td>
<td>4</td>
<td>ND</td>
</tr>
<tr>
<td>Glass Transition ($T_g$) (°C)</td>
<td>5.9</td>
<td>-1.1</td>
<td>-11.2</td>
</tr>
<tr>
<td><strong>Hard segment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melting Temperature - 1st and 2nd heating run ($T_m$) (°C)</td>
<td>ND</td>
<td>ND</td>
<td>170</td>
</tr>
<tr>
<td><strong>Polymer morphology</strong></td>
<td>Phase mixed, amorphous soft and hard segment</td>
<td>Phase mixed, semicrystalline soft segment, amorphous hard segment</td>
<td>Phase segregated, amorphous soft segment, crystalline hard segment</td>
</tr>
</tbody>
</table>

$T_g$ - Glass transition Temperature, $T_m$ - Melting Temperature, %Xc - Crystallinity, $T_g$ 1st - $T_g$ of first heating run, $T_g$ 2nd - $T_g$ of second heating run, $T_m$ 1st - $T_m$ of first heating run, ND - Not Detected
**PCL-1-Z-Ser-PU** - The DSC thermograms for the 1\(^\text{st}\) and 2\(^\text{nd}\) heating runs of PCL-1-Z-Ser-PU are shown in Figure 30.

![DSC thermograms](image)

**Figure 30**: DSC thermograms for 1\(^\text{st}\) and 2\(^\text{nd}\) heating run of PCL-1-Z-Ser-PU

1\(^\text{st}\) Heating Run - The soft segment glass transition, \(T_g\) for 1\(^\text{st}\) heating run for PCL-1-Z-Ser-PU was observed at 6.1°C. No soft segment melt endotherm \(T_m\) was observed. For the hard segment, no \(T_g\) or \(T_m\) was observed during the first heating run of the polymer. Amorphous nature for the soft and hard segment was observed.

2\(^\text{nd}\) Heating Run – The soft segment \(T_g\) was observed at 5.9°C and no soft segment melting endotherm \(T_m\) was observed. For the hard segment, no \(T_g\) or \(T_m\) was observed for the 2\(^\text{nd}\) heating run. Thermal transitions of the polymer, shows that soft and hard segments are amorphous in nature.
**PCL-1-Z-Thr-PU** – The DSC thermograms for the 1\(^{\text{st}}\) and 2\(^{\text{nd}}\) heating runs of PCL-1-Z-Thr-PU are shown in Figure 31.

1\(^{\text{st}}\) Heating Run - The \(T_g\) of the soft segment for the 1\(^{\text{st}}\) heating run was observed at 1.2°C along with soft segment crystal melt endotherm (\(T_m\)) recorded at 48°C with 4% crystallinity. The presence of a soft segment melt endotherm in the 1\(^{\text{st}}\) run of PCL-1-Z-Thr-PU shows the soft segment has some, albeit very small, semicrystalline nature as compared to the completely amorphous nature of soft segment of PCL-1-Z-Ser-PU. No hard segment transitions such as \(T_g\) or \(T_m\) was observed in the first heating run indicating amorphous nature.

2\(^{\text{nd}}\) Heating Run - During the 2\(^{\text{nd}}\) run, the soft segment \(T_g\) was observed at -1.1°C and no soft segment \(T_m\) was observed at this time. The appearance or disappearances of these melt endotherms results from the disruption of ordered segments. Van Bogart *et al.* (1981) has stated that “between the completely amorphous and perfect crystalline state, there exist a continuum of ordered segment morphologies and known as order – disorder transitions. Disruption of this ordering requires energy and thus an endotherm is observed. In the current case, the polymer sample was quenched rapidly during the cooling cycle,
the polymer chains did not get sufficient time to form any crystal structure and hence did not show any soft segment \( T_m \) in the second heating run. However, no hard segment \( T_g \) or \( T_m \) was observed for the 2\(^{nd} \) run of PCL-1-Z-Thr-PU. Based on these DSC observations, hard segment are predominantly amorphous in nature and soft segment is semicrystalline in nature.

Both the polymers, PCL-1-Z-Ser-PU and PCL-1-Z-Thr-PU show soft segment \( T_g \) values below body temperature indicating they will exhibit elastomeric properties at body temperature (37°C). However, the observed soft segment \( T_g \) values for both the PUs remain substantial higher than the \( T_g \) of pure homopolymer soft segment, PCL (- 62°C) (Bogdanov et al., 1999) indicating that a certain degree of phase mixing is present in these PUs. In other words, there must be some hard segments present within the soft-segment phase and these hard segments place restriction to the movement of chains. Camberlin et.al. (1983) has proposed that \( T_g \) value can be affected by these chain end restrictions i.e. \( T_g \) value can increase by up to 4°C for a soft segment \( T_g \). Hence, a high value of \( T_g \) obtained in current case indicate that there must be a significant amount of phase mixing present in the synthesised PUs. Similar observations for the increase in PCL based soft segment \( T_g \) due to phase mixing morphology have been recorded in the literature (Li et al., 1994).

Unlike the soft segment, the hard segment glass transition is usually poorly resolved by DSC. It is reported in the literature (Joshi, 2009) that this happens primarily due to the following reasons:

1. The sequence length of the hard segment is often broadly distributed thereby resulting in a broad \( T_g \). Due to the chemistry involved in the PU synthesis, it is difficult to achieve control over the hard segment length distribution.

2. The change in the heat capacity of the PU hard segment is often small, making it difficult to observe.

The absence of hard segment \( T_g \) and \( T_m \) for the PUs investigated here, illustrate that their hard segments are largely amorphous in nature and cannot form a crystalline structure. This is presumably due to:
(1) The short chain length distribution of the hard segment and

(2) The unsymmetrical structure of the chain extender, which prevents any ordering of the hard segments through hydrogen bonding.

It has been reported in the literature that hard segment should be of sufficient length to form the crystals and should be symmetrical in nature, to allow chains to come together at distances close enough to have effective hydrogen bonding (Pergal et al., 2011). For example, more than three MDI units are required to form stable enough hard segment crystals to show a $T_m$ in MDI based PUs (Wang et al., 1994). A lack of symmetry in the hard segment due to the presence of the bulky pendant Z groups of the serine and threonine based chain extender is another reason for lack of crystallinity. The disruption caused by the pendant group while packing the hard segment creates more free volume within the polymer chains which further results in loose packing of the hard segment and hence the absence of a hard segment $T_m$. It has been reported in the literature (Parrag and Woodhouse, 2010) that if the chain extender and the diisocyanate are not symmetrical in structure and have bulky pendant groups, they will not form an ordered structure through hydrogen bonding. Similar results have been documented in the other reports for the amorphous hard segment of PU due to the non-symmetrical structure of chain extender (Won et al., 1998), (Skarja, 2001), (Hepburn, 1991).

It can be concluded that PCL-1-Z-Ser-PU contains both amorphous soft and hard segments whilst PCL-1-Z-Thr-PU shows a very weekly semicrystalline soft segment (4% crystallinity) and an amorphous hard segment. Both polymers show phase mixed morphology. Given that L-Z-serine and L-Z-threonine amino acid based PU are synthesized here for the first time, it is difficult to compare these observed properties directly with literature results.

**PCL-1-BDO-PU**

The DSC thermograms for the 1st and 2nd heating run of the control PCL-1-BDO-PU are shown in Figure 32. Hepburn (1991) has reported that PUs containing non-linear chain extenders are usually more phase mixed, when compared to those containing linear chain
extenders such as the BDO based PU used here. The control PCL-1-BDO-PU displays phase segregated morphology, as shown in Figure 32, which is more consistent with Bagdi et.al. (2009) who reports that MDI-BDO based hard segments tend to form phase segregation morphology through strong hydrogen bonding.

**Figure 32**: DSC thermograms for 1\textsuperscript{st} and 2\textsuperscript{nd} heating run of PCL-1-BDO-PU

1\textsuperscript{st} Heating Run - The \(T_g\) of the soft segment for the first heating run was observed at -14.8 °C and no soft segment \(T_m\) was observed. For the hard segment, no \(T_g\) was observed whilst a \(T_m\) was obtained at 170°C. The observed \(T_m\) was not a sharp melting endotherm as expected for BDO based PUs, presumably because it is a mixture of several hard segment transitions, poorly resolved in DSC. Further detailed analysis of DSC, such as modulated DSC, might be useful in understanding this transition, but was considered beyond the scope of this work.

Broadly speaking, the symmetrical structure (no pendant group) of BDO as a chain extender has helped the MDI based hard segments to form a densely packed regular structure, bound via H-bonding, and thus shows a hard segment melt endotherm (\(T_m\)), albeit not a sharp one. This transition can be attributed to the melting of the hard segment crystalline domain. Similar results are reported by Chen et.al. (1997) and Caracciolo et. al. (2008) based on MDI-BDO as a hard segment for their synthesised PU.
Two other thermal transitions were also observed at 50°C and 80°C during the first heating run. Both transitions are weakly defined and broad. It is proposed in literature that these endotherms are morphological in origin. As proposed by Seymour and Cooper (1973), these transitions represent disordering of the hard segment with relatively short and long range order of hard segment. Short range order represents the interaction between the soft segment and hard segment that contributes to the phase mixing behaviour of the PU. Long range order represents ‘unspecified’ interactions within the hard segment domains. The broad nature of the two peaks shows the wide distribution of the hard segment lengths.

2nd Heating Run - During the second run, the T_g of the soft segment appeared at -11.1°C which is close to the T_g observed in the first run (-14.8°C) and no soft segment T_m was observed. It is worth mentioning that the T_g for both first and second heating run for control PU is lower than the T_g of the novel amino acid based PUs (Table 13) and remains much higher than T_g of the pure homopolymer. The low T_g value for BDO based PU shows the phase segregated morphology of the control PU as compare to phase mixed behaviour of amino acid based PU.

For the 2nd heating run, the hard segment melt endotherm (T_m) appears again at the same temperature of 170°C, showing the crystalline nature of the hard segment. Hence it was concluded that the BDO based PU shows amorphous soft segment and crystalline hard segment with phase segregated morphology.

Amino acid based PUs, are synthesized for the first time, hence it would be difficult to compare the observed thermal properties of these PUs to the PUs reported in the literature based on MDI-PCL as hard and soft segment. However, in general, it was observed that the novel amino acid based PUs are more phase mixed with semicrystalline soft segment (PCL-Z-thr-PU) and amorphous hard segment as compared to the control BDO based PU which shows phase segregated morphology with amorphous soft segment and crystalline hard segment.
4.5.2.6 Thermogravemetric Analysis

Thermogravemetric analysis (TGA) is a suitable method to evaluate the thermal stability of polymers, including PU elastomers (Hepburn, 1991). The TGA data for Series 1 PU is shown in Figure 33.

*Figure 33: TGA analysis of Series 1 PU*

The TGA analysis shows that amino acid based PUs are more thermally stable compared to the control BDO based PU. The onset of degradation for PCL-1-Z-Ser-PU and PCL-1-Z-Thr-PU is at ~ 350°C and is essentially complete by ~ 550°C. For the control PCL-1-BDO-PU, the onset of degradation is ~ 270°C and is complete by ~ 470°C. The PUs exhibit a two-step degradation processes presumably reflective of the two phase (hard and soft segment) structure of the PU. It is reported in the literature (Gupta and Adhikari, 2003) that the thermal degradation of the PU starts in the hard segment first then follows into the soft segment. The hard segment degradation involves dissociation of urethane and urea bonds presents in the hard segment.

The result indicates that amino acid based PUs are thermally stable up to a high temperature and the reason may be:

1. A higher level of aromatic components.
The increase in thermal stability of amino acid based PU is probably due mainly to the presence of more aromatic components compared to that of the control PU. Similar observations for higher thermal stability of MDI and aromatic chain extender based PU have been noted in the past. For example, Azzam et.al.(2007) has been reported that the aromatic and heterocyclic moieties present in the polymer main chain or as side group can contribute to the increased thermal stability of PU. The aromatic group present in the MDI and Z group of the chain extender present in the hard segment of amino acid based PUs provide additional intermolecular attraction through delocalised π bond interaction (Dieterich et al., 1994) with neighbouring polymer chains having similar aromatic MDI and Z groups.

Antipova et.al. (1970) has reported that the degree of phase segregation or segmental mixing has a significant effect on the thermal stability of PU, with a mutual stabilization effect present between the soft and hard segment of phase mixed morphology. DSC analysis indicates phase mixing behaviour of amino acid based PUs and these polymers also shows high thermal stability as compared with the control PU which shows phase segregation morphology. This observation supports the fact that during heating at high temperature, especially under nitrogen atmosphere, interurethane hydrogen bonding dissociate and after the dissociation, more phase mixing favours the thermal stability of PUs. A similar correlation between the phase mixed morphology and higher thermal stability of PTMG/PEG-MDI based PU has been reported by Wang et.al. (1997).

In summary, amino acid based PUs in Series 1 are more thermally stable than the control PU. This could be an advantage in thermal processing of these polymers as they offer a broader temperature range for processing.

4.5.2.7 Water Contact Angle
Contact angle are commonly used to describe surface wettability (Dou et al., 2006). Water contact angle was measured in order to obtain an idea of the relative polarity of the amino acid based PU surface. Contact angle values for Series 1PU are reported in Table 14.
Table 14: Water contact angle values for Series 1 PU

<table>
<thead>
<tr>
<th>Polyurethane</th>
<th>Contact Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Z-Ser-PU</td>
<td>72° ± 4</td>
</tr>
<tr>
<td>PCL-1-Z-Thr-PU</td>
<td>77° ± 3</td>
</tr>
<tr>
<td>PCL-1-BDO-PU</td>
<td>68° ± 2</td>
</tr>
</tbody>
</table>

There is a very slight increase in contact angle values from PCL-1-Z-Ser-PU to PCL-1-Z-Thr-PU, however the difference is minor. Both amino acid based PUs exhibited a higher contact angle than the control PCL-1-BDO PU, but again the difference is minor, although the difference between the threonine based PU and the control PU is more obvious. In all cases, the contact angle indicates a relatively hydrophobic surface with the two amino acid, non-linear chain extender based, PUs being slightly more hydrophobic than the control.

Similar observation have been reported in the literature (Guelcher et al., 2005) showing relatively hydrophilic contact angles for linear chain extenders where non-linear chain extenders are slightly more hydrophobic. For example, Skarja et al. (1998) has reported contact angle value of 71° and Sarkar et al. (2009) as reported the contact angle value of 75° for non-linear chain extender based polyurethane.

The small increase in hydrophobicity shown in Table 14 may be due to:
1. Incomplete phase separation between hard and soft segments leading to fewer and smaller dipole formation (Fernández d'Arlas et al., 2008), and
2. The hydrophobic nature of the PCL soft segment (Kavlock et al., 2007)

All these factors, the hydrophobic nature of PCL, MDI, non-linear structure of chain extender and the presence of bulky aromatic pendant Z group have made the surface of the amino acid based PU more hydrophobic in nature as compared to BDO based PU.

In summary, the novel amino acid based PUs are slightly more hydrophobic in nature than the control PU and may therefore be good alternatives where slightly more water repellent properties are required.
4.5.2.8 Mechanical Properties

Tensile properties of the PUs can be affected by different factors such as polymer overall molecular weight, polymer morphology, hard and soft segment length and their crystallinity. The stress strain graphs of the amino acid based Series 1 PUs are shown in Figure 34 and for Series 1 control PUs are shown in Figure 35. The mechanical properties are shown in Table 15 and compared to the control PU. It is clear that the Series 1 amino acid based PUs are weak materials with low mechanical strength. Specifically, they have low modulus of elasticity, low tensile strength and high percentage of elongation during tensile testing.

![Figure 34](image)

**Figure 34**: Tensile stress – strain curve of Series 1 PU

The stress-strain curve shows a significantly long region of plastic deformation, unlike conventional PUs. Both the amino acid based PUs are soft materials with near identical elongation at break values. In contrast, the control PCL-1-BDO-PU behaved as a strong and stiff polymer with a high value of modulus and tensile strength but with low percent of elongation. Weak mechanical properties shown by amino acid based PU might be due to the less ordered hard segment domain observed in amino acid based Series 1 PUs.
Since the amino acid chain extenders have a higher molecular weight than BDO, there are fewer numbers of chain extender units incorporated into the hard segment of the PUs. This leads to fewer urethane linkages in the hard segment as compared to the control PU, which in turn leads to a less ordered hard segment domain and thus a relatively more amorphous material.

**Table 15**: Mechanical properties of Series 1 PU (mean ± SD, n = 6)

<table>
<thead>
<tr>
<th>Polyurethane (PU)</th>
<th>Modulus of elasticity (MPa)</th>
<th>Ultimate tensile strength (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Z-Ser-PU</td>
<td>6.2 ± 2</td>
<td>4.8 ± 1</td>
<td>625.8 ± 35</td>
</tr>
<tr>
<td>PCL-1-Z-Thr-PU</td>
<td>4.2 ± 1</td>
<td>10.9 ± 6</td>
<td>660 ± 59</td>
</tr>
<tr>
<td>PCL-1-BDO-PU</td>
<td>52.8 ± 22</td>
<td>32.9 ± 12</td>
<td>234 ± 18</td>
</tr>
</tbody>
</table>

It is reported in the literature (Lamba et al., 1998) that phase morphology plays a vital role in determining the mechanical properties of the synthesised polymer, with amorphous materials prone to show weaker mechanical properties. As seen via DSC (Table 13), amino acid based PUs particularly PCL-1-Z-Ser-PU show phase mixed morphology in contrast to phase segregated morphology shown by control PU.

*Figure 35*: Tensile stress- strain curve of PCL-1-BDO-PU
PCL-1-Z-Thr-PU shows a semicrystalline soft segment, while PCL-1-Z-Ser-PU shows an amorphous soft segment and both polymers showed an amorphous hard segment. Lack of soft segment crystallinity in PCL-1-Z-Ser-PU has resulted in weaker mechanical properties as compared to PCL-1-Z-Thr-PU which shows semicrystalline (4% crystallinity) soft segment morphology with improved tensile properties. A similar observation has been reported by Skarja et al. (2001), and Guelcher et al. (2005) where weak and low mechanical properties of PCL based PU were observed due to the lack of soft segment crystallinity.

The overall mechanical properties of a polymer can be highly effected by the structure and symmetry of the diisocyanate present in the polyurethane. In general, it is reported in the literature (Smith et al., 1987), (Lee and Tsai, 2000) that cyclic structures such as MDI improve mechanical properties due to ordered (hydrogen bonding) and crystalline hard segments. DSC data however, indicates substantial phase mixing present in the polymer along with disordered, non-crystalline hard segment present in the amino acid based PU. This indicates the hard segment’s inability to act as a physical cross link for the soft segment domain to improve mechanical properties. Hence a low modulus of elasticity and tensile strength of the amino acid based PU was observed. An increase in the percentage elongation, as seen in stress strain graph of amino acid (Table 15) based PUs, is contrary to the general trend reported in literature. The structure of MDI is symmetrical and should contribute to improve mechanical properties of PUs. However, the presence of the unsymmetrical and bulky pendant Z group in the chain extender prevents close packing of the polymer chains (lack of hydrogen bonding) in the hard segment. The hard segment is thus loosely packed leading to more free volume within the polymer structure.

This might affect the PU mechanical property in two ways:
1. The crystallinity and ordering of the hard segment is destroyed by the presence of the bulky pendant Z group (as seen in DSC data, Table 13) resulting in lower modulus and tensile strength.
2. The ability to deform and reorient polymer chains at increasing strain levels has led to high extension before failure.
A similar observation has been reported by Sarkar et.al. (2008) where a tyrosine amino acid based chain extender was used for PU synthesis. The bulky side chains of the tyrosine amino acid prevented the close packing of the hard segment of PU and hence affected the overall mechanical properties of the synthesised PU.

The control BDO based PU, behaves as a stiff and strong material with high modulus and tensile strength. It is reported in the literature (Patterson et al., 1999), (Joshi, 2009) that MDI-BDO hard segment result in very strong hydrogen bonding that helps to attain improved mechanical properties. The mechanical properties are also depend on the phase morphology of the polymer. It was observed in the DSC, that the control PU polymer was segregated into two separated phases showing both a $T_g$ of the soft segment and a $T_m$ of the hard segment. The hard segment is crystalline in nature and the soft segment was present in a viscoelastic state. In phase segregated morphology, the hard segment may act as a physical cross link for the soft segment domains and therefore act as a reinforcing filler (McBane et al., 2007). When the polymer is pulled during mechanical testing, the polymer chains align with each other, making it very difficult to stretch. Hence the high modulus and tensile strength was obtained, as seen in control PU and due to the stiffness of the polymeric chains, it cannot be stretched for long and hence a low value of percentage elongation was observed. The other reason for enhanced mechanical properties of BDO-PU is the symmetrical structure and low molecular weight of the chain extender. BDO as a linear chain extender, does not have any pendant group in its structure to disturb the hard segment packaging which has result in very ordered structural packing of the hard segment due to strong hydrogen bonding which in turn shows improved mechanical properties.

It can be concluded that amino acid based PUs behave as mechanically weak materials with high extension to failure and low tensile strength and modulus compared to the control PU. These results show the importance of the chain extender structure and effect of polymer morphology on mechanical properties of PUs confirming the requirement of a short chain symmetrical molecule as a chain extender to achieve good mechanical property.
4.6 Deprotection of Z-group

4.6.1 Introduction

Deprotection of the Z-group from polymer backbone may be useful for future polymer applications. For example, the resultant free amine group could be used to covalently attach any given drug or biologically active compound, such as a polysaccharide or peptide, to the PU making the polymer more biocompatible. Incorporation of the charged groups (NH$_3^+$) into the PU backbone could produce material known as a PU ionomer which has been shown to have improved blood compatibility (Jinhuang et al., 1989). Several investigations on drug delivery systems based on polymers with pendant functional groups have been reported in literature (in't Veld et al., 1992), (Fiétier et al., 1990), (Zhou and Kohn, 1990). Skarja et.al. (2001) have also synthesised PU with amine groups as pendant groups on the PU backbone.

The research group (Xie et al., 2007) has reported the hydrogenation method for the deprotection reaction of the Z group from the PU backbone, resulting in the regeneration of NH$_3^+$ group as pendant group. The synthesis of deprotected PU was not the main focus and it was not further characterised. Detailed characterisation and application of deprotected PU can be considered as future work and some preliminary experiments are carried out here.

An initial experiment to demonstrate the removal of the Z group from the PCL-1-Z-Ser-PU is described in this section 4.3.3. The aim of the experiment is to demonstrate the possibility of deprotection of the Z group from the synthesised PU without affecting the PU backbone and leaving a functional pendant amine group at the polymer backbone. A new batch of PCL-1-Z-Ser-PU was synthesised, based on the two step prepolymer method as described in section 4.3. Polymer reaction conditions remained the same as describe in section 4.3 and the quantities of the reactants used for the PU synthesis are given in Table 16. The polymer contained 0.05 % stannous octoate as catalyst, an isocyanate index of 1.05 was used, and 50 weight % hard segment was present in PU.
Table 16: Composition of PCL-1-Z-Ser-PU for deprotection reaction

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Mass (g)</th>
<th>Molecular Weight</th>
<th>Moles</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>2.5</td>
<td>1057</td>
<td>0.00236</td>
</tr>
<tr>
<td>MDI</td>
<td>1.25</td>
<td>250.25</td>
<td>0.00499</td>
</tr>
<tr>
<td>CE (318)</td>
<td>1.3</td>
<td>532.31</td>
<td>0.00244</td>
</tr>
</tbody>
</table>

318 - Octane-1,8-diyl bis (2-amino-4-methylpentanoate)

CE – Chain extender

4.6.2 Deprotection reaction

An acidolysis method, using hydrogen bromide and acetic acid solution (HBr/HAc), was utilised to remove the Z group from the PU backbone. This method has been reported in the literature (Deng et al., 2007), (Katchalski et al., 1948), (Ben-Ishai and Berger, 1952), (Anderson et al., 1952) (Hernández and Klok, 2003) and results in cleavage of the carbamate (urethane) linkage and regeneration of the free amine group. An alternative has been reported in the literature involving hydrogenation of the Z- group from the polymer backbone using palladium/charcoal carbon as catalyst (Skarja, 2001). The hydrogenation method, however, has been reported as time consuming, complete elimination of the Z- group cannot be achieved and the complete separation of the residual palladium activated charcoal is very difficult from the final product. Xie et al. (2007) has reported the use of HBr/HAc method to remove the Z group from the backbone of PCL-PEG-HDI based PU. The HBr/HAc based method is fast and easy to use, and more likely to be complete, so was the chosen method here.

The general reaction for deprotection of the Z group from PCL-1-Z-Ser-PU is shown in Scheme 5. At the end, a free amine results as a salt with bromine ion (NH$_3^+$Br$^-$). The deprotected PU will be denoted as PCL-1-Ser-PU. Following deprotection, the resultant polymer was stored in a desiccator until further use.
Scheme 5: Deprotection reaction to remove Z group from PCL-1-Z-Ser-PU

The yield of the deprotection reaction was 70% and this did not vary by more than 5% on repetition of the experiment. The deprotected PU exhibits polycationic character due to its pendant NH$_3^+$ cation. The acidolysis method attacks the carbamate (urethane) linkage present between the pendant Z group and NH group of the amino acid and results in the release of benzyl alcohol and carbon dioxide as side products. The NH group of the chain extender is converted into the free NH$_3^+$ group. PU also contains a number of urethane linkages in the polymer backbone, so it was anticipated that backbone cleavage may occur during deprotection, leading to a loss in polymer molecular weight and subsequent modification of polymer properties. Deprotection reaction conditions such as reaction time were therefore first optimised in an attempt to promote deprotection but limit polymer backbone cleavage. The detailed method of the deprotection reaction is given in the experimental section 4.3.3.
4.6.3 Optimisation of deprotection reaction

For the optimisation of the deprotection reaction, the $^1$H-NMR spectrum of the deprotection reaction mixture was obtained at different time intervals. This monitors the progress of the reaction in order to attain the minimum time required for the deprotection of $Z$ group from the PU backbone. It was observed that long exposure of the polymer reaction mixture to HBr/HAc solution causes degradation of the urethane linkage present in the polymer backbone. This results in polymer degradation and loss of polymer molecular weight. Details of the degradation reaction are beyond the scope of this thesis. However it is relevant to report the observations here as it indicates that optimisation is required.

4.6.3.1 $^1$H Nuclear Magnetic Resonance Spectroscopy

Aliquots of reaction mixture were taken at 10 minutes and 30 minutes time interval from the start of the deprotection reaction. $^1$H-NMR spectroscopy was performed and compared with the protected PU $^1$H NMR. The collective $^1$H- NMR spectrum of protected PU (zero reaction time) and deprotection reaction mixtures at 10 minute and at 30 minutes are shown in Figure 36. It was observed that the proton peaks obtained in Spectrum A at 7.39ppm (due to protons of aromatic ring of the $Z$ group) and at 5.15ppm (due to benzyl methylene proton of $Z$ group) were reduced in intensity in the Spectrum B and C. The reduction in peak height related to protons associated with the $Z$ group demonstrates a clear indication of the progress of the deprotection reaction. Calculations were done on the basis of $Z$ proton (present at 5.15ppm) peak height ratio by keeping PCL peak (present at 2.2ppm) constant to monitor the percent reduction of $Z$ group after 10 and 30 minutes. It was observed that 62% reduction in $Z$ protons peak was observed after 10 minutes and a further 11% reduction in peak height ratio was observed after 30 minutes. This observation further supports the fact that $Z$ group based protons were removed during the deprotection reaction. Similar observation for $^1$H-NMR spectroscopy was recorded by Xie et.al. (2007) where the HBr/HAc method was utilised to remove the $Z$ group at the backbone of PCL-PEG-HDI based PU.
It was observed that deprotection reaction was 62% complete in first 10 minutes, and a characteristic salt peak NH$_3^+$ Br$^-$ was observed at 8.5ppm. To ensure the near complete removal of Z-group related proton peaks, the reaction was carried out for 30 minutes. From the $^1$H-NMR Spectrum C (Figure 36), it was observed that the ratio of area per aromatic peak was reduced due to the removal of aromatic Z-group from the polymer. The rest of the peaks related to PU remained intact. Moreover, no new peak was observed in Spectra B and C during the deprotection step which might have indicated the formation of new impurities due to the degradation of PU backbone. It was observed that the polymer chain scission reaction depends on the time of the deprotection reaction. The longer the depolymerisation reaction, the greater the chances of degradation of polymer backbone by acid based ester hydrolysis. 30 minutes was therefore a good compromise (the best possible time), for near complete removal of the Z group but without PU
backbone scission. The current optimisation reaction time (30 minutes) is consistent with the time used in literature (Xie et al., 2007).

In conclusion, 30 minutes reaction time was sufficient to remove most of the Z group from PU backbone but not so long as to promote the unwanted scission of the PU chain. The deprotected polymer will be referred as PCL-1-L-Ser-PU.

4.6.3.2 Solubility
The deprotected polymer, PCL-1-Ser-PU, was found to be soluble in a range of different solvents such as water, acetone, methanol, ethanol, ethyl acetate and tetrahedron furan (THF) solvent. It is worth noticing that the protected PU was insoluble in the above mentioned solvents, providing further evidence that the deprotection reaction was successful. The cationic nature of the deprotected PU has increased the solubility of the PU probably via hydrogen bonding. The deprotected PU was also soluble in Dimethylformamide (DMF), Dimethylsulphoxide (DMSO) and N- Methyl pyrrolidine (NMP) indicating solubility in both polar aprotic and protic solvents. This shows that the unprotected PU solubility and hydrophilicity has increased as compared to the protected PU.

4.6.3.3 Gel permeation chromatography
The effect of the deprotection reaction on polymer molecular weight was investigated by gel permeation chromatography (GPC). The molecular weight of protected and deprotected PU was determined by GPC and compared. Figure 37 shows the GPC traces of deprotected and protected PCL-1-Z-Ser-PU and Table 17 shows the molecular weight of PU before and after deprotection reaction.
The Z group based urethane group was expected to be prone to hydrolysis due to the proximity of the phenyl group to the urethane linkage. Similar benzyl esters are removed by hydrogenolysis in literature (Skarja, 2001). The Z group is similar in nature to benzylic ester and is known to be particularly susceptible to hydrolysis as compared to backbone urethanes groups which were expected to be much less reactive.

The weight average molecular weight (Mw) of the original polymer used for the deprotection reaction was $5.07 \times 10^4$ Dalton and after deprotection there was only a minor change in the weight average molecular weight (Mw) of polymer ($4.98 \times 10^4$ Dalton) was observed. The polymer molecular weight and its distribution did not significantly change indicating that polymer chains were intact and little altered.

The minor changes observed in the Mw of deprotected polymer shows the loss of molecular weight from the removed Z group from PU and the data is consistent with the molecular weights (The Z group should account for approximately 1000 molecular weight and there was a loss of approximately 800 molecular weight after its removal).
Table 17: Molecular weight of PCL-1-Z-Ser-PU before and after deprotection reaction

<table>
<thead>
<tr>
<th>Polyurethane (PU)</th>
<th>Weight average molecular weight (Mw) (Dalton)</th>
<th>Number average molecular weight (Mn) (Dalton)</th>
<th>Poly Dispersity Index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Z-Ser-PU</td>
<td>5.07 x 10^4</td>
<td>2.95 x 10^4</td>
<td>1.71</td>
</tr>
<tr>
<td>(Protected PU)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCL-1-Ser-PU</td>
<td>4.98 x 10^4</td>
<td>2.93 x 10^4</td>
<td>1.70</td>
</tr>
<tr>
<td>(Unprotected PU)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In summary, the molecular weight and polydispersity of the deprotected PU did not change significantly from the protected PU and hence it can be concluded that the PU chains were intact and polymer backbone cleavage did not happen during the deprotection step.

4.6.3.4 Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) was performed on PCL-1-Z-Ser-PU (protected) and PCL-1-Ser-PU (deprotected). The comparison was hoped to further illustrate the complete removal of the Z-group from the protected PU, formation of a salt peak in the deprotected polymer and to exclude the possibility of scission of the polymer backbone during the deprotection reaction. The FTIR spectra of deprotected PU (Spectrum B) is compared to the protected PU (Spectrum A) and shown in Figure 38.

The FTIR spectra of protected PU (Spectrum A) shows characteristic peaks at 3345, 3336cm⁻¹ (N-H), 1731cm⁻¹ (C=O) and 1120, 1167cm⁻¹ for the C-N bond of Z and urethane group. In spectrum B of deprotected PU, a change in the peak area of 3100 – 3600cm⁻¹ region was observed which corresponds to a change in the N-H region of PU. The peak area and shape for the N-H group (3345, 3336 cm⁻¹) region in deprotected polymer was changed. A broad N-H peak was observed at 3480cm⁻¹ (Spectrum B) which illustrates the formation of salt (ammonium bromide, NH₃⁺Br⁻) at the polymer backbone. In the FTIR spectrum B, the aromatic C-H out of plane bending absorption peaks observed at 749cm⁻¹ and 697cm⁻¹ due the Z- benzyl group disappeared. Similar FTIR observation for the Z group peak removal has been reported by Deng et.al. (2007) and Won et.al. (1998) where
a Z group was removed from the polymer backbone and FTIR technique was used to confirm this.

Figure 38: FTIR absorption spectra of (A) Protected PCL-1-Z-Ser-PU (B) Deprotected PCL-1-Ser-PU

The results from FTIR here clearly indicate that the benzyloxycarbonyl group (Z) has been removed completely from the PU backbone. It was also observed that the rest of the polymer peaks stayed intact and were present in their expected position, proving the integrity of the polymer backbone. For example, the ester C=O peak present at 1731 cm\(^{-1}\) and C-N urethane polymer linkage was observed at 1220 cm\(^{-1}\) and 1167 cm\(^{-1}\) respectively. The appearance of an ammonium band at 3480 cm\(^{-1}\) and disappearance of Z (Benzyloxycarbonyl) group peaks at 749 and 679 cm\(^{-1}\) indicate the significant removal of Z-group from the polymer and the formation of free pendant amine group. It was also observed that the polymer backbone was not degraded during the course of the deprotection reaction.

In summary, it was concluded that the pendant Z group present at the PU backbone was removed successfully as illustrated by \(^1\)H-NMR, GPC and FTIR characterisation. The
formation of NH$_3^+$ as a pendant group shows the potential of the synthesised novel amino acid based PU to be used in applications where the presence of a cationic group may be advantageous, for example in delivery systems for gene based therapeutics. Future work may include a physiochemical characterisation of the deprotected PU to further test its biomedical applications.

4.7 Polyurethane urea

4.7.1 Polyurethane urea synthesis

A series of polyurethane ureas (PUUs) were prepared using the two step prepolymer method. The incorporation of diamine chain extender into the polymer results in the formation of a urea linkage with the NCO group in the hard segment of the polymer. PCL (Mw 1057) was used as the polyol, MDI was used as the diisocyanate and the novel synthesised compounds 311, 312, 313 and 314 were used as diamine chain extenders for PUU synthesis. The general chemical structure of the leucine diamine chain extender (311) based PUU as an exemplar, is shown in Figure 39 where the urethane and urea linkages are highlighted.

![Figure 39](image_url)

**Figure 39**: The chemical structure of PCL-1-Leu-PUU showing urea and urethane linkages

The synthesised series of PUU based on PCL 1057 (Mw) is referred as Series 1 PUU. The synthesis, characterisation and chemical structure of the novel amino acid based diamine chain extenders were detailed in Chapter 3. The prepolymer method for PUU synthesis is described in detail in the experimental section (4.3).

The resultant polymers were slightly yellow in colour, opaque and rubbery in appearance. The final polymer was stored in a sealed plastic bag for future research work. The net yield of the polymerisation reaction was ~70% (in all cases) and the result was
reproducible within range of ± 5%. A loss of low molecular weight species during solvent precipitation may account, in part, for the lower yield of the purified polymer.

The chemical structure of all the novel synthesised PUU is shown in Figure 40 and Table 18 shows the abbreviations followed for PUUs reported in this chapter. The nomenclature is based on the type and molecular weight of the soft segment and chain extender.

**Table 18 : Nomenclature for Series 1 PUU**

<table>
<thead>
<tr>
<th>Polyol Molecular Weight</th>
<th>Diisocyanate</th>
<th>Chain extender (Diamine)</th>
<th>Polyurethane urea (PUU) representative codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycaprolactone (PCL-1) (Mw=1057)</td>
<td>4,4-methylene diphenyl diisocyanate (MDI)</td>
<td>L-Leucine diamine ester (311)</td>
<td>PCL-1-Leu-PUU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Isoleucine diamine ester (312)</td>
<td>PCL-1-Ileu-PUU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Valine diamine ester (313)</td>
<td>PCL-1-Val-PUU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Tyrosine diamine ester (314)</td>
<td>PCL-1-Tyr-PUU</td>
</tr>
</tbody>
</table>

For example, PCL-1-Leu-PUU refers to PUU consisting of 1057 molecular weight of PCL and with L-Leucine based diamine chain extender (311, chapter 3). All the PUUs described in this and the following chapters contained MDI as the diisocyanate component and follow this nomenclature.

The quantities of starting material used in the synthesis of Series 1 PUUs are given in Table 19. All synthesised PUUs contained 0.05% stannous octoate as catalyst, had an isocyanate index of 1.05, with 50 weight% hard segment.
Figure 40: Chemical structure of Series 1 PUU

Table 19: Composition of Series 1 PUU

<table>
<thead>
<tr>
<th>PUU</th>
<th>PCL-1-Leu-PUU</th>
<th>PCL-1-Ileu-PUU</th>
<th>PCL-1-Val-PUU</th>
<th>PCL-1-Tyr-PUU</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDI (Mw)</td>
<td>250.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDI mass (g)</td>
<td>14.21</td>
<td>14.4</td>
<td>14.6</td>
<td>13.1</td>
</tr>
<tr>
<td>MDI moles</td>
<td>0.056</td>
<td>0.057</td>
<td>0.058</td>
<td>0.052</td>
</tr>
<tr>
<td>PCL (Mw)</td>
<td>1057</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCL mass (g)</td>
<td>25.03</td>
<td>25.02</td>
<td>25.02</td>
<td>25.03</td>
</tr>
<tr>
<td>PCL moles</td>
<td>0.023</td>
<td>0.023</td>
<td>0.023</td>
<td>0.023</td>
</tr>
<tr>
<td>CE (Mw)</td>
<td>372.5</td>
<td>358.5</td>
<td>344.4</td>
<td>472.5</td>
</tr>
<tr>
<td>CE mass (g)</td>
<td>11.42</td>
<td>11.25</td>
<td>11.07</td>
<td>12.48</td>
</tr>
<tr>
<td>CE moles</td>
<td>0.03</td>
<td>0.031</td>
<td>0.032</td>
<td>0.026</td>
</tr>
</tbody>
</table>
4.7.2 Characterization of polyurethane ureas

4.7.2.1 Gel Permeation Chromatography

The measured average molecular weight (Mw), number average molecular weight (Mn) and polydispersity index of Series 1 PUUs is shown in Table 20 and GPC traces are shown in Figure 41. All PUU molecular weights reported in this thesis are polystyrene equivalent molecular weights.

A typical GPC chromatograph of Series 1 PUUs shows a uni modal peak (Figure 41). Series 1 PUUs synthesised to a relatively high molecular weight, typical for experimental amino acid based polyurethanes reported in the literature (Skarja, 1998), (Marcos-Fernández et al., 2006), (Kavlock et al., 2007), (Fromstein and Woodhouse, 2002). PCL-1-Leu-PUU shows the highest of number average molecular weight and the aromatic PCL-1-Tyr-PUU shows the lowest of the number average molecular weight for Series 1 PUUs.

![Figure 41: GPC traces of Series 1 PUU](image-url)
Table 20: Molecular weight of Series 1 PUU

<table>
<thead>
<tr>
<th>Polyurethane Urea (PUU)</th>
<th>Number Average Molecular Weight ($M_n$) (Dalton)</th>
<th>Weight Average Molecular Weight ($M_w$) (Dalton)</th>
<th>Polydispersity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Leu- PUU</td>
<td>6.09 x 10^4</td>
<td>10.23 x 10^4</td>
<td>1.67</td>
</tr>
<tr>
<td>PCL-1-Ileu- PUU</td>
<td>7.40 x 10^4</td>
<td>13.43 x 10^4</td>
<td>1.81</td>
</tr>
<tr>
<td>PCL-1-Val- PUU</td>
<td>5.02 x 10^4</td>
<td>8.25 x 10^4</td>
<td>1.64</td>
</tr>
<tr>
<td>PCL-1-Tyr- PUU</td>
<td>4.88 x 10^4</td>
<td>8.35 x 10^4</td>
<td>1.71</td>
</tr>
</tbody>
</table>

Overall, it was observed that:

1. Polyurethanes with moderately high molecular weight were achieved using all four chain extenders used in this study.
2. The chain extender reactivity and slight variations (such as monomer weighing) in experimental technique may be the reason for the slight variation in molecular weight of Series-1-PUUs. It is not expected that their physical properties will be significantly affected by these slight variations. Molecular weight particularly for two PUUs (Valine and Tyrosine based PUU) was slightly lower than the other two PUUs (Leucine and Isoleucine based PUU).

The data indicates that Series 1 PUUs molecular weight distribution is relatively narrow (polydispersity less than 2) as compared to most literature values (Marcos-Fernández et al., 2006), (Guelcher et al., 2005). It is therefore assumed that properties of these polymers are determined by compositional morphology rather than molecular weight or its distribution.

4.7.2.2 Solubility

The solubility results for Series 1 PUUs in different organic solvents were tested at room temperature (25°C). The results indicate that PUUs are soluble in polar aprotic solvent such as Dimethylsulphoxide (DMSO), Dimethylacetamide (DMAc), N-Methyl pyrrolidine (NMP), and Dichloromethane (DCM) and are insoluble in water and protic solvent such as chloroform, acetone, ethanol, methanol, ethyl acetate and partially soluble in Tetrahydrofuran (THF). The insolubility of amino acid based PUU in most of the
organic solvents is probably due to the high molecular weight of the polymers. It has also been reported in the literature (Okuto, 1966), (Chao and Tian, 2002) that the insolubility of PU/PUUs in organic solvents might be attributed to the allophanate linkage formation when the isocyanate functionality is used in excess, as is the case here (NCO/OH - 1.05) for Series 1 PUUs.

4.7.2.3 $^1$H Nuclear Magnetic Resonance Spectroscopy

To avoid repetition of $^1$H-NMR spectrum and its peak assignment for all the PUUs, only leucine based PCL-1-Leu-PUU $^1$H-NMR spectra is describe here in detail and is shown in Figure 42. The choice of PCL-1-Leu-PUU for full description was random. The $^1$H-NMR spectrum of the rest of Series 1 PUUs are reported in the Appendix B. Note that DMSO-d$_6$ was chosen as the NMR solvent.

PCL-1-Leu-PUU

The $^1$H-NMR spectrum for PCL-1-Leu-PUU is shown in Figure 42 along with the $^1$H-NMR spectrum of the starting materials PCL and MDI.

Figure 42: $^1$H-NMR spectra (Spectrum A) PCL-1-Leu-PUU, (Spectrum B) MDI and (Spectrum C) PCL
In spectrum A, the peak present at 0.90 – 0.95ppm represents the terminal methyl (CH$_3$) protons of leucine diamine chain extender (311) and appears as a doublet. Peaks in the region of 1.4 – 1.6ppm are related to the diastereotopic hydrogen of methylene (CH$_2$), protons of PCL and diamine chain extender, and appear as a multiplet. The six methylene protons of neopentyl glycol (used for the ring opening of caprolactone to make PCL) appear at 0.87 and 0.19ppm. Methylene protons (CH$_2$) of PCL are present at 1.1 – 1.5 ppm. At 2.2ppm, terminal methylene protons (CH$_2$) present next to the carbonyl bond of PCL are present as a multiplet. The protons of CH$_2$ of the MDI moiety and CH$_2$ of the urethane linkage appear in the region of 3.8 – 4.2ppm respectively. The aromatic protons of MDI appear in the region of 7.1 – 7.4ppm. Similar observations were recorded by Rafiemanzelat, et.al. (2010) for $^1$H-NMR spectrum of poly ether urethane urea having MDI as diisocyanate and leucine based cyclopeptide as chain extender. Due to the presence of multiple peaks in the polymer spectrum, it is sometimes difficult to analyse each peak.

The presence of major protons associated with urethane and urea linkages are present to confirm the PUU synthesis. Thus the successful synthesis of PCL-1-Leu-PUU can be concluded from the $^1$H-NMR data.

The $^1$H-NMR spectroscopy peaks related to the PCL-1-Ileu- PUU, PCL-1-Val- PUU , PCL-1-Tyr-PUU are reported in Appendix B and display all the expected structural features of the targeted polymer. This demonstrates the successful formation of Series 1 PUUs.

### 4.7.2.4 Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed to determine the presence of specific functional groups in the PUU particularly ester, urethane and urea groups. To avoid repetition, only the PCL-1-Leu-PUU, FTIR data is discussed here, with the main FTIR peaks reported in Table 21. FTIR spectrum of PCL-1-Leu-PUU is shown in Figure 43. FTIR data for the rest of the Series 1 PUU is reported in the Appendix B.
A strong peak at 1735 cm\(^{-1}\) can be attributed to the carbonyl group (C=O) of urethane linkage indicating successful synthesis of the polymer. The broader shoulder around 3330 cm\(^{-1}\) is indicative of hydrogen bonded N-H stretching. It is worth noting the absence of the 2260 cm\(^{-1}\) band that is characteristic of the unreacted isocyanate group.

Stretching vibrations of the ester groups are assigned to the 1060, 1160, 1239 cm\(^{-1}\) bands. The peak at 1536 cm\(^{-1}\) is characteristic of the carbonyl stretching vibration, which is very intense. The urea carbonyl (hydrogen bonded) peak around 1640 – 1629 cm\(^{-1}\) indicates the presence of the urea group. The characteristic bands of ester and urethane free carbonyl overlap between 1735 and 1710 cm\(^{-1}\).

The absorption peaks at 2854 and 2924 cm\(^{-1}\) correspond to symmetric and asymmetric CH\(_2\) groups respectively. Bands at 1458, 1420, 1394 and 1365 cm\(^{-1}\) correspond to various modes of CH\(_2\) vibrations.

Figure 43 : FTIR spectra of PCL-1-Leu-PUU
Table 21: FTIR peak assignment for PCL-1-Leu-PUU

<table>
<thead>
<tr>
<th>Wavenumber (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3339, 3330</td>
<td>ν (N-H) (urethane H-Bonded), ν (N-H) (urea H-Bonded)</td>
</tr>
<tr>
<td>3191, 3125</td>
<td>ν (C-C) aromatic ring</td>
</tr>
<tr>
<td>2924, 2854</td>
<td>νₘ (C-H) in CH₂, νₘ (C-H) in CH₂</td>
</tr>
<tr>
<td>1735</td>
<td>ν (C=O) urethane non-bonded</td>
</tr>
<tr>
<td>1645</td>
<td>ν (C=O) urea H-Bonded</td>
</tr>
<tr>
<td>1597</td>
<td>ν (C-C) aromatic ring</td>
</tr>
<tr>
<td>1530</td>
<td>ν (C-N) + δ (N-H) Amide II</td>
</tr>
<tr>
<td>1448 - 1365</td>
<td>ν (C-C) aromatic ring</td>
</tr>
<tr>
<td>1239, 1060</td>
<td>ν (C-N) + δ (N-H)</td>
</tr>
<tr>
<td>1067, 1311</td>
<td>ν(C-O-C) in hard segment, (O=C-O-C) in soft segment</td>
</tr>
</tbody>
</table>

ν=stretching mode, νₘ=asymmetric stretching, νₘ=symmetric stretching, δ= in-plane bending, ρ=in-plane bending or rocking

In summary, it was observed that the FTIR spectral characteristic of the synthesised polyurethane PCL-1-Leu-PUU was found to be in full agreement with the expected structure. Thus the formation of PUU was concluded. Similar FTIR observations for the structural identity of PCL-1-Ileu-PUU, PCL-1-Val-PUU and PCL-1-Tyr-PUU are reported in the Appendix B. In all cases, successful formation of Series 1 PUUs was concluded.

4.7.2.5 Thermal Analysis

The Differential scanning calorimetry (DSC) thermograms of Series 1 PUUs were obtained and to avoid repetition, only the aliphatic leucine amino acid based PCL-1-Leu-PUU and aromatic tyrosine amino acid based PCL-1-Tyr-PUU DSC thermograms are described in detail here. DSC results for Series 1 PUUs are shown in Table 22.
Table 22: Thermal properties of Series 1 PUU

<table>
<thead>
<tr>
<th>Soft Segment</th>
<th>PCL-1-Leu-PUU</th>
<th>PCL-1-Ileu-PUU</th>
<th>PCL-1-Val-PUU</th>
<th>PCL-1-Tyr-PUU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass transition ($T_g$) (°C)</td>
<td>-6.5</td>
<td>-3</td>
<td>-7.2</td>
<td>13.2</td>
</tr>
<tr>
<td>Melting Temperature ($T_m$) (°C)</td>
<td>47</td>
<td>47</td>
<td>46.5</td>
<td>ND</td>
</tr>
<tr>
<td>Crystallinity (%)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>ND</td>
</tr>
<tr>
<td>Glass Transition ($T_g$) (°C)</td>
<td>-10.5</td>
<td>-15.1</td>
<td>-7</td>
<td>13.3</td>
</tr>
<tr>
<td>Tm &amp; Crystallinity (%)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table 22: Thermal properties of Series 1 PUU

$T_g$: Glass transition temperature, $T_m$: Melting temperature, $\%X_c$: Crystallinity, $T_g^{1\text{st}}$: $T_g$ of first heating run, $T_g^{2\text{nd}}$: $T_g$ of second heating run, $T_m^{1\text{st}}$: $T_m$ of first heating run, ND: Not Detected

PCL-1-Leu-PUU

The DSC thermogram for the 1st and 2nd heating run of PCL-1-Leu-PUU is shown in Figure 44.

Figure 44: DSC thermograms for 1st and 2nd heating run of PCL-1-Leu-PUU
1st Heating Run - The soft segment glass transition \( (T_g) \) observed in the first heating run for PCL-1-Leu-PUU was at -6.5°C which is much higher than the \( T_g \) of pure PCL (-60°C) (Bogdanov et al., 1999). During first heating run, the polymer shows soft segment crystal melt endotherm \( (T_m) \) at 47°C with crystallinity of 5% indicates semicrystalline nature of the soft segment. The increase in soft segment \( T_g \) as compare to respective homopolymer (PCL) \( T_g \) might be due to the mixing of the hard segment chains with soft segment chains of the polymer which decreases soft segment chain flexibility and mobility and consequently increases \( T_g \) of the soft segment. It is reported in literature (Gunatillake and Adhikari, 2011) that the presence of short chain hard segments units in soft segments can increase the \( T_g \) of the soft segment. The another reason for higher \( T_g \) might be due to the presence of less perfect crystals of PCL in the polymer soft segment which is again due to soft and hard segment phase mixing behaviour.

Based on the above observations it can be concluded that phase mixing has occurred in the synthesised PUU. However, the leucine based diamine chain extender introduces a urea group into the polymer, which is more polar than the urethane groups introduced by dihydroxy chain extender and this can promote phase separation through increased urea based hard segment interchain interactions (Oprea, 2002) (Azzam et al., 2007). It seems like that the disruptive effect of the pendant aliphatic side chains of the chain extender present in the hard segment may have offset the phase separation phenomena due to the urea group and hence polymer shows phase mixed behaviour. It is reported in literature (Hepburn, 1982, Bae et al., 1999) that polyurethanes that contain non-linear or side chain containing chain extenders are usually more phase mixed, when compare to those containing linear chain extenders. No hard segment \( T_g \) or \( T_m \) was observed for PCL-1-Leu-PUU for the first heating run indicate amorphous nature of the hard segment.

2nd Heating Run - The soft segment \( T_g \) observed during second heating run was at -10.5°C which is close to \( T_g \) observed in first heating run. But the soft segment \( T_m \) was not observed during the second heating run indicate amorphous nature of the soft segment. The reason might be that the enough time was not provided to PUU chains to relax properly. Once PUU gets heated during first heating run, the polymer gets cool down very rapidly and the polymer chains has not get enough time to relax and organise. That’s why
\( T_m \) was not observed during second heating run. This type of behaviour is typical for polyurethanes. No hard segment \( T_g \) or \( T_m \) was observed for PCL-1-Leu-PUU for the second heating run. It shows non crystalline (amorphous) hard segment is present in the polymer.

Overall, the presence of pendant aliphatic chains of chain extender in the hard segment has prevented the efficient packing necessary for crystallization and leads to amorphous hard segment. Guelcher et al. (2005) has reported the hindered hard segment packing due to the presence of aliphatic ethyl ester as pendant group or short chain branches (SCBs) into the backbone of the synthesised polyurethane. Thus there is a good agreement between the observation made with the current polymer and the examples reported in literature and hence it can be concluded that aliphatic side chains present at the polymer backbone have prevent the efficient packing of hard segment and this has led to non-crystalline hard segment. Overall, DSC analysis showed that PCL-1-Leu-PUU was a phase mixed, semicrystalline polymer with amorphous hard segment.

**PCL-1-Tyr- PUU**

The DSC thermograms for the 1st and 2nd heating run of PCL-1-Tyr-PUU is shown in Figure 45.

![DSC thermograms for 1st and 2nd heating run of PCL-1-Tyr-PUU](image)

**Figure 45** : DSC thermograms for 1st and 2nd heating run of PCL-1-Tyr-PUU
1st Heating Run - The soft segment glass transition temperature ($T_g$) for the first heating run was observed at 13.2°C, which is substantially higher than $T_g$ of pure homopolymer PCL (-60°C). It has been reported in the literature (Umare and Chandure, 2008), (Changhong et al., 2008), (Vermette, 2001) that the interactions between the soft and hard segment can influence the glass transition temperature of the polymer.

DSC shows phase mixing behaviour between the hard and soft segment of the polymer. The observed soft segment glass transition temperature is lower than the body temperature 37°C, indicating that PUUs can maintain elasticity at body temperature. Similar observation has been recorded by Zhang (2006) where MDI-PCL based polyurethane was synthesised. No soft segment crystal melt endotherm temperature ($T_m$) was observed for PCL-1-Tyr-PUU during the 1st heating run showing the amorphous nature of the soft segment as compared to semi crystalline nature of PCL-1-Leu-PUU.

2nd Heating Run – The soft segment $T_g$ was observed at 13.3°C which is quite close to the 1st heating run soft segment $T_g$. No $T_m$ was observed for the soft segment showing amorphous nature. In a same way, hard segment also did not show any thermal transitions such as $T_g$ or $T_m$ indicating amorphous nature. The absence of a melting endotherm ($T_m$) for hard segment based on MDI based PU was also reported by Changhong et.al. (2008), (Lamba et al., 1998). The absence of hard segment transition might be due to the presence of the bulky phenyl group of chain extender as pendant group which can disturb the packing of the hard segment to form any crystals and hence does not show a crystal melt endotherm ($T_m$). Skarja et.al. (1998) and Parrag et.al. (2010) have reported the similar observation of the presence of non crystalline hard segment due to the presence of bulky phenyl pendant group which has prevented the ordered structural arrangement of the hard segment and this has led to amorphous hard segment.

Overall, it was concluded that aromatic amino acid based PCL-1-Tyr-PUU shows phase mixed morphology with amorphous hard and soft segment. In general, it was observed that hard segment showed the non crystalline behaviour in Series 1 PUUs. This might be due to the presence of pendant group in the chain extender which have resulted in disordered hard segment arrangement.
4.7.2.6 Thermogravimetric Analysis

Thermogravimetric curves provide information about the decomposition mechanism of various materials. Thermogravimetric Analysis (TGA) traces illustrating the polymer weight loss as a function of temperature for Series 1 PUUs is shown in Figure 46.

All the aliphatic amino acid based PUUs showed the onset of degradation at temperature between 250 and 280°C. Weight loss in the first degradation step occurs mainly due to depolycondensation of urethane groups. The second step shows the degradation of the soft segment of PUUs which occurs between 300 and 420°C. Up to 80% degradation occurs at this stage. For the aromatic amino acid based PCL-1-Tyr-PUU, the onset of degradation starts at 285°C and completes by 435°C (the highest of the series 1 PUUs). Valine, leucine and isoleucine based PUU shows a similar thermal degradation trend, whilst the aromatic tyrosine based PCL-1-Tyr-PUU exhibits a slightly broader slope. This might be due to the presence of phenyl group in the hard segment of the PUU. These aromatic groups maybe involved in non-covalent π - π interaction with each other (Hinrichsen, 1994) or with the benzyl group of the MDI present in the hard segment. Phenyl groups are reported to strengthen inter chain hard segments and thus provides
tyrosine based PUU more thermal stability as compared to other aliphatic chain extender based PUU. Indeed, Guelcher et.al. (2005) has reported the higher melting temperature of aromatic tyramine and tyrosine amino acid based PU due to phenyl group inter chain attraction.

Sarkar et.al. (2009) reports the onset of degradation at 300°C for a tyrosine based HDI-PCL-PU. It demonstrating that the observed TGA results, here, are consistent with literature. For the control PCL-1-BDO-PU (Figure 33), the onset of degradation is around 270°C and is complete by 470°C. It shows that control PU is more thermally stable as compare to Series 1 PUUs. Although the observed difference in the degradation temperature in insignificant. However it is also reported in the literature that the urea linkage is not thermally stable at high temperature and is considered as a weak link for polyurea thermal degradation studies (Yilgör et al., 2000). It might be the reason for obtaining the low thermal stability of Series 1PUUs as compared to control BDO-PU which lacks urea linkage.

Fernandez et.al. (2006) has also reported the low thermal stability of diamine chain extender based PCL-PUUs. Ornithine and lysine amino acids were used to make diamine chain extender. They reported the onset of thermal degradation temperature for ornithine based PUUs as 195 to 218°C and for lysine based PUUs, 172 to 215°C. Both are less than the current Series 1 PUUs, indicating that the Series 1PUUs, here, exhibit improved thermal stability. Tyrosine based PUUs also show improved thermal stability as compared to aliphatic chain extender based PUUs in Series 1.

In summary, it was observed that Series 1 PUUs have shown a potential range of thermal stability which might be useful for thermal processing of these polymers.

4.7.2.7 Water Contact Angle

Static contact angle measurements of the materials was obtained at room temperature (25°C) in order to obtain an indication of the relative polarity of the polymer surface. Deionised water was used and five different contact angle readings were taken at different places on each polymer film. It was observed that synthesised PCL-MDI-based
polyurethane urea have high contact angle value, indicating lower surface polarity which is consistent with literature (Fernández d'Arlas et al., 2008). The value of the water contact angle for Series 1 PUUs was in the range from 82° to 94° and is shown in Table 23.

Table 23: Contact angle values for Series 1 PUU

<table>
<thead>
<tr>
<th>Polyurethane Urea (PUU)</th>
<th>Contact Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Leu-PUU</td>
<td>83° ± 2</td>
</tr>
<tr>
<td>PCL-1-IsoLeu-PUU</td>
<td>82° ± 2</td>
</tr>
<tr>
<td>PCL-1-Val-PUU</td>
<td>85° ± 1</td>
</tr>
<tr>
<td>PCL-1-Tyr-PUU</td>
<td>94° ± 3</td>
</tr>
</tbody>
</table>

The obtained PUUs can be considered somewhat hydrophobic. This behaviour is due to the very low surface energy of PCL, which is expected to migrate to the surface. The hydrophobic nature of the hard segment (aromatic diisocyanate (MDI)) and high hydrocarbon content of the chain extender also contributes to the high value of contact angle for the synthesised PUUs. The aromatic amino acid based PCL-1-Tyr-PUU shows the highest water contact angle (94°±3) (i.e. hydrophobic), probably due to the presence of the benzene ring in the side chain of the tyrosine amino acid. It is reported in the literature (Sarkar, 2007) (Guan et al., 2004, Guan and Wagner, 2005) that the water contact angle value of up to 75° for tyrosine amino acid based PCL-HDI polyurethane considered as hydrophobic polymer surface. In summary, it was observed that the incorporation of the novel amino acid based chain extenders into the PUUs has made the polymer surfaces more hydrophobic.

4.7.2.8 Mechanical properties

The stress strain curves for Series 1 PUUs are shown in Figure 47 and the values are shown in Table 24. The results show that the PUUs have high extension to failure, exhibiting very low modulus and tensile strength. Tyrosine based PUU had the highest tensile strength in Series 1 PUUs.

Aliphatic amino acid based PUUs containing leucine, isoleucine and valine amino acid display very similar mechanical properties, such as high elongation to failure and low
values of tensile strength and modulus of elasticity as compared to the aromatic tyrosine amino acid based PUU and the control PCL-1-BDO-PU.

Figure 47: The Stress strain curves for Series 1 PUU

PCL-1-Tyr-PUU displays much improved tensile properties, with a tensile strength three times higher than leucine-PUU and two times higher than Isoleucine-PUU. This may be due to the ability of the pendant phenyl group of the tyrosine based chain extender to make π - π bond interactions with other similar groups in neighbouring polymer chains of hard segments. PCL-1-Leu-PUU was the weakest material in the series despite having a high molecular weight, exhibiting very high extension (>900%) before failure indicating very poor molecular interactions. DSC of this material also exhibited a highly phase mixed morphology indicating the mixed phase may contribute to poor tensile properties.

Several factors can impact the mechanical properties of the PUUs. However, it is the chain extender’s non-linear structure and its role in polymer hard segment packing (as observed in DSC, Table 22) which may be one of the main factors for obtaining such low mechanical properties of the synthesised PUU. Hard segment packing has been shown to have a pronounced effect on the mechanical properties of polymers (Puskas et al., 2003). Simultaneously, MDI based polymers showed improved mechanical properties due to the symmetric and cyclic structure of the MDI (Prisacariu, 2011). The observed weak
mechanical property for Series 1 PUUs based on MDI does not match with this literature finding. DSC scans revealed hard segment was not packed efficiently due to bulky side chains of the chain extender which may have restrict the hard segment mobility and reduce its packing to observe any thermal transitions. This shows that the mechanical properties or the synthesised PUUs are mainly governed by the soft segment and the hard segment does not act as physical cross linker to improve the tensile properties.

**Table 24** : Mechanical properties of Series 1 PUU (mean ± SD, n = 6)

<table>
<thead>
<tr>
<th>Polyurethane Urea (PUU)</th>
<th>Modulus of Elasticity (MPa)</th>
<th>Tensile Strength (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Leu-PUU</td>
<td>4.3 ± 1</td>
<td>0.52 ± 0</td>
<td>982.2 ± 11</td>
</tr>
<tr>
<td>PCL-1-Ileu-PUU</td>
<td>5.1 ± 1</td>
<td>1.1 ± 0</td>
<td>337.0 ± 46</td>
</tr>
<tr>
<td>PCL-1-Val-PUU</td>
<td>7.2 ± 2</td>
<td>2.0 ± 1</td>
<td>563.7 ± 22</td>
</tr>
<tr>
<td>PCL-1-Tyr-PUU</td>
<td>11.6 ± 8</td>
<td>7.3 ± 3</td>
<td>421.7 ± 58</td>
</tr>
<tr>
<td>PCL-1-BDO-PU</td>
<td>52.8 ± 22</td>
<td>32.9 ± 12</td>
<td>234 ± 18</td>
</tr>
</tbody>
</table>

*Sample did not break during tensile testing.

Moreover, the presence of a pendant aliphatic carbon atom chain on the chain extender probably leads to lose packing of the hard segment and hence the loss of crystallinity and disordered packing (less hydrogen-bonding) (Teo et al., 1997) leading to poor mechanical properties. Parrag et al. (2010) has also reported the low mechanical properties of phenylalanine based PU due to a pendant bulky aromatic side chain of phenylalanine amino acid based chain extender present in the hard segment. Similarly Sarkar et al. (2007) has reported observations of obtaining low mechanical properties for aromatic tyrosine based PUs.

The observed weak mechanical properties of synthesised PUU as compared to BDO based PU are further supported by DSC data as phase mixed morphology was observed for Series 1 PUUs. When the polymer was stretched during tensile testing, the ability of loosely packed hard segments to deform and reorient at increasing strain could lead to high elongation properties.

In summary, the importance of phase morphology and role of chain extender chemical structure on phase morphology was demonstrated with weak mechanical properties.
obtained for the Series 1 PUUs. PUUs based on novel amino acid based chain extenders showed significantly lower mechanical properties as compared to BDO based PU, due to the non-linear chemical structure of the amino acid based chain extenders used for PUU synthesis. As compared to the control BDO, the synthesised chain extenders are non-linear with bulky pendant groups, and hence form very weak H-bonding interactions with other polymeric chains which in turns lower the tensile properties of the material. The effect of this weak interaction was reflected in attaining poor mechanical properties of the synthesised polymers. The synthesised polymers behaved as low modulus highly extendable materials with very poor tensile strength.

4.8 Conclusion

This Chapter was focused on the synthesis and characterisation of Series 1 PU and Series 1 PUU based on novel L-amino acid based chain extenders. It was found that the synthesised PUs and PUUs were light yellow colour in appearance with low modulus and highly extendable materials as compared to the hard and strong BDO based polyurethane. The Series 1 PUs and Series 1 PUUs all show moderately high molecular weights with relatively narrow polydispersity. The polymers were soluble in polar aprotic solvents with potential to use solvent based processing techniques. DSC analysis of synthesised polymers showed a phase mixed morphology with an amorphous hard segment. The synthesised polymers were thermally stable up to ~285°C. Series 1 PUs exhibited high thermal stability due to the presence of aromatic moieties within the hard segment derived from the new chain extenders. Mechanical properties as observed by stress strain curves, were weak as compared to the BDO based PU. Amino acid based PUs and PUUs exhibited properties of high extensibility and low tensile strength. Overall the amino acid based Series 1 PUs and Series 1 PUUs exhibited weak mechanical properties as compared to the control PU. The lack of hydrogen bonding within the hard segment and the non-linear structure of the novel chain extender may be the possible reasons for the low mechanical properties observed. The surface hydrophobicity, as measured by water contact angle, increased with incorporation of the novel amino acid based chain extenders indicating their potential for use as implant material in future biomedical applications.
The results in this chapter show that the composition of the polymer plays a crucial role in determining the properties of the resultant polymeric material, thus future work could focus on optimisation of formulation to give improved mechanical properties. The polyurethanes synthesised in this work may be suitable for applications such as scaffolds for soft tissue regeneration, drug delivery systems and other applications where high mechanical strength is not required.

Since the aim of the experimental work was to create a range of polyurethanes that exhibit different properties (physical, thermal and mechanical), it is logical to investigate the effect of changing the chemical composition of the soft segment, which has been kept constant in this study (PCL) and which will be discussed in the following chapter.
Chapter 5: Effect of soft segment molecular weight on the structure property relationship of polyurethanes (PUs) and polyurethane urea’s (PUUs)
5.1 Introduction

The structure property relationship of polyurethanes prepared from conventional chain extenders, diisocyanate and polyols, are very well documented in the literature (Gunatillake et al., 2003). The amino acid based chain extenders reported in this thesis are, however, novel and detailed understanding of their influence on polyurethane properties is important to explore. In chapter 4, the effect of (amino acid based) chain extender structure on PU and PUU properties was investigated, keeping the polyl type, molecular weight and the diisocyanate constant. In this chapter, an investigation to study the effect of the polyl molecular weight will be discussed.

A series of PUs and PUUs were prepared using polycaprolactone (PCL) of molecular weight 2,000 Daltons and their properties were compared with those prepared using PCL of molecular weight 1,000 Dalton (Chapter 4). This molecular weight (Mw) range represents that generally used for polyurethane formulations (Król, 2007). The synthesis and characterisation of the chain extenders used here was described in detail in chapter 3 and the synthesis and characterisation of polyurethanes (PUs) and polyurethane ureas (PUUs) based on 1,000 Dalton (Mw) PCL were described in chapter 4. The synthesis of the 2,000 Dalton (Mw) PCL based PUs and PUUs followed the same procedure. The diisocyanate used was 4,4’-methylene diphenyl diisocyanate (MDI) and the weight percent of hard to soft segment was kept constant at 50 weight %. Since PUs and PUUs exhibit different structure property relationships due to their functional group differences, it is reasonable to assume that the use of a different molecular weight soft segment (PCL 2,000 Dalton) may impact the physio mechanical properties of the two classes of polymers in different ways. This work aims to describe the effect of soft segment structural variation on the properties of amino acid based PUs and PUUs with a long term goal of helping to assess their suitability for biomedical applications.
5.2 Materials

Polycaprolactone (PCL) as polyol (Mw 2,000) was dried at 60°C for 48 hr under vacuum (0.1 torr) prior to use and 4,4-methylenediphenyl diisocyanate (MDI), was used as received from Sigma Aldrich, USA. Since MDI can react with moisture in the air, pure MDI was distributed into small dry bottles and fresh MDI was used for each reaction. Anhydrous DMF was obtained from sigma Aldrich, USA. The solvents were analytical grade and were used as received. Deionised water was used for all purposes. Stannous octaoate (Aldrich) was obtained from Sigma Aldrich and kept moisture free and used as received. All other solvents and chemicals used for purification methods were of analytical grade. Unless stated otherwise, all reactions were carried out in oven dried glassware. An inert atmosphere of nitrogen was used while working with anhydrous solvents. Chemical reaction temperatures were controlled using an IKA brand magnetic temperature modulator.

5.3 Experimental Procedure

5.3.1 Synthesis of polyurethane

Polycaprolactone (PCL, Mw-2,000) was used as polyol and 4,4-methylenediphenyl diisocyanate (MDI) was used as diisocyanate for polymer synthesis. The amino acid based dihydroxy diester compounds (318, 319) synthesised and reported in chapter 3 are used as chain extender here. Synthesised PUs will be referred as Series 2 PU where 2 refers to the 2,000 molecular weight of the PCL. A two step or prepolymer method was adopted for the synthesis of polyurethane and described in detail in chapter 4. Prepolymer method followed is illustrated in Scheme 4, chapter 4. Stannous octaoate was used as catalyst and N,N-dimethylformamide (DMF) was used as solvent for the polymerization. The polymerisation reaction was carried out under a completely dry environment under inert (dry N₂ was used) atmosphere. PUs were synthesised with equivalent hard and soft segments (50 weight % each) in all the cases. In brief, the method of synthesis of Series 2 PU is described below.
Polymerization was conducted in a 250 mL, 3 neck round bottom flask equipped with magnetic stirrer, nitrogen gas inlet and outlet fitted to the two necks of the flask, and an additional funnel fitted to the third neck. PCL was added to the flask. Reaction solvent, anhydrous DMF was added to the flask using a syringe. Few drops of stannous octaoate as catalyst was added to the reaction mixture under dry and inert atmosphere with continuous stirring. MDI was charged to the reaction flask at room temperature. The temperature was increased to 80°C and the reaction was allowed to proceed for 3 hours at this temperature, and slowly cooled to room temperature (25°C) with continuous stirring. The temperature of the reaction was carefully maintained within the range of ± 3°C. Chain extension step was then carried out by simultaneous addition of amino acid based dihydroxy chain extender to the prepolymer solution under vigorous stirring. The NCO/OH ratio was maintained at 1.05. The temperature of the reaction was then gradually increased to 80°C and stirred for another 12 hours at the same temperature. After 12 hours, the reaction was quenched by pouring the reaction mixture into a cold concentrated aqueous solution of sodium chloride. At this point, solid polyurethane polymer precipitates out from the reaction mixture. The final polymer was filtered out and washed with distilled water. The polymer was dried under vacuum at 40°C for 48 hours. Synthesised PUs will be referred as Series 2 PU where 2 refers to the 2,000 molecular weight of the PCL. In total, two PUs were synthesised based on two (318 and 319) amino acid based dihydroxy chain extender. The net yield of the polymerisation reaction was ~70% (in all cases for Series 2 PU). The polyurethane synthesised were stored in a sealed plastic bag for the purpose of characterisation and future experiments.

5.3.2 Synthesis of polyurethane urea

The method of synthesis of polyurethane urea is similar to PU synthesis as described in section 5.3.1. The same two step solution polymerization method was used for the preparation of PUU containing equivalent hard and soft segments (approximately 50 weight % each). Stannous octaoate was used as catalyst and anhydrous DMF act as reaction solvent. Amino acid based diamine compounds (311, 312, 313, 314) act as chain extenders to synthesise PUUs. The reaction conditions and procedure was the same as for Series 2 PU (Section 5.3.1). Polycaprolactone (PCL, Mw-2,000) was used as polyol and aromatic diisocyanate 4,4-methylene diphenyl diisocyanate (MDI) was used as diisocyanate for polymer synthesis. Synthesised PUUs will be referred as Series 2 PUU
where 2 refers to the 2,000 molecular weight of the PCL. The method of the synthesis of Series 2 PUUs is described as follow.

Briefly, Polymerization was conducted in a 250 mL, 3 neck round bottom flask equipped with magnetic stirrer, nitrogen gas inlet and outlet fitted to the two necks of the flask, and an additional funnel fitted to the third neck. PCL (Mw 2,000 Dalton) and reaction solvent (DMF) was added to the flask. Few drops of stannous octaoate as catalyst was added to the reaction mixture under dry and inert atmosphere with continuous stirring. Following that, MDI was charged to the reaction flask at room temperature. The temperature was increased to 80°C and the reaction was allowed to proceed for 3 hours at this temperature, and slowly cooled to room temperature with continuous stirring. Chain extension step was then carried out by simultaneous addition of amino acid based diamine chain extender to the prepolymer solution under vigorous stirring. The NCO/OH ratio was maintained at 1.05. The temperature of the reaction was then gradually increased to 80°C and stirred for another 12 hours at the same temperature and then, the reaction was quenched by pouring the reaction mixture into a cold concentrated aqueous solution of sodium chloride. At this point, solid polyurethane urea polymer precipitates out from the reaction mixture. The final polymer was filtered out and washed with distilled water. The polymer was dried under vacuum at 40°C for 48 hours. Synthesised PUUs will be referred as Series 2 PUU where 2 refers to the 2,000 molecular weight of the PCL. In total 4 PUUs were synthesised based on four (311, 312, 313, 314) amino acid based diamine chain extender. The net yield of the polymerisation reaction was ~70% (in all cases for Series 2 PUU). The polyurethane urea synthesised were stored in a sealed plastic bag for the purpose of characterisation and future experiments.

5.4 Characterisation of polymers

5.4.1 Gel permeation chromatography
The molecular weights of polymers were determined by Gel Permeation Chromatography (GPC). A Waters 515 HPLC pump attached to Waters 590 equipped with a refractive index detector (a Waters 410 differential refractometer) which uses dimethyl formamide (DMF) as solvent (containing Lithium Bromide (LiBr), 0.05 M). The flow rate of the
mobile phase was maintained at 1mL/min and the operating temperature was 30°C. Polyurethane sample was dissolved in DMF (approx. 2 mg/mL) and filtered through a 0.5 m (MFS Advantech) syringe filter and injected (50 µl) into an HPLC pump. Calibration of the apparatus was routinely performed with monodisperse polystyrene as standard. Data was analysed using Empower Pro software to determine the average number molecular weight (Mn), weight average molecular weight (Mw) and polydispersity (Mw/Mn) of the polymer. All molecular weights reported here are in terms of polystyrene standard.

5.4.2 Thermal characterization

The thermal behaviour of the PUs and PUUs was characterised by Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). The DSC was performed using a DSC30 (Mettler Toledo) instrument. For the DSC measurements, the samples were vacuum dried for 48 hours at room temperature in the presence of phosphorous pentaoxide. A specimen of approximately 5 mg was cut from compression moulded sheet and was encapsulated in an aluminium pan. Samples were scanned in alternating DSC mode from -50°C to 200°C at a rate of 10°C min⁻¹ under nitrogen atmosphere. A three stage heating and cooling method was used in which the sample was first heated from -50°C to 200°C and then quenched immediately to -50°C and heated again to 200°C. First heating run provides information about any crystallinity present in the soft segment which might disappear in the second heating run due to the rapid cooling of the polymer sample. DSC thermograms were analysed using Mettler: STAR EV.9.00 software to determine polyurethane glass transition (Tg) and melting temperature (Tm). Melting point (Tm) was taken as the peak temperature of the observed endothermic transition, whereas glass transition temperature (Tg) was measured at the half width of the transition after plotting tangents on the curve. The percent crystallinity (% Xc) of the polymer was calculated according to the melting peak area of the DSC graph by assuming that a perfect PCL crystal has a melting enthalpy of 139.5 J/g [Enthalpy was determined using the values (ΔHm / ΔHm100) where Δ Hm100 is Δ Hm is enthalpy of melting temperature of 100% crystalline PCL] (Pitt et al., 1990). Thermo-gravimetric analysis (TGA) was performed with a Mettler Toledo TGA/STDA851 from 0°C to 800°C under nitrogen at a rate of 20°C min⁻¹. TGA graphs were analysed using Mettler: STAR EV.9.00 software. The
specimens were placed in a ceramic pan and an average of 10 mg of solid sample was used for the experiments.

5.4.3 Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) spectra were obtained using a ThermoNicolet 6700 spectrometer using a SmartATR (attenuated total reflectance) attachment fitted with a diamond window. The sample was analysed (32 scans) at 25°C in the transmission mode over 4000-700 cm⁻¹.

5.4.4 Water contact angle measurement

Water contact angles were measured using a contact angle meter (KSV CAM200 Instruments Ltd.). Thin films of polyurethane were prepared on thoroughly cleaned and dried glass slides by spin coating of 5 wt% solution of polymer in DMF. The films were dried initially at room temperature for 24 hours followed by vacuum drying at 50°C for another 48 hours to remove the residual solvents. Static water contact angles were measured by depositing a drop of 2 µl of deionised water (Millipore, Elix 5, 15 MΩ) at room temperature (25°C) and then a picture of the surface was taken through a camera (Sony). The contact angle was measured manually on the picture. To verify the contact angle reproducibility, an average of five readings (± standard deviation) from different parts of the each film was taken.

5.4.5 Compression moulding

Compression moulding was performed on a hydraulic press (with a thermostat and water-cooling capability) to obtain polymer films of the synthesised polyurethanes. The polymer was cut into small pieces using clean tin snips and pressed into a 1 mm thick plaque at a temperature above the melting point of the polymer (typically). The temperature was held constant for a period of 5 minutes before cooling in a standard manner under the flow of cold water. A standard mould was used which consisted of a rectangular cavity 100 mm x 60 mm x 1 mm deep cut into a metal plate. Teflon fabric sheet was used on both sides of mould to prevent adhesion of the polymer to the metal. The press used was a model 12-10-1T Wabash hydraulic press. In order to avoid unnecessary degradation of the material, the polyurethanes were not subjected to annealing. Polymer films thickness was
measured with digital callipers. The polymer films were stored in plastic bags in a desiccator prior to characterization.

5.4.6 Mechanical properties

The mechanical properties of the polyurethane films were measured using an Instron model 4468 universal testing machine according to the ASTM D-882 method. The compression moulded plaques were cut into thin strips with a straight section of 40 mm x 5 mm x 0.4 mm. A 100 N load cell was used with a crosshead speed of 100 mm/min at room temperature. At least seven replicate measurements were taken and averaged (± standard deviation). Strong pneumatic grips were used to prevent slippage. Data was analysed using Blue hill v.2.5 software.

5.5 Result and Discussion

5.5.1 Polyurethane synthesis

A series of amino acid based polyurethanes (PUs) was synthesised using the two-step method described in the experimental section 5.3. Here, polycaprolactone (PCL) of molecular weight 2,000 Dalton was used as the soft segment and the series is thus referred as Series 2 PU. 4,4-methylene diphenyl diisocyanate (MDI) was used as diisocyanate. Z-serine and Z-threonine amino acid based diester dihydroxy compounds (318 and 319, chapter 3) were used as the chain extender. The polymer abbreviation used is based on the type and molecular weight of chain extender and polyol used respectively. For example, PCL-2-Z-Ser-PU, refers to PU consisting of 2,000 Dalton molecular weight PCL, chain extended with an L-Z-Serine based dihydroxy chain extender (318, chapter 3), Z is a benzyloxycarbonyl group; an amine group protecting agent present in the chain extender and acting as a pendant group in the PU structure. Table 25 shows the abbreviations used for Series 2 PUs in this chapter.
Table 25: Polyurethanes abbreviations used for Series 2 PUs

<table>
<thead>
<tr>
<th>Polyol</th>
<th>Diisocyanate</th>
<th>Chain extender (dihydroxy)</th>
<th>Polyurethane Representative Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycaprolactone (PCL-2) (Mw=2,000 dalton)</td>
<td>4,4’-methylene diphenyl diisocyanate (MDI)</td>
<td>Z-Serine dihydroxy ester (318)</td>
<td>PCL-2-Z-Ser-PU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z-Threonine dihydroxy ester (319)</td>
<td>PCL-2-Z-Thr-PU</td>
</tr>
</tbody>
</table>

In general, synthesised Series 2 PUs were yellowish-white and rubbery in appearance. They were stored in sealed plastic bags for future use. The percent yield of the Series 2 PUs was ~ 70% in each case. The slight loss in yield may be due, in part, to the loss of low molecular weight polymer during the polymer purification (precipitation) process.

Series 2 PUs were compared to Series 1 PUs in order to evaluate the effect of soft segment molecular weight on the physiochemical properties of the synthesised PU. A 50 weight % hard segment (HS)/50 weight % soft segment (SS) ratio was kept constant for all polyurethane synthesis here. It has been reported in the literature (Prisacariu and Scortanu, 2010, Caracciolo et al., 2009) that changes to the soft segment / hard segment ratio can affect the mechanical properties of the resultant polyurethane and therefore needs to be kept constant.

5.5.2 Characterization of Polyurethanes

5.5.2.1 Gel permeation chromatography

Table 26 shows the GPC results obtained for Series 2 PUs and compared with Series 1 PUs. The molecular weights reported here are polystyrene equivalents. As shown in Table 26, GPC data indicates a relatively low polydispersity (< 2) for all PUs, consistent with values typically reported in the literature (Guelcher et al., 2005, Parrag and Woodhouse, 2010).
Table 26: Comparison of molecular weight of Series 1 and 2 PUs

<table>
<thead>
<tr>
<th>Polyurethane (PU)</th>
<th>Number Average Molecular Weight (Mn) (Dalton)</th>
<th>Weight Average Molecular Weight (Mw) (Dalton)</th>
<th>Polydispersity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Z-Ser-PU</td>
<td>$6.67 \times 10^4$</td>
<td>$12.76 \times 10^4$</td>
<td>1.91</td>
</tr>
<tr>
<td>PCL-2-Z-Ser-PU</td>
<td>$4.89 \times 10^4$</td>
<td>$7.70 \times 10^4$</td>
<td>1.57</td>
</tr>
<tr>
<td>PCL-1-Z-Thr-PU</td>
<td>$5.86 \times 10^4$</td>
<td>$8.70 \times 10^4$</td>
<td>1.48</td>
</tr>
<tr>
<td>PCL-2-Z-Thr-PU</td>
<td>$7.69 \times 10^4$</td>
<td>$14.33 \times 10^4$</td>
<td>1.86</td>
</tr>
</tbody>
</table>

The observed molecular weight of Series 2 PUs is also consistent with similar GPC observations reported for PUs based on PCL 2000 as the soft segment (Skarja, 2001, Sarkar, 2007). As shown in Table 26, the molecular weight of PCL-2-Z-Ser-PU decreased and PCL-2-Z-Thr-PU increased as compared to Series 1 PUs. The exact reason for observed change in molecular weight of the PUs is not clear but may simply reflect side reactions (chapter 2, Figure 6) which can cause a change in the molecular weight of the synthesised polymer. The differences are, however, small and probably not overly significant. Overall, the GPC results show that the polymerization reaction resulted, as expected, in moderate molecular weight polyurethanes.

5.5.2.2 Differential scanning calorimetry

The Differential Scanning Calorimetry (DSC) thermograms for 1st and 2nd heating run of Series 2 PUs are shown in Figure 48 and 49. Two heating runs (first and second) for the DSC thermograms were obtained. The first heating run was performed in order to eliminate any previous thermal history of the PUs sample and the second heating run was obtained so that PUs could be compared having the same thermal history. Both heating runs provide useful information and are worth reporting. The first heating run provides information on any crystallinity present in the soft segment which may disappear in the second heating run due to the rapid cooling of the polymer sample. The second heating run allows comparison of PUs having similar starting thermal histories. The values of glass transition temperature ($T_g$) and melting temperature ($T_m$) obtained in both heating runs were used, when appropriate, for the overall thermal analysis of the polymer sample.
The thermal properties obtained via DSC for Series 2 PUs are shown in Table 27. The DSC result for Series 1 PUs are also shown in Table 27 for easy comparison. Series 1 PUs were originally shown and discussed in detail in section 4.5.2.5 of chapter 4. The DSC analysis shows a significant effect of increasing the soft segment (PCL) molecular weight on the thermal properties of PUs.

**Figure 48**: DSC thermograms for the 1st and 2nd heating run of PCL-2-Z-Ser-PU

**Figure 49**: DSC thermograms for the 1st and 2nd heating run of PCL-2-Z-Thr-PU
Table 27: Thermal properties of Series 2 and 1 PUs

<table>
<thead>
<tr>
<th>Polyurethane (PU)</th>
<th>Soft Segment</th>
<th></th>
<th></th>
<th></th>
<th>Polymer Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_g$-1st ($°C$)</td>
<td>$T_m$-1st ($°C$)</td>
<td>%Xc</td>
<td>$T_g$-2nd ($°C$)</td>
<td></td>
</tr>
<tr>
<td>PCL-1-Z-Ser-PU</td>
<td>6.1</td>
<td>ND</td>
<td>ND</td>
<td>5.9</td>
<td>Phase mixed, SS &amp; HS=amorphous</td>
</tr>
<tr>
<td>PCL-2-Z-Ser-PU</td>
<td>1.3</td>
<td>47</td>
<td>19</td>
<td>-7.7</td>
<td>Phase segregated, SS = semicrystalline &amp; HS = amorphous</td>
</tr>
<tr>
<td>PCL-1-Z-Thr-PU</td>
<td>1.2</td>
<td>48</td>
<td>4</td>
<td>-1.1</td>
<td>Phase mixed, SS = semicrystalline &amp; HS = amorphous</td>
</tr>
<tr>
<td>PCL-2-Z-Thr-PU</td>
<td>-2.9</td>
<td>48</td>
<td>15</td>
<td>-9.2</td>
<td>Phase segregated, SS = semicrystalline and HS = amorphous</td>
</tr>
</tbody>
</table>

$T_g$ – Glass transition temperature, $T_m$ – Melting temperature, %Xc – Percent crystallinity, HS=Hard segment, SS= Soft segment, $T_g$ 1st- $T_g$ of first heating run, $T_g$ 2nd- $T_g$ of second heating run, $T_m$ 1st- $T_m$ of first heating run, ND- Not Detected

**PCL-2-Z-Ser-PU**

DSC thermograms for the 1st and 2nd heating run of PCL-2-Z-Ser-PU were shown in Figure 48. For the 1st run, the soft segment glass transition, ($T_g$) was observed at 1.3°C, the soft segment melting temperature ($T_m$) was observed at 47°C and 19% crystallization was noted. No hard segment $T_g$ or $T_m$ was observed for the 1st heating run. During the second heating run, the soft segment $T_g$ appeared at -7.7°C and no $T_m$ or % Xc was observed, indicating a semi crystalline nature of the soft segment. Again, no hard segment $T_g$ and $T_m$ was observed indicating an amorphous nature for the hard segment.

**PCL-2-Z-Thr-PU**

DSC thermograms for the 1st and 2nd heating run of PCL-2-Z-Thr-PU were shown in Figure 49. The $T_g$ and $T_m$ observed for the soft segment during the 1st heating run was at -2.9°C and 48°C respectively. The crystallinity observed for the soft segment was 15% in the first heating run. During the second heating run, only soft segment $T_g$ was observed at -9.2°C and no $T_m$ (or % Xc) was obtained showing the semicrystalline nature of the soft segment. For both the heating runs, no hard segment transitions such as $T_g$ or $T_m$ were observed, indicating amorphous nature of the hard segment (similar to PCL-2-Z-Ser-PU).
The DSC thermograms for Series 1 PUs (PCL-1-Z-Ser-PU and PCL-1-Z-Thr-PU) were shown in chapter 4, Figure 30 and 31 respectively. On comparing the DSC results of Series 2 with Series 1 PUs, it can be observed that the soft segment $T_g$ decreases with increasing soft segment (PCL) molecular weight. This shift is indicative of differences of the morphology within the polymers, with a stronger phase separation in Series 2 PUs compared to the more phase mixed morphology shown by Series 1 PUs. Similar results have been reported previously (Seefried et al., 1975, Gorna et al., 2002, Garrett et al., 2003, Gisselfält and Helgee, 2003) in regards to decreases in soft segment $T_g$ with increasing soft segment molecular weight. The more the soft domains are contaminated with dissolved hard segments (as in the phase mixing morphology shown by Series 1 PUs), the higher will be the soft segment $T_g$. Similar observations for the soft segment morphology in relation to soft segment molecular weight have also been reported in the literature (Hu et al., 1982, Paik Sung et al., 1980b, Van Bogart et al., 1981).

Series 2 PUs also show soft segment crystallization ($\% X_c$), which increases with increasing PCL molecular weight as compared to Series 1 PUs. For example, 19\% $X_c$ was observed for PCL-2-Z-Ser-PU and 0\% $X_c$ was observed for PCL-1-Z-Ser-PU. Similarly, 15\% $X_c$ was observed for PCL-2-Z-Thr-PU and only 4\% $X_c$ was shown by PCL-1-Z-Thr-PU. A similar observation was recorded by Fernandez et al. (2006) with PCL based PUs.

No hard segment $T_g$ or $T_m$ was observed for either Series 1 or Series 2 PU. This is consistent with both series having an amorphous hard segment domain. Usually, as reported in the literature (Camberlin and Pascault, 1984, Yilgör et al., 1982, Joshi, 2009), in the segmented PUs, change in heat capacity of the amorphous hard blocks is often too small to be observed. Possible reasons for obtaining non-crystalline hard segment of the PUs might be:

1. Disruption of hard segment packing caused by a non-linear chain extender with bulky pendant Z group may inhibit polymer chain packing in the hard segment resulting in the absence of hard segment $T_g$ or $T_m$. A similar observation was reported by Zhang et al. (2006) for PUs having their hard segment based on MDI and using a non-linear chain extender.
2. The length of the hard segment unit is not long enough to make an ordered structure and does not, therefore, exhibit a $T_m$. It has been reported in the literature that MDI based hard segments need at least 2-3 hard segment units in a sequence to form an ordered enough structure to show any $T_m$.

Based on the DSC results shown in Table 27, the following observations can be made for Series 2 PUs:

1. In the first heating run, Series 2 PUs showed a soft segment glass transition ($T_g$) and melting endotherm ($T_m$) characteristic of PCL based soft segment with percent crystallinity of 19% (PCL-2-Z-Ser-PU) and 15% (PCL-2-Z-Thr-PU).
2. In the second heating run, the soft segment $T_m$ disappeared due to rapid cooling of the polymer sample before the second run was completed.
3. In the second heating run, Series 2 PUs exhibited a soft segment $T_g$ with no other notable soft segment thermal transitions observed indicating that the soft segment of Series 2 PUs are largely semi crystalline in nature.
4. No hard segment thermal transitions ($T_g/T_m$) were observed in the first or second heating run indicating that hard segments are non-crystalline or amorphous in nature.

The overall thermal characteristic features observed by comparing DSC result of Series 1 and 2 PUs indicate that:

1. PUs formed with higher molecular weight soft segments are more phase separated. This means longer soft segment length allowed high degree of phase separation resulting in lower soft segment $T_g$.
2. As PCL molecular weight increases, soft segment glass transition ($T_g$) decreases.
3. The soft segment crystallinity increases as PCL length increases.
4. The presence of a non-linear and bulky chain extender tends to destroy the ordered packing of the hard segment which results in amorphous hard segments having no hard segment $T_g$ or $T_m$. 
5.5.2.3 Fourier Transform Infrared Spectroscopy

The DSC results show clear differences in morphology between Series 1 and Series 2 PUs. This is further investigated in this section using Fourier Transform Infrared Spectroscopy (FTIR). FTIR can reveal information about hydrogen bonding within the hard and soft segments respectively. Here only the serine amino acid based Series 1 and 2 PUs (PCL-1-Z-Ser-PU and PCL-2-Z-Ser-PU) are reported in detail. The threonine amino acid based Series 1 and 2 PUs were similar and to avoid repetition of text, threonine amino acid based polymers FTIR data is not discussed here. The FTIR spectrum of PCL-1-Z-Ser-PU and PCL-2-Z-Ser-PU is shown in Figure 50 and the carbonyl, C=O (approx. 1600 – 1800 cm\(^{-1}\)) stretching region and amine, N-H (approx. 3100 -3500 cm\(^{-1}\)) region of the PCL-1-Z-Ser-PU and PCL-2-Z-Ser-PU is shown separately in Figure 51 and important FTIR absorption bands for MDI-PCL based polyurethane are reported in Table 28.

**Table 28 : Assignment of the major absorption bands in the FTIR spectra of the PCL-MDI based PU(Mattia and Painter, 2007), (Yilgor and Yilgor, 2007)**

<table>
<thead>
<tr>
<th>Frequency (cm(^{-1}))</th>
<th>Assignment</th>
<th>Frequency (cm(^{-1}))</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>~3440</td>
<td>v(NH),free</td>
<td>1598</td>
<td>v(C=O),benzene ring</td>
</tr>
<tr>
<td>3340</td>
<td>(NH) H-bonded, urethane</td>
<td>1528, 1508, 1530</td>
<td>(\delta) (N-H) + v(C-N), amide II</td>
</tr>
<tr>
<td>2940, 2863, 2862</td>
<td>v(_a)(CH(_2)), v(_s)(CH(_2)), v(_s)(CH(_3))</td>
<td>1464, 1370, 1365</td>
<td>(\delta)(_a)(C-H) in CH(_3), (\omega)(C-H) in CH(_2), (\delta)(C-H) in CH(_3)</td>
</tr>
<tr>
<td>1730</td>
<td>v(C=O),free urethane, ester carbonyl (soft segment)</td>
<td>1240,1202,1219, 1191</td>
<td>(\delta) (N-H) + v(C-N), amide III</td>
</tr>
<tr>
<td>1710</td>
<td>v(C=O),H-bonded urethane</td>
<td>1216, 1065,1063, 1046, 1016</td>
<td>v(CO-O-C)</td>
</tr>
</tbody>
</table>

\(v\) = stretching mode, \(v_a\) = asymmetric stretching, \(v_s\) = symmetric stretching, \(\delta\) = in-plane bending or scissoring, \(\rho\) = in-plane bending or rocking, \(\omega\) = wagging
Figure 50: FTIR absorption spectrum of polyurethane (A) PCL-1-Z-Ser-PU (B) PCL-2-Z-Ser-PU

Figure 51: FTIR absorption spectra of carbonyl and N-H region of polyurethane (A) PCL-1-Z-Ser-PU and (B) PCL-2-Z-Ser-PU
In PUs, hydrogen bonding can occur between the urethane groups, hard and soft segment carbonyl oxygen. Indication of the level of hydrogen bonding and hence the information about the overall polymer phase morphology can be obtained from the ratio of the absorbance of hydrogen bonded to absorbance of free carbonyl group present in the polymer. Each spectrum in Figure 50 was normalized using the specific peak area (1413 cm\(^{-1}\) peak of C-C stretching mode) of aromatic ring (Srichatrapimuk and Cooper, 1978). This peak has been used previously (McCarthy et al., 1997, Ishihara et al., 1974) as a reference. Each spectra were adjusted so that the relative peak areas of the absorbance at 1413 cm\(^{-1}\) are the same.

Different types of hydrogen bonding present in PUs/PUUs and the functional group involved in this interaction are shown in Figure 60 (section 5.5.3.2.3). Figure 52 shows the different phase morphologies which can exist in polyurethane structures, and has been “adapted from Prisacariu” (2011).

**Figure 52**: Different types of phase morphology present in polyurethane structure
As shown in Figure 50, the characteristic carbonyl group peak for PCL based PU is present at 1719 cm\(^{-1}\) (spectrum B, Figure 50, PCL-2-Z-Ser-PU) and at 1726 cm\(^{-1}\) (spectrum A, Figure 50, PCL-1-Z-Ser-PU) representing the ester carbonyl (C=O) group of PCL. This peak is very strong and dominating and hence it has overlapped the peaks due to free urethane carbonyl group which otherwise would have appeared at 1730 cm\(^{-1}\). In Figure 51, the appearance of an additional peak at 1709 cm\(^{-1}\) in spectrum A, Series 1 PU is indicative of the presence of H-bonded carbonyl group in low molecular weight based PU. This indicates that with lower molecular weight of PCL, there is certain degree of phase mixing present. This peak (1709 cm\(^{-1}\)) is either absent or merged with the strong ester carbonyl absorbance at 1719 cm\(^{-1}\) in Series 2-PU in Figure 50.

For PCL based PU, significant phase mixed morphology is observed with the low molecular weight soft segment, which is consistent with literature findings (Petrovic and Ferguson, 1991, Wang and Cooper, 1983). The phenomena of phase mixing behaviour is further supported by the appearance of asymmetric stretch (Figure 50) at 1216 cm\(^{-1}\) and 1065 cm\(^{-1}\) (CO-O-C) in Series 1 PUs which is less intense for Series 2 PUs. Similar observation for FTIR studies with different molecular weight of PCL based polyurethane are recorded in the literature (Sarkar et al., 2008). This indicates that the ester oxygen of carbonyl group of PCL forms an H-bonded structure with urethane linkages to give rise to asymmetric C-O stretch indicative of a higher degree of phase mixed behaviour.

The carbonyl stretching region (1600 – 1800 cm\(^{-1}\)) is dominated by an strong soft segment ester carbonyl band located at 1726 cm\(^{-1}\) (spectrum A) to 1719 cm\(^{-1}\) (spectrum B). It has been reported in the literature (Rao, 1963, Sarkar, 2007) that the presence of a high percentage of soft segment crystallinity can shift the frequency of the ester C=O peak towards a lower frequency. A similar observation was noted here as well when the soft segment crystallinity was found to shift the main PCL ester band from 1726 cm\(^{-1}\) (Series 1 PU) to a lower frequency at 1719 cm\(^{-1}\) (Series 2 PUs), (Figure 51). This shift characterizes the more crystalline nature of the high molecular weight of soft segment (Skarja and Woodhouse, 2000). The observation is in agreement with the DSC results (Table 27) which also support the semicrystalline behaviour of high molecular weight PCL, along with phase segregated morphology.
The N-H stretching region of 3100 - 3500\text{cm}^{-1} of the synthesised PU is also shown in Figure 51. The N-H stretching region shows a broad peak at 3330\text{cm}^{-1} (Figure 51, spectrum \textbf{A}) and at 3334\text{cm}^{-1} (Figure 51, spectrum \textbf{B}) representing the hydrogen bonded N-H group. The intensity of this peak appears to increases in Series 1-PU indicating more hydrogen bonding in soft and hard segments leading to some degree of phase mixing which is relatively less in Series 2-PU.

Finally it can be concluded that low molecular weight of the soft segment encourage more phase mixed polymer morphology. The molecular weight of the soft segment is also critical for the crystallization of the soft segment, with the lower molecular weight being mainly amorphous in nature due to the shorter chain length. This is supported by DSC data (Table 27, Section 5.5.2.2) where the low molecular weight soft segment based polymer showed phase mixed morphology and soft segment was amorphous in nature. The absence of enough soft segment crystallization acts as a driving force for the mixing of the hard and soft segment. The less cohesive amorphous soft segment tends to form a phase mixed morphology. However, the increase in soft segment crystallinity in Series 2 PUs (Table 27) favours formation of phase separated morphology. Thus the higher the molecular weight of PCL, the lower phase mixing occurs and vice versa. The appearance of hydrogen bonded carbonyl and high intensity asymmetric C-O stretch bands in FTIR analysis supports the formation of urethane – ester H-bonding interactions to form phase mixed morphology in Series 1 PUs.

5.5.2.4 Thermogravimetric analysis

Thermogravimetric Analysis (TGA) analysis of Series 2 PUs was performed (under nitrogen) and the results are given in Figure 53. The results for Series 1 PUs are shown in Figure 53 for easy comparison.

The onset of degradation for Series 2 PUs started at a temperature (T_{on}°\text{C}) of \sim 250°\text{C} and is essentially complete by \sim 480°\text{C}. This level of thermal stability is consistent with literature, for example Vlad \textit{et al.}(2008) reports thermal stability of PUs based on PCL (2,000)/MDI within the range of \sim 200°\text{C} to \sim 550°\text{C}. Comparison of TGA results for Series 1 with Series 2 PUs, however, shows that Series 1 PUs are significantly more
thermally stable. The onset temperatures ($T_{on}$ °C) of degradation for Series 1 PUs were ~350°C whereas, for Series 2 PUs they were ~250°C. This difference may be attributed to different phase morphology observed between the materials in the two series. It was indicated during discussion of the DSC results (Section 5.5.2.2) that Series 1 PUs exhibit phase mixed morphology (Section 4.5.2.5, chapter 4) whilst Series 2 PUs exhibit phase segregated behaviour (Table 27).

![TGA analysis of Series 1 PUs and Series 2 PUs](image)

**Figure 53**: TGA analysis of Series 1 PUs and Series 2 PUs

Increased thermal stability through phase mixed morphology may be established via the formation of hydrogen bonds between the soft and hard segments. It has been previously postulated (Wang and Hsieh, 1997) that the thermal stability of PUs can be highly stimulated by the degree of phase separation/phase mixed morphology and thus there is a mutual stabilization effect present between the soft and hard segment leading to improved thermal stability of the material.

Antipova *et.al.* (1970), has stated that “the urethane group can behave as an antioxidant having a stabilizing effect on the soft segment. Therefore the presence of hard segments within the soft segments can increase the stability of soft segment at high temperature, while the soft segment may have a protective function on the hard segment and hence
increase the thermal stability of segmented PU. This can be interpreted as a mutual stabilization effect of phase mixed segmented PUs”. The low thermal stability of Series 2 PUs, as compared to Series 1 PUs, is presumably due to less H-bonding between hard and soft segment as both are present as separate domains which in turn leads to early thermal degradation. Even though the thermal stability of Series 2 PUs is lower than Series 1 PUs, they still can be considered thermally stable based on their thermal degradation limit (> 300° C), and therefore have potential use in, for example, biomedical applications.

Overall, the effect of soft segment (PCL) molecular weight on thermal degradation can be seen during thermogravimetric analysis. It was observed that as the molecular weight of the soft segment increases, phase segregated morphology dominates, making the polymer less thermally stable. It is probably due to poor intramolecular interactions such as hydrogen bonding. Presumably, this will place a limit to the amount of soft segment (PCL) content which is advisable for such polymers. An optimisation process was considered outside the scope of this thesis.

5.5.2.5 Contact Angle
Advancing contact angle measurements of the polymers with water were performed in order to study their relative hydrophobicity. Static contact angle results for Series 2 PUs are given in Table 29. The results for Series I PUs are also shown in the Table 29 for easy comparison.

<table>
<thead>
<tr>
<th>Polyurethane (PU)</th>
<th>Series 1 PU</th>
<th>Series 2 PU</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-L-Z-Ser-MDI-PU</td>
<td>72° ± 4</td>
<td>75° ± 3</td>
</tr>
<tr>
<td>PCL-L-Z-Thr-MDI-PU</td>
<td>77° ± 3</td>
<td>80° ± 2</td>
</tr>
</tbody>
</table>

The data in Table 29 shows that the contact angle increases slightly as PCL molecular weight increases. It was noted during DSC experiments that phase separation increases as PCL molecular weight increases, and hence soft segment crystallinity also increases.
Thus increasing phase separation may be expected to generate more pure PCL surface regions. Since PCL is hydrophobic compared to the polar hard segments, increasing its content at the surface would be expected to increase the contact angle, as observed. The effect, as expected, is however very slight and indicates data which is consistent with an expected increase in contact angle rather than proof that it genuinely does. It has been reported in the literature (Skarja, 2001, Sarkar, 2007) that high contact angle with increasing PCL molecular weight results from an increase in the phase separation of the polymer, which in turns increases as the molecular weight of PCL increases. The contact angle values obtained for Series 1 and 2 PUs are close to literature findings. For example, Vlad et.al.(2010) has reported contact angle values up to 89° based on MDI-PCL-PU and Skarja et.al.(2001) has reported a contact angle value of 71° for PCL 1250-Z-lysine amino acid based polyurethane. Increase in contact angle value with increasing PCL molecular weight is also reported by Marija et.al.(2011).

Overall it can be concluded that there is a very slight increase in contact angle with increasing PCL molecular weight, consistent with an increases in phase segregation of the polymer.

5.5.2.6 Mechanical Properties

The stress strain curves for Series 2 PUs are shown in Figure 54 and 55. The mechanical properties of Series 2 PUs are summarised, and compared with Series 1, in Table 30. In general, it was observed that Series 1 and 2 PUs show poor tensile properties as compared to literature findings (Sarkar, 2007, Marcos-Fernández et al., 2006, Guelcher et al., 2005) for other similar types of polyurethanes. These polymers can therefore be described as relatively weak material.
Figure 54: Tensile stress – strain curve for PCL-2-Z-Ser-PU

Series 2 PUs based polymers were hard and stiff with high values of modulus of elasticity and low values of percent elongation and tensile strength. Series 1 PUs behaved as more flexible materials, with higher values of tensile strength and percent elongation and lower values of modulus of elasticity. The molecular weights (Table 26) of Series 1 and 2 PUs were significantly different and this presumably is a major contributor to the variation in their tensile properties.

Figure 55: Tensile stress – strain curve for PCL-2-Z-Thr-PU

The mechanical properties observed for Series 2 PUs (Table 30) indicate that the modulus of elasticity increases and elongation at break and ultimate tensile strength decreases with increasing molecular weight of soft segment. The elongation at break values for both the series, as shown in Table 30, might suggest an elastic material but this is somewhat misleading. None of the materials, and particularly the Series 1 PUs, exhibited any
elastomeric behaviour but instead behave similar to “chewing gum” with a high percent of elongation. It was observed, following tensile testing, that the polymer did not return to its original shape. Hence it would be inappropriate to call them elastomers. They are more correctly described as flexible, stretchy – chewing gum type materials. The reason for this type of behaviour is not fully known.

**Table 30 : Mechanical Properties of Series 1 and 2 PUs (mean ±SD, n=6)**

<table>
<thead>
<tr>
<th>Series 1 and 2 PU</th>
<th>Modulus of Elasticity (MPa)</th>
<th>Ultimate tensile strength (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Z-Ser-PU</td>
<td>6.2 ± 2</td>
<td>4.8 ± 1</td>
<td>625.8 ± 35</td>
</tr>
<tr>
<td>PCL-2-Z-Ser-PU</td>
<td>362.4 ± 89.1</td>
<td>2.5 ± 0.5</td>
<td>32.1 ± 4.7</td>
</tr>
<tr>
<td>PCL-1-Z-Thr-PU</td>
<td>4.2 ± 1</td>
<td>10.9 ± 6</td>
<td>660 ± 59</td>
</tr>
<tr>
<td>PCL-2-Z-Thr-PU</td>
<td>22.6 ± 6.2</td>
<td>0.61 ± 0.2</td>
<td>454.1 ± 49.7</td>
</tr>
</tbody>
</table>

The tensile strength of the PUs can be affected by several factors including hard and soft segment cohesion energy, packing of macromolecules, phase morphology and chain extender structure (Gunatillake et al., 2003). The stress-strain curves for Series 1 and 2 PUs show that these factors have resulted in relatively weak materials. Additional factors may also play significant roles in determining tensile properties for Series 2 PUs. Firstly, the absence of hard segment chains packing as ordered structure reduces the tendency for the hard segment to act as a reinforcing filler or physical crosslinking site, and this results in poor mechanical properties for the polymer in both the series. Secondly, the presence of soft segment (PCL) crystallization at 2,000 Dalton molecular weight may have overruled any effect on the overall polymer tensile strength and stiffness due to disordered hard segment. Soft segment crystal structures may act as physical cross-linkers in a manner similar to that normally ascribed to the hard segment for Series 2 PUs which has shown somewhat improved tensile properties as compared to Series 1 PUs. As was noted in the DSC data (Table 27), increasing the PCL molecular weight leads to increasing soft segment crystallinity and thus apparently improving tensile properties such as modulus of elasticity in Series 2 PUs. It has been reported in literature (Hepburn, 1982, Lamba et al., 1998, Van Bogart et al., 1983) that the crystallizable material tends to show better mechanical properties as compare to non-crystallizable material. Yen *et al.* (1994) have also reported that enhanced tensile properties depends on the soft segment crystallinity.
The role of soft segment crystallinity in mechanical properties of PU is described in the literature by Bogart *et al.* (1983).

Overall, with the exception of PCL-1-Z-Thr-PU, all materials have poor tensile strength throughout both series. The reason for obtaining such weak materials might be the lack of hard segment crystallization in both series due to the presence of non-linear chain extender containing a pendant Z group. It is possibly the bulkiness of the chain extender which prevents hard segment domain formation and also decreases hydrogen bonding. Hence there is a reduction in ability of the hard segments to act as reinforcement fillers or as physical cross linking sites, that impacts the overall mechanical properties. Therefore, the PUs containing non-linear chain extenders seem to have quite different overall morphology to those with linear chain extenders. Bae *et al.* (1999) for example, found that when non-linear chain extenders were used, the hard segments did not produce an ordered structure due to the bulkiness of the chain extender. Hence the PU prepared with non-linear chain extenders may have non-crystalline hard segment domains (as seen in DSC Table 27) with less degree of order and resulting in weak mechanical properties.

A substantial difference was observed in tensile properties for Series 2 PUs (hard and stiff) as compared to Series 1 PUs (soft and flexible). As a result of an amorphous hard segment, the tensile properties of the synthesised PUs are controlled by the soft segment rather than the hard segment. In addition, the structure of the non-linear chain extender has shown to impact the overall mechanical properties. The observed tensile properties of the synthesised polymer showed the importance of ordered hard segment structure and soft segment crystallization in determining the mechanical properties of the polymer.

### 5.5.3 Polyurethane urea

#### 5.5.3.1 Polyurethane urea synthesis

A series of amino acid based polyurethane ureas (PUUs) were synthesised based on the two-step method described in the experimental section 5.3.2. Polycaprolactone (PCL) of molecular weight 2,000 Dalton was used as the soft segment and the resultant series of
polymers are referred to as Series 2 PUUs. 4,4-methylene diphenyl diisocyanate (MDI) was used as the diisocyanate. Amino acid based diamine compounds (311, 312, 313 and 314) were used as chain extenders. The polymer nomenclature used in this thesis is based on the type and molecular weight of chain extender and polyol used respectively. The abbreviations used for Series 2 PUUs are shown in Table 31.

**Table 31: Abbreviations used for PCL 2,000 based Series-2 PUUs**

<table>
<thead>
<tr>
<th>Polyol molecular weight</th>
<th>Diisocyanate</th>
<th>Chain extender (diamine)</th>
<th>Polyurethane Urea (PUU) codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycaprolactone (PCL-2) (Mw=2000Dalton)</td>
<td>4,4-methylene diphenyl diisocyanate (MDI)</td>
<td>L-Leucine diamine ester (311)</td>
<td>PCL-2-Leu-PUU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Isoleucine diamine ester (312)</td>
<td>PCL-2-Ileu-PUU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Valine diamine ester (313)</td>
<td>PCL-2-Val-PUU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Tyrosine diamine ester (314)</td>
<td>PCL-2-Tyr-PUU</td>
</tr>
</tbody>
</table>

For example, PCL-2-Leu-PUU refers to polyurethane urea consisting of 2,000 Dalton PCL, and chain extended with L-Leucine based diamine chain extender (311, chapter 3). In general, synthesised Series 2 PUUs were yellowish-white and rubbery in appearance. Following synthesis, they were stored in sealed plastic bags for future use. The percent yield of the Series 2 PUUs was ~70% in each case. The slight loss in yield might be due to the loss of low molecular weight polymers during the purification process. Series 2 PUUs was compared to Series 1 PUUs to evaluate the effect of soft segment molecular weight on the physiochemical properties of the synthesised PUUs. The hard segment/soft segment ratio (50 weight %), was kept constant for both series of PUUs for comparison purpose.

5.5.3.2 Characterization of polyurethane ureas

5.5.3.2.1 Gel permeation chromatography

Gel Permeation Chromatography (GPC) results for Series 2 PUUs are given in Table 32 and compared with Series 1 PUUs. The number average molecular weights (Mn) of Series
2 PUUs varied from approximately 33,000 to 69,000 Dalton with a polydispersity index varying from approximately 1.6 to 2.2. All molecular weights reported here are in terms of polystyrene as standards.

Table 32: Molecular weight of Series 2 and Series 1 PUU

<table>
<thead>
<tr>
<th>Series 1 and 2 PUU</th>
<th>Number Average Molecular Weight (Mn) (Dalton)</th>
<th>Weight Average Molecular Weight (Mw) (Dalton)</th>
<th>Polydispersity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Leu-PUU</td>
<td>6.09 x10^4</td>
<td>10.23 x10^4</td>
<td>1.67</td>
</tr>
<tr>
<td>PCL-2-Leu-PUU</td>
<td>3.30 x10^4</td>
<td>5.34 x10^4</td>
<td>1.61</td>
</tr>
<tr>
<td>PCL-1-Ileu-PUU</td>
<td>7.40 x10^4</td>
<td>13.43 x10^4</td>
<td>1.81</td>
</tr>
<tr>
<td>PCL-2-Ileu-PUU</td>
<td>6.87 x10^4</td>
<td>14.22 x10^4</td>
<td>2.06</td>
</tr>
<tr>
<td>PCL-1-Val-PUU</td>
<td>5.02 x10^4</td>
<td>8.25 x10^4</td>
<td>1.64</td>
</tr>
<tr>
<td>PCL-2-Val-PUU</td>
<td>4.81 x10^4</td>
<td>8.47 x10^4</td>
<td>1.76</td>
</tr>
<tr>
<td>PCL-1-Tyr-PUU</td>
<td>4.88 x10^4</td>
<td>8.35 x10^4</td>
<td>1.71</td>
</tr>
<tr>
<td>PCL-2-Tyr-PUU</td>
<td>4.61 x10^4</td>
<td>9.88 x10^4</td>
<td>2.14</td>
</tr>
</tbody>
</table>

The observed GPC data was consistent with literature values for PUUs (Skarja, 2001, Sarkar, 2007, Marcos-Fernández et al., 2006, Caracciolo et al., 2008). On closer inspection, there are some apparent trends observed which are contradictory. Most polymers decreased in their number averaged molecular weight (Mn) but increased in their weight averaged molecular weight (Mw), in moving from Series 1 to Series 2; an apparent contradiction. In all cases, however, the polydispersity value also increased (albeit slightly). A slightly increased polydispersity represents a wider distribution of particle sizes. Weight averaged molecular weight, which are biased towards larger particles, will therefore be slightly higher whilst number averaged molecular weight, which are biased towards smaller molecular weight will be slightly lower. The bias, in this case, appears to lead to the contradiction of molecular weight increasing (from Series 1 to Series 2) when based on weight averages, but decreasing (from Series 1 to Series 2) when based on number averages. The exception was the leucine based polymers which decreased in both weight and number averaged particles sizes. This polymer was also the
exception to the increase in polydispersity. The polydispersity in this case did not increase (it was essentially unaltered) and therefore the decrease in number averaged molecular weight was also reflected in a decrease in weight averaged molecular weight.

Notwithstanding the above argument, the molecular weight in all cases were similar, given that the particle size of polymers can vary by many orders of magnitude. It is therefore assumed that the properties of these polymers will be determined by compositional morphology rather than the effect of molecular weight or its distribution.

5.5.3.2.2 Differential scanning calorimetric
Differential Scanning Calorimetric (DSC) results for Series 2 PUUs are given in Table 33 along with Series 1 PUUs result for comparison. DSC thermograms for the 1\textsuperscript{st} and 2\textsuperscript{nd} heating run for Series 2 PUUs are shown in Figures 56 and 57. The PCL molecular weight appears to have a significant effect on the thermal properties of the polymers.
Figure 56: DSC thermograms for the 1st and 2nd heating run of (A) PCL-2-Leu-PUU and (B) PCL-2-Ileu-PUU
Figure 57: DSC thermograms for the 1st and 2nd heating run of (C) PCL-2-Val-PUU and (D) PCL-2-Tyr-PUU

Table 33: Thermal properties of Series 2 PUUs and its comparison with Series 1 PUUs

<table>
<thead>
<tr>
<th>Series 1 and 2 PUU</th>
<th>Soft Segment</th>
<th>Glass transition (T_g (°C))</th>
<th>Melting Temperature (T_m (°C))</th>
<th>Crystallinity (% X_c)</th>
<th>Glass Transition (T_g (°C))</th>
<th>Tm &amp; Crystallinity (%X_c)</th>
<th>Polymer morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Leu-PUU</td>
<td>-6.5</td>
<td>47</td>
<td>5</td>
<td>-10.5</td>
<td>5</td>
<td>Phase mixed, SS = Semi Crystalline, HS = Amorphous</td>
<td></td>
</tr>
<tr>
<td>PCL-2-Leu-PUU</td>
<td>-39.8</td>
<td>42</td>
<td>3</td>
<td>-30.2</td>
<td>40°C and 3% total of 6.0%</td>
<td>Phase separated, SS= Crystalline, HS = Amorphous</td>
<td></td>
</tr>
<tr>
<td>PCL-1-Ileu-PUU</td>
<td>-3</td>
<td>47.5</td>
<td>5</td>
<td>-15.1</td>
<td>5</td>
<td>Phase mixed, SS = Semi Crystalline, HS = Amorphous</td>
<td></td>
</tr>
<tr>
<td>PCL-2-Ileu-PUU</td>
<td>-35.4</td>
<td>52</td>
<td>16</td>
<td>-27.7</td>
<td>16</td>
<td>Phase separated, SS = Semi Crystalline, HS = Amorphous</td>
<td></td>
</tr>
<tr>
<td>PCL-1-Val-PUU</td>
<td>-7.2</td>
<td>46.5</td>
<td>5</td>
<td>-7</td>
<td>5</td>
<td>Phase mixed, SS = Semi Crystalline, HS = Amorphous</td>
<td></td>
</tr>
<tr>
<td>PCL-2-Val-PUU</td>
<td>-43.9</td>
<td>46.7</td>
<td>2</td>
<td>-17.3</td>
<td>2</td>
<td>Phase separated, SS = Semi Crystalline, HS = Amorphous</td>
<td></td>
</tr>
<tr>
<td>PCL-1-Tyr-PUU</td>
<td>13.2</td>
<td>ND</td>
<td>ND</td>
<td>13.3</td>
<td>ND</td>
<td>Phase mixed, SS &amp; HS = Amorphous</td>
<td></td>
</tr>
<tr>
<td>PCL-2-Tyr-PUU</td>
<td>-10</td>
<td>46</td>
<td>10</td>
<td>-12.1</td>
<td>10</td>
<td>Phase separated, SS = Semi Crystalline, HS = Amorphous</td>
<td></td>
</tr>
</tbody>
</table>

T_g – Glass transition temperature, T_m – Melting temperature, %X_c – Percent crystallinity, HS=Hard segment, SS= Soft segment, T_g 1st- T_g of first heating run, T_g 2nd- T_g of second heating run, T_m 1st- T_m of first heating run, ND- Not Detected
For Series 2 PUUs, the soft segment glass transition temperature ($T_g$) decreases and crystallinity increases with increasing polyol molecular weight. It specifies increasing phase segregation morphology. Similar observations for soft segment $T_g$ and crystallinity based on the different molecular weight of PCL-MDI based PUU are also recorded in literature (Gisselfält and Helgee, 2003). However, in case of PCL-2-Val-PUU, soft segment crystallinity decreased as compared to the rest of the Series 2 PUUs. For Series 2 PUU, $T_m$ for the soft segment appears in the 1st heating run only indicating the semi crystalline nature of the soft segment. Series 2 PUUs appear to be more phase segregated than the corresponding Series 1 PUUs, with a lower glass transition temperature ($T_g$). Similar results have been observed and reported in the literature (Gunatillake and Adhikari, 2011) for other PUUs with high molecular weight soft segment.

Leucine and Isoleucine based Series 2 PUUs also exhibited higher phase separated morphologies, and lower $T_g$ values, for their soft segments, as compared to Series 1 PUUs. PCL-2-Leu-PUU showed an additional endotherm, at 60°C, in the 1st heating run (Figure 56 (A)). This probably corresponds to disruption of short and long range order of the hard segment. Similar thermal transitions have been reported by Skarja et.al. (2001) for PCL based PUUs. Bogart et.al. (1983) has also described similar thermal transition between 50 – 60°C in PCL based PUs.

PCL-2-Leu-PUU showed no hard segment crystallinity, thus the endotherm present in the 1st heating run at 60°C is assumed to be due to the disruption of ordered, non-crystalline hard segment aggregates present within the soft segment. In literature, several reports have ascribed similar transitions in this particular region to hard segments dispersed throughout the soft segment region (Brunette et al., 1981). The appearance of these transitions suggest the existence of the amorphous hard segment (Frontini et al., 1993).

For PCL-2-Val-PUU, soft segment $T_g$ appears at -43.9°C (1st heating run) and appeared again at -17.3°C (2nd heating run). The $T_m$ for soft segment appeared at 46.7°C in 1st heating run and 2% crystallinity observed. The observed $T_g$ for PCL-2-Val-PUU is much lower than PCL-1-Val-PUU. Similar observations were noticed for PCL-2-Tyr-PUU. The
soft segment $T_g$ (-10°C) was lower than PCL-1-Tyr-PUU ($T_g$ - 13.2°C). 10% crystallinity appeared in PCL-2-Tyr-PUU with no crystallinity for PCL-1-Tyr-PUU.

No hard segment $T_g$ or $T_m$ was found for Series 2 PUUs indicating a non-crystalline or amorphous nature for the hard segment, similar to Series 1 PUUs. The main reason for the amorphous nature of hard segment is probably the presence of pendant methyl group side chains attached to the (non-linear) chain extenders. The non-linear structure of chain extenders results in a less ordered arrangement of polymer chains within the hard segments, which in turn results in the absence of a $T_g$ or $T_m$. Bae et al. (1999) has also argued that the use of non-linear chain extenders with MDI as hard segment results in non-crystalline and less ordered hard segments.

By comparing the DSC results for Series 1 and 2 PUUs, it was observed that PCL soft segments with higher molecular weight exhibit higher soft segment crystallinity. For example, 6%, 16% and 10% of soft segment crystallinity was observed for PCL-2-Leu-PUU, PCL-2-Isoleu-PUU, and PCL-2-Tyr-PUU respectively, as compared to 5% for PCL-1-Leu-PUU, 5% for PCL-1-Isoleu-PUU, 0% for PCL-1-Tyr-PUU for Series 1 PUU. This increase may be due to dipolar interactions of the ester carbonyls in the caprolactone units (Sarkar, 2007).

The increase in soft segment crystallinity reflects incompatibility between the soft and hard segments of the polymer and this, in turn, results in a more cohesive soft segment and phase segregated behaviour shown by Series 2 PUUs (Skarja, 2001). Hu et al. (1982) have reported an explanation for the co-relation between the soft segment crystallization and polymer phase segregated morphology. In Hu’s study, as well as in the results presented here, it was shown that an increase in crystallinity with increased PCL molecular weight associate with improved micro phase separation behaviour of Series 2 PUUs.

Overall, it was observed that a higher molecular weight of the soft segment encourages phase segregation morphology along with an increase in the soft segment $T_g$ (in both the heating runs) and in crystallinity. The non-linear nature of the chain extender (with
pendant group) prevents the hard segment from packing in an ordered fashion, which results in the absence of a hard segment glass transition temperature ($T_g$) or a melt endotherm temperature ($T_m$); hence it is amorphous in nature in Series 2 PUU.

5.5.3.2.3 Fourier Transform Infrared Spectroscopy

The two main areas of the FTIR spectra of the synthesised polymer are of specific interest in the investigation of the effect of soft segment molecular weight in phase morphology (phase segregation/phase mixed) of segmented PUUs. The first is the carbonyl (C=O) region and the second is the N-H region. It is expected that upon formation of hydrogen bonds, the absorption vibrations precisely associated with N-H and C=O group would be shifting toward lower energy based on the environment present around each functional group. The magnitude of these shifts, as well as change in the absorption characteristics reflects hydrogen bond strength. To avoid repetition, only the PCL-1-Leu-PUU and PCL-2-Leu-PUU FTIR spectra are described here in detail. FTIR spectra of Series 1 and 2 PUU are shown in Figure 58 and the major FTIR peak positions are reported in Table 34. The carbonyl stretching region is located between $1630 - 1780 \text{ cm}^{-1}$ and the N-H region is located at $3100 - 3500 \text{ cm}^{-1}$. Both regions are shown with an expanded scale in Figure 59.
Figure 58: FTIR absorption spectra of polyurethane (A) PCL-1-Leu-PUU and (B) PCL-2-Leu-PUU

Figure 59: FTIR absorption spectra of carbonyl and N-H region of polyurethane (A) PCL-1-Leu-PUU and (B) PCL-2-Leu-PUU
Table 34: Assignment of the major absorption bands in the FTIR spectrum of the MDI-PCL based Polyurethane

<table>
<thead>
<tr>
<th>Frequency (cm⁻¹)</th>
<th>Assignment</th>
<th>Frequency (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3340</td>
<td>ν(NH), H-bonded urethane</td>
<td>1666</td>
<td>ν(C=O), H-bonded disordered urea</td>
</tr>
<tr>
<td>3340-3320</td>
<td>ν(NH), H-bonded, ordered urea</td>
<td>1645-1628</td>
<td>ν(C=O), H-bonded ordered urea</td>
</tr>
<tr>
<td>~3340</td>
<td>ν(NH), H-bonded, disordered urea</td>
<td>1600</td>
<td>ν(C=C), aromatic</td>
</tr>
<tr>
<td>2939, 2866, 2794</td>
<td>ν(CH₃)</td>
<td>1540</td>
<td>δ (N-H) + ν(C-N), amide II</td>
</tr>
<tr>
<td>1732, 1723</td>
<td>ν(C=O), free urethane, ester carbonyl (soft segment)</td>
<td>1450, 1370, 1365</td>
<td>δ₉(C-H) in CH₃, ω(C-H) in CH₂, δ₆(C-H) in CH₃</td>
</tr>
<tr>
<td>1706 - 1713</td>
<td>ν(C=O), hydrogen bonded urethane</td>
<td>1240</td>
<td>δ (N-H) + ν(C-N), amide III</td>
</tr>
<tr>
<td>1690-1700</td>
<td>ν(C=O), free urea</td>
<td>1161</td>
<td>ν(CO-O-C)</td>
</tr>
</tbody>
</table>

ν = stretching mode, νₐ = asymmetric stretching, νₛ = symmetric stretching, δ = in-plane bending or scissoring, ρ = in-plane bending or rocking, ω = wagging

Polyurethane urea are extensively hydrogen bonded. It has been reported in the literature (Blackwell et al., 1982, Teo et al., 1997, Pitt et al., 1990, Coleman, 1997) that MDI based PUUs show the presence of three-dimensional hydrogen bonding via the urea group within the hard segment. A variety of hydrogen bonds are possible and shown in Figure 60. In all cases, hydrogen bonding involves the N-H donor group present in urethane and urea linkages and the carbonyl or ether oxygen acceptor groups. The hydrogen bond donor is present exclusively in the hard segment of the polymer while the acceptor may be present either in the hard segment (carbonyl of urethane or urea) or the soft segment (ester carbonyl or ether oxygen). Degree of phase separation define the ratio of hard and soft segment hydrogen bonding. Phase separation favours interurethane, inter urea or urethane urea hydrogen bonding.
The carbonyl region in FTIR is most applicable to the analysis of the hydrogen bonding properties in polyurethanes (Luo et al., 1997). The carbonyl region comprises five distinct bands. These bands are located between ~1620 cm\(^{-1}\) and 1760 cm\(^{-1}\). The absorbance at 1640 cm\(^{-1}\) in spectrum A (PCL-1-Leu-PUU) and at 1645 cm\(^{-1}\) in spectrum B (PCL-2-Leu-PUU) of Figure 58 represents hydrogen bonded carbonyl, C=O, group. The hydrogen bond is formed between C=O group to urea (referred as ‘ordered’ urea) and N-H group. Urea C=O groups may also be hydrogen bonded in disordered manner and located at 1666 cm\(^{-1}\). The non-hydrogen bonded urea C=O is located at approximately 1700 cm\(^{-1}\) (Coleman et al., 1988). It seems likely that both of these peaks contributes to the intensity between 1710 cm\(^{-1}\) and 1690 cm\(^{-1}\). The hydrogen bonded and non-hydrogen bonded urethane C=O peak was located at 1715 cm\(^{-1}\) and 1732 cm\(^{-1}\) respectively and appear together to make it difficult to undertaken more detailed analysis. It has been proposed by Coleman et al. (1986) that “the relative degree of micro phase separation in amine chain-extended polyurethanes can be assessed by determining the degree of urea C=O hydrogen bonding (Bummer and Knutson, 1990) and that an increase in the extent of micro phase separation is accompanied by an increase in absorbance of the ordered urea C=O peak” (Teo et al., 1997). There are couple of bands present in this region and are...
overlapping each other and hence cannot be properly examined due to poor resolution of bands (Figure 58). Therefore spectra was analysed by qualitative peak analysis. Each spectrum in Figure 58 was normalized using the area of the 1413 cm\(^{-1}\) peak, assigned to the C–C stretching mode of the aromatic ring (Srichatrapimuk and Cooper, 1978). This peak has been used previously (McCarthy et al., 1997, Ishihara et al., 1974) as a reference and its assignment is routine. The spectra were adjusted so that the relative peak areas of the absorbance at 1413 cm\(^{-1}\) are the same.

The main peak to investigate in the carbonyl region is that due to the hydrogen bonded C=O portion of the urea group located at 1640 cm\(^{-1}\) in spectrum A (PCL-1-Leu-PUU) and at 1645 cm\(^{-1}\) in spectrum B (PCL-2-Leu-PUU) of Figure 58. It has been reported in the literature (Garrett et al., 2003) that this peak increases with an increase in the urea group concentration, and this represents increased interaction (hydrogen bonding) between the hard segments which, in turn, indicates hard-soft segment phase segregation. On comparing the Series 1 and 2 PUU FTIR spectra, it was observed that intensity of the bonded urea C=O peak is increased in Series 2-PUU. The high intensity of this peak in Series 2 PUU (spectrum B) indicates a high level of hydrogen bonding present within the hard segment of the PUU which in turns indicates phase segregation morphology. It was also observed that the absorbance in the region of the disordered and free C=O is increased and decrease in the ratio of the absorbance of the ordered urea C=O peak at 1640 cm\(^{-1}\) was noticed for in Series 1 PUU. This further suggests a decrease in polymer phase segregation morphology. It was detected that an increase in the absorbance of the ordered C=O peak is associated with phase segregation morphology.

Other major bands present in the carbonyl region are at 1732 cm\(^{-1}\) and at 1723 cm\(^{-1}\) for spectrum A and B respectively (Figure 59). This represents free (non-hydrogen bonded) urethane C=O bond and the soft segment ester C=O group respectively. The bonded urethane carbonyl ester band may appear between 1706 – 1713 cm\(^{-1}\), but is not clearly visible and could be masked by the free urethane C=O peak (McCarthy et al., 1997, Teo et al., 1997). It seems likely that the high intensity of the urethane free ester peak might be due to the combination of bonded and non-bonded urethane carbonyl group. Hence, there are two diverging effects observed and the overall effect is not clear, may be hidden.
and it is hard to distinguish these peaks separately. Hence it would be difficult to use these peaks for hydrogen bond elucidation in PUU structure.

The second area of interest is located between 3100cm\(^{-1}\) and 3500cm\(^{-1}\) as shown in Figure 59 and belongs to N-H stretching region. The very broad band at 3320cm\(^{-1}\) (spectrum A and B), has been allocated to the hydrogen bonded N-H group present in urethane and urea segment of the polymer. It indicate amorphous and disordered hard segment with subsequently less phase segregation morphology (Coleman, 1997, Ishihara et al., 1974). The peak shape of the absorbance in N-H region is similar to that reported for amorphous polyurea (Garrett et al., 2003) which specify to the primarily disordered domain and lower phase separation. No sign of free (non-hydrogen bonded) N-H group was observed, otherwise that would have appeared as sharp peak at 3450 cm\(^{-1}\). No significant shift or change in peak for N-H region was observed. Hence, not much information was collected from the N-H region of PUU.

Based on this FTIR study, complemented by the earlier DSC results, it can be concluded that low molecular weight PCL based PUUs show phase mixed morphology and high molecular weight PCL based PUUs show phase segregated characteristics. Excellent agreement between the FTIR study and DSC results (Section 5.5.3.2.2) was noted.

### 5.5.3.2.4 Thermo Gravimetric Analysis

Thermogravimetric analysis (TGA) was performed, under nitrogen, on Series 2 PUUs and compared with that for Series1 PUUs in an effort to understand the effect of soft segment polyol molecular weight on the thermal stability of polyurethane ureas. The TGA curves for Series 2 and 1 PUUs are shown in Figure 61 and 62 respectively.
Figure 61: TGA analysis of Series 2 PUUs

Figure 62: TGA analysis of Series 1 PUUs
The thermal stability parameters including the onset decomposition temperature ($T_{on}$ °C) (which is defined as the initial temperature at which polymer weight loss starts) and 10% weight loss temperature ($T_{10%}$ °C) (Temperature at which 10% of polymer weight loss was observed) was observed for both the series and are shown in Table 35.

Table 35: Characteristic temperature on TGA curves for Series 1 and 2 PUUs

<table>
<thead>
<tr>
<th>Series 1 and 2 PUU</th>
<th>$T_{on}$ (°C) (Onset Temp.)</th>
<th>$T_{10%}$ (°C) (10% Weight loss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Val-PUU</td>
<td>280</td>
<td>308</td>
</tr>
<tr>
<td>PCL-2-Val-PUU</td>
<td>300</td>
<td>315</td>
</tr>
<tr>
<td>PCL-1-Tyr-PUU</td>
<td>285</td>
<td>320</td>
</tr>
<tr>
<td>PCL-2-Tyr-PUU</td>
<td>263</td>
<td>290</td>
</tr>
<tr>
<td>PCL-1-Leu-PUU</td>
<td>265</td>
<td>285</td>
</tr>
<tr>
<td>PCL-2-Leu-PUU</td>
<td>290</td>
<td>314</td>
</tr>
<tr>
<td>PCL-1-Ileu-PUU</td>
<td>271</td>
<td>304</td>
</tr>
<tr>
<td>PCL-2-Ileu-PUU</td>
<td>297</td>
<td>315</td>
</tr>
</tbody>
</table>

It was observed that PCL-2-Val-PUU exhibited the highest, and PCL-2-Tyr-PUU showed the lowest, onset temperature in Series 2 PUUs. For tyrosine PUU, the result for PCL-2-Tyr-PUU, is somehow reversed as compared to PCL-1-Tyr-PUU. For example, PCL-1-Tyr-PUU ($T_{on}$ (285°C)) showed higher thermal stability among the rest of the Series 1 PUU. But in case of Series 2 PUU, PCL-2-Tyr-PUU ($T_{on}$ (263°C)), showed least thermal stability among Series 2 PUU. The reason for this observation is not known.

In all cases, Series 2 PUUs have a higher onset, and 10% weight loss, temperature (except PCL-2-Tyr-PUU) than Series 1 PUUs. Again, this is probably due to the longer soft segment in Series 2 PUUs favouring phase separation morphology and enhancement of inter urethane hydrogen bonding. The enhancement of hydrogen bonding then provides a higher thermal stability. The higher micro phase separation in Series 2 PUUs may be due to the crystallization effect of PCL 2000 Dalton which was also confirmed by the presence of $T_m$ and $\% X_c$ in the DSC analysis (Table 33).
The results (Figure 61) show that Series 2 PUUs are more thermally stable in the initial stages and degrade rapidly later on as compared to Series 1 PUUs (Figure 62). It appears that PCL 2,000 has a higher mutual stabilization effect than PCL 1,000 due to the hard segment being protected by a higher soft segment concentration in the polymer and hence an increase in its degradation temperature (V.F. Antipova, 1970). Although the weight percent of soft and hard segment was the same for both the series, the use of a higher molecular weight PCL and hence longer soft segment polymer chains has provided thermal stability to Series 2 PUUs.

Wang et.al. (1997) has reported similar results for the effect of polyol (polyether) molecular weight on the thermal stability of segmented PUUs. Ferguson et al. (1976) has also reported the stabilizing effect of soft segment concentration on the thermal stability of PTHF-MDI based polyether PUU. They have also reported that the presence of urea, urethane and amine groups have a stabilizing effect on PUUs during oxidative degradation. It is worth mentioning that Series 1 and 2 PUs and PUUs are based on different amino acid based chain extenders (i.e. dihydroxy versus diamine), hence it would not be appropriate to directly compare the TGA behaviour between the PUs and PUUs series. The use of different functionality of chain extender would be expected to impart different thermal characteristic to the polymer.

Overall, the thermal characteristic of all polymers synthesised here indicate a wide range of temperatures within which the polymer can be thermally processed, for example as scaffold fabrication in various biomaterial applications.

5.5.3.2.5 Contact angle
Contact angle values for Series 2 PUUs are shown in Table 36. The results for Series 1 PUUs are also shown in the Table 36 for easy comparison. Series 2 PUUs show slightly higher contact angles compared to Series 1 PUUs. Although the difference is barely significant, it is consistent with, and supports, the main findings of polymer characterisation to date. Lamba et.al. (1998), for example, has reported that a higher contact angle results from increasing the molecular weight of the soft segment and is related to phase separation morphology within the polymer.
Table 36: Contact angle values for Series 1 and 2 PUUs (mean ± SD, n = 5)

<table>
<thead>
<tr>
<th>Polyurethane urea (PUU)</th>
<th>Advance contact angle (θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Series 1 PUU</td>
</tr>
<tr>
<td>PCL-L-Leucine-MDI-PUU</td>
<td>83° ± 2</td>
</tr>
<tr>
<td>PCL-L-Isoleucine-MDI-PUU</td>
<td>82° ± 2</td>
</tr>
<tr>
<td>PCL-L-Valine-MDI-PUU</td>
<td>85° ± 1</td>
</tr>
<tr>
<td>PCL-L-Tyrosine-MDI-PUU</td>
<td>94° ± 3</td>
</tr>
</tbody>
</table>

In Series 2 PUUs, DSC results (Table 33), it was observed that phase separation and soft segment crystallinity increase as the molecular weight of PCL increases. An increase in phase separation would lead to more PCL migrating to the surface. Since PCL is hydrophobic, increasing its content would in turn increase the contact angle of the polymer. Hence, Series 2-PUUs have higher contact angles as compared to Series 1 PUUs. PCL- L-Tyrosine-MDI-PUU exhibits the highest and L-Isoleucine-PCL-MDI-PUU exhibits the lowest (just) contact angles in both series. For tyrosine based PUU, the aromatic structure of the tyrosine amino acid might have contributed to the higher contact angle. Sarkar et.al. (2009) has reported the synthesis of PCL-Tyrosine-HDI based polyurethane and a contact angle value of 80° was reported.

Overall, it was concluded that the obtained higher contact angle values for Series 2 PUUs are consistent with literature findings given that the (hydrophobic) soft segment dominates the hydrophobicity of the surface.

5.5.3.2.6 Mechanical Properties
The stress strain curve for Series 2 PUUs is shown in Figure 63 and 64. The resultant mechanical properties for Series 2 PUUs are reported, and compared to those for Series 1 PUUs, in Table 37. By increasing the molecular weight of soft segment in Series 2 PUUs, the mechanical properties of the polymer have improved as compared to Series 1 PUUs. An obvious cause for this is the increased phase segregation morphology observed in Series 2 PUUs. It has been reported in the literature (Lamba et al., 1998) that phase separation morphology tends to impart better tensile properties on segmented polymers.
Overall, however, compared to literature finding, Series 2 PUUs would still be classified as weak materials showing poor mechanical properties.

**Figure 63**: The Stress strain curves for Series 2 PUU

**Figure 64**: The Stress strain curves for PCL-2-Tyr-PUU
Table 37: Mechanical properties of Series 1 and 2 PUUs (mean ± SD, n = 6)

<table>
<thead>
<tr>
<th>Series 1 and 2 PUU</th>
<th>Modulus of Elasticity (MPa)</th>
<th>Tensile Strength (MPa)</th>
<th>Elongation at Break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-1-Leu-PUU</td>
<td>4.3 ± 1</td>
<td>0.52 ± 0</td>
<td>982.2 ± 11</td>
</tr>
<tr>
<td>PCL-2-Leu-PUU</td>
<td>13.8 ± 2</td>
<td>2.1 ± 1</td>
<td>700 ± 66</td>
</tr>
<tr>
<td>PCL-1-Ileu-PUU</td>
<td>5.1 ± 1</td>
<td>1.1 ± 0</td>
<td>337.0 ± 46</td>
</tr>
<tr>
<td>PCL-2-Ileu-PUU</td>
<td>32.6 ± 3.2</td>
<td>12.0 ± 3.6</td>
<td>479.2 ± 55.7</td>
</tr>
<tr>
<td>PCL-1-Val-PUU</td>
<td>7.2 ± 2</td>
<td>2.0 ± 1</td>
<td>563.7 ± 22</td>
</tr>
<tr>
<td>PCL-2-Val-PUU</td>
<td>41.8 ± 11.9</td>
<td>5.4 ± 1.1</td>
<td>533.7 ± 46.9</td>
</tr>
<tr>
<td>PCL-1-Tyr-PUU</td>
<td>11.6 ± 8</td>
<td>7.3 ± 3</td>
<td>421.7 ± 58</td>
</tr>
<tr>
<td>PCL-2-Tyr-PUU</td>
<td>2.0 ± 1</td>
<td>0.03 ± 0</td>
<td>350.1 ± 1</td>
</tr>
</tbody>
</table>

*Sample did not break during tensile testing.

A very low value for the tensile properties of PCL-2-Tyr-PUU (Figure 64) was obtained, consistent with the material being weak and tacky to handle. The reason for obtaining such low tensile properties is not known, however the improvement in mechanical properties (in most cases) from Series 1 to Series 2 suggests that optimising the soft segment may be a useful future research direction. The low molecular weight and high polydispersity (Table 32) in comparison to typical segmented PUUs is probably the main reason for obtaining low tensile properties for PCL-2-Tyr-PUU. It has been reported in the literature (Schollenberger and Dinbergs, 1973) that tensile properties increase as molecular weight of the segmented PUs increases. For most polymers, properties such as tensile strength, modulus and elongation are affected by molecular weight increases since longer chains are more likely to act as connectors between the crystalline or glassy state, allowing greater toughness along with soft segment crystallinity present in the polymer (R.B. and Carraher, 1984, Skarja, 2001).

The relatively high values for the moduli of elasticity suggests that Series 2 PUUs are stiffer than Series 1 PUUs and the tensile strengths for Series 2 PUUs are higher than Series 1 PUUs (except for PCL-2-Tyr-PUU which was very low for Series 2 PUU). Elongation properties were more robust for Series 2 PUUs as compared to Series 1 PUUs, possibly due to the change from phase mixed to phase segregated morphology as the PCL molecular weight increases. Again, the tensile properties for Series 2 PUUs appear improved as compared to Series 1 PUUs, but remain on the poor side compared to
literature findings ((Azzam et al., 2007, Skarja, 2001). Another major factor which may contribute in the attaining poor tensile properties for Series 2 PUUs is the use of non-linear and bulky chain extenders as compared to conventional small, linear chain extenders such as BDO (Caracciolo et al., 2008, Bagdi et al., 2009, Caracciolo et al., 2009). The non-linear structure of the chain extender has resulted in a lack of hard segment crystallization, which reduces the tendency of the hard segment to act as reinforcing filler or physical cross link site. Hence the overall mechanical properties of the polymer is reduced. It was also observed during DSC analysis (Section 5.5.3.2.2) that the higher percent of soft segment crystallization in Series 2 PUUs (compared to Series 1 PUUs) improved the mechanical properties of Series 2 PUUs. Soft segment crystal structure may act as physical cross links in a manner normally attributed to the hard segments. Increasing phase segregation morphology is accompanied by increasing PCL content which may yield superior mechanical properties for polyester based PUUs (Foks et al., 1990).

Overall, it was observed that the mechanical properties of the Series 2 PUUs were much improved as compared to Series 1 PUUs but still low as compared to the literature. The main reason for the improved mechanical properties for Series 2 PUUs might be the increase in the soft segment crystallinity and the existence of phase segregated morphology for Series 2 PUUs. The importance of both soft segment crystallinity and phase separation is highlighted by the low tensile properties of Series 1 PUUs. Overall, it was observed that PUUs made from these bulky and non-linear chain extenders have generally poor mechanical properties as compared to the literature findings and as expected no hard segment ordering was observed to contribute toward obtaining good mechanical properties.

5.6 Conclusion

A series of PUs and PUUs synthesised using 2,000 Dalton (Mw) PCL as soft segment and using novel chain extenders exhibited a variable degree of phase behaviour depending on the chemistry of the soft segment, structure and composition of the polymer. Detailed
analysis of the characterization results shows that the properties of these polymers vary over a wide range. The main observations recorded are as follows:

1. From DSC analysis, it was observed that as the soft segment molecular weight increases, phase segregation and percent crystallinity increases and the glass transition temperature of the soft segment decreases. This trend was seen in both PUs and PUUs series.

2. FTIR studies shows that more hydrogen bonding is present in phase mixed PUs and phase segregated PUUs shows hard segment urea bonding.

3. Phase segregated PUs are thermally less stable as compared to phase mixed PUs and vice versa for PUUs Series, although the difference was much less pronounced for PUUs.

4. Phase segregation improved the mechanical properties of the polymer producing a stiff polymer as compared to phase mixed morphology where the polymer was much weaker. The higher molecular weight of the soft segment has tended to improve the overall tensile properties of the synthesised PUs and PUUs. Overall, however, the tensile properties of both the Series 2-PU and PUU series are poor.

5. Contact angle increases (very marginally) as the soft segment molecular weight increases. This is consistent with the soft segment dominating the surface region.

The structural characterization, along with analysis of the biphasic nature of the final polymer, directly indicates the structure - property relationship of the synthesised polymer and this should provide important information for designing appropriate material for specific applications. The library of PUs and PUUs synthesised using two different molecular weights of soft segment and using novel amino acid based chain extenders shows that physical properties can be manipulated to provide tailored mechanical properties for biomaterial application including tissue engineering. However it was observed that changes in the soft segment molecular weight changes the polymer phase morphology which in turns highly affects the thermal, surface and mechanical properties of the synthesised polymer, making predictions of the resultant properties somewhat challenging.
Chapter 6: Cells Cytotoxic studies on polyurethane and polyurethane urea films
6.1 Introduction

This chapter describes cytotoxicity screening of the amino acid based Series 1 and 2 polyurethanes and polyurethane ureas. Mouse Fibroblast L929 (ATCC) cells were chosen as the test cells for the study and the LIVE/DEAD® Viability/Cytotoxicity Assay Kit (Molecular Probes L-3224) was used to investigate short term cytotoxicity of the synthesised polymer films. Fibroblast cells have been used in literature (Rechichi et al., 2008), (Henry et al., 2007), (Harris et al., 2006), (Li, 2009) to examine the biocompatibility of the synthesised polyurethane. This assay examines basic cytotoxicity and hence the actual cell type used is not crucial. It is possible that the polymer may remain cytotoxic to specific cell type not tested here and hence further testing would be required for specific applications. The test here, however, will determine whether or not there are substantial generic cytotoxic concerns with the use of these polymers as biomaterials.

It is reported in the literature that polyurethanes based on 4,4-methylene diisocyanate (MDI) produce the highly toxic aromatic amine, 4,4-methylenedianiline (MDA) upon degradation , (Vermette, 2001). MDA is mutagenic and carcinogenic in animals and is therefore not desirable for in vivo applications (Kavlock et al., 2007), (Szycher and Siciliano, 1991). There is, however, some doubt as to whether the concentration of these harmful degradation products reach any physiologically relevant level upon in vivo applications (Blais, 1990), (Coury, 2004). The ill effects of MDI for polyurethane synthesis can also be ameliorated by:

- changing the concentration of MDI used in polymer synthesis,

- choosing a final application (in vitro rather than in vivo) where toxicity is less problematic,

- changing the duration (short/long term) of the polymer, and/or its degradation products, for in vivo applications

It is reported in literature (Guelcher, 2008) that the data currently available for toxicity of MDI and its by-product (MDA) suggest that the physiological effects of aromatic
polyamines depend on the degradation rate of the material, as well as the clearance rate of the degradation product from the tissue.

In the present study, MDI as diisocyanate was chosen for PU/PUU synthesis due to its high reactivity and ability to impart superior physical properties to the polymers. There will therefore be a balance between toxicity and polymer properties. Despite the drawbacks, several researchers (de Groot et al., 1996) have pioneered the use of MDI in polyurethane and polyurethane urea products. They cast doubt over magnitude of the toxicity problem and argue that the risk benefit of improved polymer properties may outweigh those problems (which would hopefully be eventually ameliorated in any case). For example, Jun et al. (2004) and Taite et al. (2008) have described the use and modification of MDI based polyurethane to develop scaffolding for vascular graft application. In this work, they have demonstrated the successful attachment and proliferation of bovine aortic endothelial cells on MDI based polyurethanes. The use of aromatic polyurethane plotting compounds for biomedical devices (Vermette, 2001) and in biomedical research (Hsu and Lin, 2004), (Liljensten et al., 2002), (Gisselfält et al., 2002) are further examples reported in the literature. In another report, aromatic diisocyanate based polyurethanes have been used for the fabrication of extracorporeal medical devices such as haemodialyzers, oxygenators, haemo-concentrators (Guelcher et al., 2005), (Reed et al., 1994).

Nevertheless, based on the uncertainty of cytotoxicity of MDI based polyurethane and polyurethane ureas, it was felt important to perform basic cell toxicity tests on the polymers synthesised in this thesis, to determine if that is likely to be a significant drawback to the novel polymers produced.

To perform the cell toxicity test, Mouse fibroblast cells were seeded onto the amino acid based PUs and PUUs films and the cells viability was evaluated after short period of time (2 days) through LIVE/DEAD® assay kit. Tissue culture polystyrene (TCPS) was used as a positive control substrate. TCPS is the most commonly used in vitro cell culture surface and has often been used as a primary reference material for the evaluation of new biomaterials (Bonfield et al., 1989), (Yun et al., 1995), (Bélanger and Marois, 2001), (Bélanger and Marois, 2001),
1, 4-butanediol (BDO) based polyurethane (chapter 4) was used as a secondary control due to its closer similarity in chemical structure to the synthesised polymer samples. Several authors have reported that BDO based polyurethanes supports cell growth (Liu et al., 2012a).

The mouse fibroblast L929 (ATCC) cell line was chosen as the model test cell, because it is one of the most common cell lines used in cell culture and is known to be strongly adhesive to TCPS surface (Vashi et al., 2013).

The LIVE/DEAD® Viability/Cytotoxicity assay Kit (L-3224) (Invitrogen, Carlsbad, CA) was used to perform the test. It provides a two colour fluorescence viability assay that is based on the simultaneous determination of live and dead cells. Calcein acetoxyethyl ester (calcein AM) and ethidium homodimer (EthD-1) are the dyes used for this assay. Simply, metabolically active cells permit calcein acetoxyethyl ester (calcein AM) to enter through the intact plasma membrane of the cells, where the dye is cleaved by cytoplasmic esterase yielding green fluorescence in live cells (ex/em ~ 495nm /~515nm). By contrast, ethidium homodimer – 1 (EthD-1), which is membrane impermeable, binds to the DNA of membrane of the compromised cells, yielding bright red fluorescence (ex/em ~ 495nm /~635nm) in dead, or damaged (membrane compromised), cells.. Live/dead assay results provide information regarding cell adhesion and viability on the various materials after a specified time (in present case, two days) of cell culture. Note that strictly, the DEAD cell determination is really a measure of compromised cells rather than simply dead cells, however it is assumed that compromised cells will not be viable and it is common practise to include them as “dead”.

The use of the LIVE/DEAD® assay to assess the cell viability and cytotoxicity of cells on polyurethane surfaces has been previously reported in literature. For example, Parrag (2010) used this method to visualise the live and dead cells present on peptide based polyurethane materials. Other reports are available in literature which demonstrate the use of this technique includes (Park et al., 2011), (Lee et al., 2009), (Blit et al., 2012), (Fromstein et al., 2008), (Shah and Yun, 2011).
6.2 Materials

LIVE/DEAD® Viability/Cytotoxicity Kit (L-3224, Molecular Probes, MoBiTec GmbH, Göttingen, Germany) was used to perform cell viability assays. Polymer films were prepared by the method describe in section 4.4.7, to act as substrate for cell culture. PUs and PUUs from Series 1 and 2 were chosen randomly for the cell cytotoxicity experiment. Specifically, PCL-1-Z-Ser-PU, PCL-2-Z-Ser-PU, PCL-1-Z-Thr-PU, PCL-2-Z-Thr-PU, PCL-1-Leu-PUU, PCL-2-Leu-PUU, PCL-1-Val-PUU and PCL-2-Val-PUU samples were selected from both the series as exemplars for this experiment, tissue culture plates (Nunc, Denmark), Mouse Fibroblast (L929, ATCC) cells, faetal calf serum (FCS), phosphate buffer solution (PBS), Antibiotic- Antimycotic (Gibco-Invitrogen), minimum essential medium (MEM) media.

6.3 Experimental Procedure

Polymer films were cut into small discs using an 8 mm diameter puncher. The discs were sterilized by soaking in 0.1M phosphate buffer solution (PBS) containing 2X Antibiotic-Antimycotic (Gibco-Invitrogen) for two days in the bottom of a 96-well tissue culture plate (Nunc, Denmark) at 37°C in 5% CO₂. Following this and prior to cell seeding, polymer discs were soaked again in PBS culture media for 2 hours. Mouse Fibroblast (L929, ATCC) cells were seeded on each polymer disk at the bottom of the 96 well tissue culture plates at a density of 50,000 cells/cm² in 10% faetal calf serum (FCS) containing minimum essential medium (MEM) media. Seeded polymer films were incubated for two days at 37°C, in a humidified atmosphere of 5% CO₂ and 95% air. Cells were then washed with PBS twice. The viability of attached and spread cells was assessed using the LIVE/DEAD® Viability/Cytotoxicity Kit assay (Molecular Probes) at 48 hours according to the manufacturer’s instructions, using a Nikon TE2000-U fluorescent microscope equipped with digital camera. Live cells were stained green by Calcein AM while dead cells stained red with EthD-1. Both Calcein AM and EthD-1 were excited at 654 nm and 530 nm, respectively. Images were viewed and photographed using a Colour View Soft/Imaging system connected to a Nikon T2000 microscope. Viable and non-viable cells were observed for each well at a magnification of X 200.
6.4 Result and Discussion

An ideal biomaterial should enhance cell growth, attachment, proliferation and other cellular functions, all of which can be determined, to a first approximation by the Live/Dead Cell assay. Figure 65 and 66 shows mouse fibroblast L929 (ATCC) cells attachment onto the PUs and PUUs surface for Series 1 and 2 respectively. In both cases, all images produced green fluorescence showing live cells with only a few spots of red fluorescence indicating dead cells.

![Figure 65](image)

**Figure 65**: Fluorescence images of mouse fibroblast cells on Series 1 polymers surfaces (A) 1,4-Butanediol (Control), (B) & (C) Series 1 PU, (D) TCPS control, (E) and (F) Series 1 PUU (magnification x 200). Circle showing dead cells with red fluorescence.

The results demonstrate that the cells seeded on Series 1 and 2 of amino acid based PUs and PUUs films were substantially viable, with excellent cell attachment onto the synthesised polymer films. Furthermore, the cell growth on amino acid based PUs/PUUs samples were equally comparable to the cell growth on the positive test control (TCPS) sample and the polymer control (BDO based PU) sample. This shows that the synthesised polymers have retained a similar cell growth response to the commercially viable and well known BDO based PU and TCPS.
Figure 66: Fluorescence images of mouse fibroblast cells on Series 2 polymers surfaces (A) PCL-1-BDO-PU (Control), (B) & (C) Series 2 PU, (D) TCPS control, (E) and (F) Series 2 PUU (magnification x 200). Circle showing dead cells with red fluorescence.

The cells spreading pattern on synthesised PUs/PUUs resembles that of spindle morphology. The attached cells show normal flattened appearance. Active adhesion is shown by cells, as cells grow on the polymer surface and spread on these samples. The cells consumed metabolic energy during this process, which is indicative of active adhesion. These observations suggest that the chemical and/or physical structure of the substrate controls the degree of cell adhesion and proliferation. This is further evidence that the cell culture experiments shows good cell attachment in all the PUs and PUUs samples used in this experiment, indicating that the scaffolds have a high level of biocompatibility.

Closer evaluation of cell growth in Series 1 and 2 PUs/PUUs indicates that PCL-1-Z-Ser-PU and PCL-2-Z-Ser-PU display a high, and even, level of cell attachment and viability,
i.e. fibroblast cells proliferated well and were evenly distributed on the serine based polyurethane surface. This, however, was not the case for the threonine, leucine and valine amino acid based PUs/PUUs where cell growth was patchy and cells were unevenly distributed onto the polymer surface. The reason for this uneven distribution is not clear, however it might be that the cells which grow on these polymer surfaces may need little longer to spread evenly. It may also be a simple reflection of unusual growth pattern on amino acid based polymer surface. Regardless of the reason for patchy growth, all the Series 1 and 2 PUs/PUUs based materials supported cells growth (~90%) with a little or few dead cells. It was observed that the cell growth was healthy and comparable to the cell growth on the TCPS and BDO based PU controls. Overall, there was no evidence of significant cytotoxic response observed by cultured cells on any polymer surface. The cells appear well attached on all polymer substrates, with no apparent orientation. In general, a fairly confluent layer of cells was identified for the culture surfaces with cells in close contact or on top of each other in many spots. This phenomenon likely reflective of high cell density, which in turn shows good cell compatibility.

It is possible that dead cells may be washed off the polymer surface during medium changes and washing carried out during the assay and this may result in an artificially good result. All the tested polyurethanes and polyurethane ureas, however, supported attachment, proliferation, and high viability of fibroblast cells \textit{in vitro}. The major observations obtained from the cell study experiment can be summarised as:

1. Cell viability test using LIVE/DEAD\textsuperscript{®} assay kit provides no evidence of a significant cytotoxic response by cultured fibroblast to any of the PUs and PUUs synthesised in this thesis. The lack of cytotoxicity indicates that the polyurethanes can be considered relatively cell compatible and have a cell adhesive surface. This result may prove very useful to the intended end polymer application as an implanted device that is designed to degrade.

2. While no cytotoxic response was detected, there was variation in cell viability depending on the polyurethane chemistry. For example, cells were evenly proliferated on PCL-Z-Ser-PU surface in both series 1 and 2, presumably due to the hydrophilic nature of the serine amino acid supporting cell growth. By contrast, the others synthesised polymer surfaces showed uneven distribution of cell growth. It might be the case that the cells on these surfaces needs little longer
to fully cover the surface evenly, or even that the patchy areas could result from tiny air bubbles present in the polymer film while preparing the polymer sample for cell study. Surfaces which are less strongly adhesive would overcome and force out such air bubbles slower and there may not have been enough time for this to occur which the polymers investigated here. It should, however, be kept in mind that the amount of dead cells detected was insignificant, strongly suggesting a good biocompatible surface even if adhesion is slightly slower than on other biocompatible surfaces.

3. Cells grown on the synthesised amino acid based PUs/PUUs surface did not differ phenotypically from cells grown on the tissue culture polystyrene (TCPS) plates or the control BDO based PU (as assessed by the cell growth, pattern and proliferation).

4. PUs and PUUs, from both Series 1 and 2, used in this experiment, all supported cell growth and proliferation, showing that biocompatibility is irrespective of the molecular weight of the soft segment.

6.5 Conclusion

Polyurethanes are promising materials for soft tissue engineering applications. The cytotoxicity assay employed here indicates that the amino acid based polymers synthesised in this thesis do not show any significant cytotoxic response. Specifically, the results from this study indicate that the polymer films can support a high density of viable cells. Fibroblast cells growth, attachment and proliferation onto the polymer samples were comparable to the positive TCPS and BDO based polymer controls. Seeded cells appeared as a fairly confluent layer on all the substrate and were adherent and spread out on the polymer surface, with no apparent orientation. The study is a suitable, but rapid and basic experiment to test the cytocompatible behaviour of the synthesised polymers. Further study, for example using other cell lines, are required before it can be said with certainty that the polymers are fully biocompatible, however the results presented here show that further development of the novel compounds developed in this thesis is worth pursuing, i.e. continued improvement of physical polymer properties whilst minimising any cytotoxic effect.
Chapter 7: Conclusion and future work
7.1 Conclusion

The focus of this dissertation was to design, synthesise and characterise segmented polyurethanes and polyurethane ureas containing L-amino acids as chain extenders. Novel L-amino acids based diamine/dihydroxyl groups containing organic compounds were successfully synthesised and subsequently used as chain extenders to formulate segmented polyurethanes and polyurethane urea. A detailed physical, mechanical and chemical characterisation, including cell study, was performed on synthesised polymers and analysis of the resultant properties showed that the L-amino acid containing polyurethanes and polyurethane ureas have good potential to use as a biomaterial in future. A study of structure property correlation was performed by developing a library of polyurethanes and polyurethane ureas. The main outcome of this dissertation is briefly summarized as follow:

Synthesis and characterisation of L-amino acid based chain extenders: Five different L-amino acids such as L-isoleucine (301), L-leucine (302), L-valine (303), L-tyrosine (304) and L-phenylalanine (305) (Figure 10, Chapter 3) were used to synthesise diamine ester compounds such as 311, 312, 313, 314 and 315 (Figure 11, Chapter 3). The purpose to synthesise these diamine diester compounds was to use them later as chain extender for polyurethane urea synthesis. Out of the total five synthesised diamine ester compounds, L-phenylalanine based compound 315 was not used as chain extender for polyurethane urea synthesis in this thesis. Compound similar in structure to 315 is already reported in literature and used for polyurethane urea synthesis (Skarja, 2001). Hence it was not worth pursuing it further. Three amine group protected L-amino acids such as L-Z-serine (306), L-Z-threonine (307) and L-Z-Tyrosine (308) (Figure 10, Chapter 3) were used to synthesise dihydroxyl ester compounds such as 318, 319, 320 (Figure 20, Chapter 3) to act as chain extender for polyurethane synthesis. PU synthesised based on compound 320 as chain extender was of very low molecular weight and was very tacky to handle. Hence this PU was not used for further detail polymer characterisation. Rest of the L-amino acid based synthesised compounds (311,312,313,314,318,319) were incorporated into polyurethane and polyurethane urea as chain extender.
Fischer esterification reaction was employed to synthesise novel diamine diester compounds (311, 312, 313, 314 and 315 – section 3.3, Figure 11. The reaction yield was ~80% in all cases. The final compounds were characterised, and confirmed, by NMR, FTIR and MS spectroscopy.

Esterification reaction with caesium salt was employed to synthesise amine group protected L-amino acid based dihydroxyl ester compounds. Reaction conditions were optimised and a purification scheme was developed to remove impurities and to obtain a pure product. The amine group of the L-amino acid used for this reaction was protected by a benzyloxycarbonyl (Z) group. L-Z-amino acids were used to synthesise dihydroxy ester compounds 318, 319 and 320. The percent yield of reaction in all cases was approximately 85%. The final products were analysed, and confirmed, by NMR, FTIR and MS spectroscopy.

A series of segmented polyurethanes (PUs) and polyurethane ureas (PUUs) were synthesised using the L-amino acid based dihydroxyl and diamine chain extenders, polycaprolactone (PCL, Mw=1000 Dalton) act as soft segment, 4,4-methylene diphenyl diisocyanate (MDI) was used as diisocyanate. Synthesised PUs/PUUs series with PCL1000 as soft segment were called Series-1-Chain Extender-PUs/PUUs. Their structure property relationships were investigated, and compared with a control polyurethane synthesised with 1,4-butanediol as the chain extender and polycaprolactone (PCL, Mw=1000 Dalton) was used for soft segment and 4,4-methylene diphenyl diisocyanate (MDI) was used as diisocyanate and abbreviated as PCL-1-BDO-PU. The deprotection of the Z group of the chain extenders was optimised to obtain minimum polymer backbone cleavage. The structure of synthesised polymers were confirmed by NMR, GPC and FTIR technique. The thermal, surface and mechanical characterisation of the synthesised PUs and PUUs were done by DSC, TGA, contact angle and tensile testing. In general, compared to the control PCL-1-BDO-PU, the synthesised novel L-amino acid based PCL-1- PUs/PUUs were:

- Weak in mechanical strength. The L-amino acid based PUs/PUUs were soft materials with high elongation at break values and with low values of tensile strength and modulus of elasticity. In contrast, the control polyurethane behaved as a strong and stiff polymer with a high value of modulus and tensile strength but
with low percent of elongation. The reason for obtaining poor mechanical properties is due to the less ordered hard segment domain observed in L-amino acid based PUs/PUUs.

- More phase mixed with semicrystalline soft segment and amorphous hard segment as shown by DSC. The reason for the absence of hard segment $T_g$ and $T_m$ for the PUs/PUUs investigated here, is the unsymmetrical structure of the chain extender, which prevents any ordering of the hard segments through hydrogen bonding. Control polyurethane act as strong material with high modulus and tensile strength. BDO based polyurethane showed phase segregated morphology with amorphous soft segment and semi crystalline hard segment.

- More thermally stable (PUs only) as compared to the control BDO based PU as observed by TGA study. However, TGA result is reverse for Series-1-PUUs, which is less thermally stable then control PU.

- More hydrophobic in nature as shown by contact angle values. Series-1-PUs/PUUs showed higher contact angle values as compared to control PU which implies to the hydrophobic surface of the polymer.

- Overall, it was observed that the properties shown by Series 1-PCL-L-amino acid based-PUs/PUUs clearly demonstrate the effect of the unsymmetrical structure of the chain extender on the physiochemical properties of the synthesised polymers.

- Structure property relationship of the synthesised polymers (PUs/PUUs) was studied by making another series with higher molecular weight of the soft segment (PCL). The PCL of 2000 Dalton molecular weight was used and this series is called Series 2-PU/PUU. L-amino acids based diamine and dihydroxy chain extenders were used and MDI act as diisocyanate. FTIR, DSC and TGA techniques were used for the primary characterisation. Series-2-PUs/PUUs were compared with Series-1-PUs/PUUs to see the effect of increased molecular weight of the soft segment (PCL) on physiochemical properties of the synthesised polymers. The
results shows that domain morphology of the polyurethanes changes considerably with the change in the molecular weight of the soft segment. Depending on the molecular weight of soft segment, polymers tends to show the phase mixed and phase segregated morphology. For example, PUs formed with higher molecular weight (PCL=2000 Mw) soft segments are more phase segregated and the soft segment crystallinity increases as PCL molecular weight increases. However, PUs/PUUs based on low molecular weight PCL showed phase mixed morphology. For thermal stability, it was observed that as the molecular weight of the soft segment increases, phase segregated morphology dominates and makes the polymer less thermally stable presumably due to poor intramolecular interactions such as hydrogen bonding. A different trend was also observed in mechanical properties of both the series. On comparing both the Series 1 and 2 PUs/PUUs, it was observed that Series 2 PUs/PUUs based polymers are hard and stiff with high values of modulus of elasticity and low values of percent elongation and tensile strength. Series 1 PUs behave as more flexible materials with higher values of tensile strength and percent elongation and lower values of modulus of elasticity.

- No evidence of cell cytotoxicity was observed when initial mouse fibroblast L929 (ATCC) cell line based cell compatibility studies were carried out on synthesised Series 1 and 2 PUs/PUUs films. Hence it can be assume that the incorporation of L-amino acids into the PUs and PUUs has retained the similar cell response as it is with commercial available and well known test control i.e. tissue culture polystyrene (TCPS) and polymer control, PCL-1-BDO-PU. This observation suggests that the synthesised PUs/PUUs may pave their way for possible use in tissue engineering applications.

7.2 Key contributions of research

- Design and synthesis of novel L-amino acid based diester compounds with dihydroxy and diamine groups that were later used as chain extender for polyurethane and polyurethane urea synthesis.
• Development of a series of segmented polyurethane containing L-amino acid based chain extenders and determination of the structure property relationship of these newly synthesised materials.

• Building a library of polyurethanes and polyurethane ureas with different molecular weight of polyl used as soft segment and investigation of structure property relationship of synthesised polymers in the design of appropriate polyurethane.

• Characterisation, and confirmation, of polymer structure using NMR, FTIR and GPC technique.

• Analysis of thermal, surface and mechanical properties of the synthesised polymer.

• Determination of initial cell toxicity behaviour of novel synthesised polyurethanes and polyurethane ureas.

• Demonstration that the structure of the chain extender (linear/non-linear) has a profound effect on overall property of synthesised polyurethane and polyurethane ureas.

### 7.3 Future Work

• Expanding the library of polyurethanes and polyurethane ureas by developing new polyurethane with bio-friendly diisocyanate. Reported novel L-amino acid based diester compounds in this thesis can act as chain extender.

• Expanded cell compatibility studies to further investigate the suitability of these polyurethanes and polyurethane ureas for in vivo applications.
• An in-depth analysis of polyurethanes and polyurethane ureas degradation (especially enzyme mediated degradation) aimed at identifying the degradation product released as a result of different degradation method used would be very useful to elucidate the site of cleavage during degradation process.

• Investigation of alternative protection/deprotection chemistry to remove the Z-group from the L-amino acid present in the polyurethanes chains. Use of other protecting group and different protection chemistry may increase the deprotection efficiency and reduce the risk of polymer backbone cleavage.

• Developing blends of polyurethanes and polyurethane ureas of different composition and structure such as a combination of different hard/soft segment ratio can be examined to observe any change in physiochemical properties of the polyurethanes and polyurethane ureas.

• Development of new polyurethanes and polyurethane ureas through the copolymerisation of soft segment. For example, blending of polycaprolactone and polyethylene oxide based polyurethane to obtain an intermediate range of material and degradation properties.

• Fabrication and characterisation of three dimensional scaffold for tissue engineering applications.
References


CHAN-CHAN, L., TKACZYK, C., VARGAS-CORONADO, R., CERVANTES-UC, J., TABRIZIAN, M. & CAUICH-RODRIGUEZ, J. 2013. Characterization and
biocompatibility studies of new degradable poly(urea)urethanes prepared with arginine, glycine or aspartic acid as chain extenders. *Journal of Materials Science: Materials in Medicine, 24*, 1733-1744.


CHANGHONG, Z. 2006. *Elastic degradable polyurethanes for biomedical applications*. Master of Science, Clemson University, USA.


GORMA, K. & GOGOLEWSKI, S. 2002. In vitro degradation of novel medical biodegradable aliphatic polyurethanes based on [epsilon]-caprolactone and
Puronics® with various hydrophilicities. Polymer Degradation and Stability, 75, 113-122.


JOSHI, V. P. 2009. *Studies on synthesis and characterisation of thermoplastic polyurethane urea copolymers*. Doctor of Philosophy, PhD, University of Pune.


KORLEY, L. S. T., JAMES, P., BRIAN, D., THOMAS, E. L. & HAMMOND, P. T. 2006. Effect of the degree of soft and hard segment ordering on the morphology
and mechanical behavior of semicrystalline segmented polyurethanes. Polymer, 47, 3073-3082.


234


244


WILLIAMS, D. F. 2014. There is no such thing as a biocompatible material. *Biomaterials*, 35, 10009-10014.


Appendix A

Octane-1, 8-diyl bis (2-amino-4-methylpentanoate) (311)
The synthesis process for 311 is described in detail in chapter 3, section 3.3 and based on amino acid 301. A light yellow color oil was obtained as final product (311). Yield 80%. ¹H-NMR (400MHz, d₆-DMSO): δ (ppm) = 0.9 (6H, dd, i, h); 1.4 – 1.7 (9H, m, b, c, d, f, g); 3.3 (1H, m, C-H, e); 4.0 (2H, m, a). ¹³C-NMR (200MHz, d₆-DMSO): δ (ppm) = 13.5(2C), 15.2, 21.4, 24.3, 24.6, 28.2, 55.8, 59.9, 171.4. MS (ESI)-Found m/z 373.4 [M+H]⁺. C₂₀H₄₀N₂O₄ requires 372.54. FTIR (neat, cm⁻¹): 3376, 3305, 2953, 2930, 2857, 1730, 1728, 1380, 1168 cm⁻¹.

Octane-1, 8-diyl bis (2-amino-3-methylpentanoate) (312)
302 based titled compound 312 was synthesised following the reaction conditions described for 311 chapter 3, section 3.3. The crude product was purified by washing and extracted to yield 312 as pure light yellow coloured oil. Yield 80%. ¹H-NMR (400MHz, d₆-DMSO): δ (ppm) = 0.9 (6H, dd,); 1.2 (1H, m,); 1.3 (4H, m,); 1.4 (1H, m,); 1.5 (3H, m,); 3.1 (1H, d, N-H,); 4.0 (2H, m,). ¹³C-NMR (200MHz, d₆-DMSO): δ (ppm) = 15.9, 25.5, 25.6, 28.3, 28.7(2C), 58.6, 64.0, 175.5. MS (ESI)- Found m/z 359.52 [M+H]⁺. C₁₉H₃₈N₂O₄ requires 358.52. IR (neat, cm⁻¹): 3384, 3315, 2960, 2931, 2875, 2858, 1728, 1389, 1174 cm⁻¹.

Octane-1, 8-diyl bis (2-amino-3-methylbutanoate) (313)
Compound 313 was synthesized following the method outlined for 311 chapter 3, section 3.3. Compound 303 was used as starting material. The product as light yellow colored oil was obtained after purification. Yield 80%. ¹H-NMR (400MHz, d₆-DMSO): δ (ppm) = 0.8 (6H, dd,); 1.2 (4H, m,); 1.5 (2H, m,); 1.8 (1H, m,); 3.1 (1H, N-H,); 4.0 (2H, m,). ¹³C-NMR (200MHz, d₆-DMSO): δ (ppm) = 17.8, 19.4, 25.8(2C), 28.6, 28.9, 59.7, 64.5, 171.4. MS (ESI)- Found m/z 345.3 [M+H]⁺. C₂₀H₄₀N₂O₄ requires 344.4. FTIR (neat, cm⁻¹) 3348, 3315, 2929, 2856, 1728, 1389, 1174 cm⁻¹.

Octane-1, 8-diyl bis (2-amino-3-(4-hydroxyphenyl) propanoate (314)
The aromatic 304 based compound 314 was synthesized along the general procedure outlined for compound 311 chapter 3, section 3.3. The crude white product isolated was
purified to obtain colorless solid as pure product. Yield 80%. $^1$H-NMR (400MHz, d$_6$-DMSO): $\delta$ (ppm) = 1.2 (4H, m); 1.4 (2H, m); 1.7 (2H, N-H); 2.7 (2H, d); 3.5 (1H, t C-H); 4.0 (2H, m); 6.6 (2H, d, Ar-H); 6.8 (2H, d Ar-H). $^{13}$C-NMR (200MHz, d$_6$-DMSO): $\delta$ (ppm) = 22.5, 25.2, 28.0, 28.5, 55.9, 63.8, 114.9, 127.7, 130.0, 155.8, 171.5, 175.0. MS (ESI) - Found m/z 473.5 [M+H]$^+$: C$_{20}$H$_{40}$N$_2$O$_4$ requires 472.5. FTIR (neat, cm$^{-1}$): 3333, 3059, 3032, 2973, 2947 1737, 1540, 1525, 1480, 1461, 1355, 1334, 801cm$^{-1}$.

Octane-1, 8-diyl bis (2-amino-3-phenyl) propanoate (315)

The titled compound was synthesized following the procedure outlined for compound 311 chapter 3, section 3.3. Aromatic 305 was used as starting material to obtain final 315 compound. The crude white product isolated was purified to obtain yellow color solid as pure product. Yield 80%. $^1$H-NMR (400MHz, d$_6$-DMSO): $\delta$ (ppm) = 1.2 (4H, m); 1.5 (2H, m); 1.7 (2H,s, N-H,); 2.8 (2H, m); 3.6 (1H, m); 4.0 (2H, t); 7.2 (5H, m Ar-H). $^{13}$C-NMR (200MHz, d$_6$-DMSO): $\delta$ (ppm) = 25.66, 28.47, 28.92, 56.23, 64.33, 126.65, 128.49, 129.59, 138.34, 175.41. MS (ESI) - Found m/z 463.3 [M+Na]$^+$: C$_{26}$H$_{36}$N$_2$O$_4$ requires 440.5. FTIR (neat, cm$^{-1}$): 3333, 3059, 3032, 2973, 2947 1737, 1540, 1525, 1480, 1461, 1355, 1334cm$^{-1}$.

Butane-1, 4-diylbis (2-(benzyloxycarbonylamino)-3-hydroxypropanoate) (318)

The synthesis process for 318 is described in detail in chapter 3, experimental procedure section 3.3. 306 amino acid was used for esterification. A colorless solid was obtained as final product (318). Yield 85%. $^1$H-NMR (400MHz, d$_6$-DMSO): $\delta$ (ppm) = 1.6 (2H); 3.6 (2H, t); 4.0 (2H, m); 4.1 (2H, t); 7.3 (5H, m Ar-H). $^{13}$C-NMR (200MHz, d$_6$-DMSO): $\delta$ (ppm) = 25.0, 57.2, 61.6, 64.4, 65.9, 128.1, 128.2, 128.7, 137.4, 156.4, 171.1. MS (ESI) - Found m/z 571.3 [M+K]$^+$: C$_{26}$H$_{32}$N$_2$O$_{10}$ requires 532.54. FTIR (neat, cm$^{-1}$): 3492, 3337, 3356, 3059, 3032, 2973, 2947, 1737, 1540, 1525, 1480, 1461, 1355, 1334cm$^{-1}$.

Butane-1, 4-diylbis (2-(benzyloxycarbonylamino)-3-hydroxybutanoate) (319)

Compound 319 was synthesized following the method outlined for 318 in section 3.3, chapter 3, and 307 was used as an amino acid. The crude solid was chromatographed (hexane: acetone in 6:4 ratio) to obtain the colourless solid 319. Yield 85%. $^1$H-NMR (400MHz, d$_6$-DMSO): $\delta$ (ppm) = 1.1 (2H, d); 1.6 (2H, m 4.0 (4H, m); 5.0 (2H, s, Ar-H);
7.3 (5H, m, Ar-H). $^{13}$C-NMR (200MHz, d$_6$-DMSO): $\delta$ (ppm) = 20.5, 25.0, 60.6, 64.3, 66.0, 66.8, 128.0, 128.2, 128.7, 137.3, 156.8, 171.2. MS (ESI)- Found m/z 583.3 [M+ Na]$^+$. C$_{28}$H$_{36}$N$_2$O$_{10}$ requires 560.59. FTIR (neat, cm$^{-1}$): 3395, 3059, 3032, 2973, 2947, 1737, 1684, 1669, 1540, 1525, 1480, 1461, 1355, 1334, 1220, 1190, 697, 752cm$^{-1}$.

**Butane-1, 4-diylbis (2-(benzyloxycarbonylamino)-3(4-hydroxyphenyl) propanoate) (320)**

Compound 320 was synthesized following the method outlined for 318 in section 3.3, chapter 3. This compound is based on 308 as starting material. The crude product was purified by column chromatography (hexane: acetone in 6:4 ratio) and obtain a colourless solid. Yield 85%. $^1$H-NMR (400MHz, d$_6$-DMSO): $\delta$ (ppm) = 1.3 (2H, m); 2.8 (2H, dd, Ar-H); 3.9 (2H, m); 4.1(IH, m N-H); 5.0 (2H, s Ar-H); 6.6 (2H, d); 7.0 (2H, d); 7.3 (5H, m). $^{13}$C-NMR (200MHz, d$_6$-DMSO): $\delta$ (ppm) = 24.9, 36.2, 56.4, 64.3, 65.8, 115.4, 127.7, 127.9, 128.1, 128.7, 130.4, 137.3, 156.3, 156.4, 172.4. MS (ESI)- Found m/z 708.3 [M+ Na]$^+$. C$_{38}$H$_{40}$N$_2$O$_{10}$ requires 684.73. FTIR (neat, cm$^{-1}$) 3333, 3059, 3032, 2973, 2947, 1737, 1684, 1669, 1540, 1525, 1480, 1461, 1355, 1334, 1220, 1190, 801cm$^{-1}$. 

256
Appendix B

Series 1 PU - \textsuperscript{1}H-NMR and FTIR Spectroscopy data

**PCL-1-Z-Ser-PU:** \textsuperscript{1}H- NMR (400MHz, DMSO-d\textsubscript{6}): \( \delta \text{(ppm)} = 0.8 - 0.9 \) (-CH\textsubscript{3} in neopentyl glycol in PCL), 1.1 – 1.5 (-CH\textsubscript{2} in PCL, serine chain extender), 2.24 (-CH\textsubscript{2}- in PCL), 3.7-3.8 (-CH\textsubscript{2}- PCL, MDI), 4.0 - 4.2 (-CH\textsubscript{2}-C=O PCL, serine chain extender and -CH- \( \alpha \) proton of amino acid), 4.4 (-CH\textsubscript{2}- in side chain of serine chain extender), 5.02 (-CH\textsubscript{2} - \( \phi \) in Z- group of amino acid), 7.0 (-C\textsubscript{6}H\textsubscript{4}- MDI), 7.3 (-C\textsubscript{6}H\textsubscript{4}- MDI, Z-group), 7.8 (N-H- urethane link).

FTIR (neat, cm\textsuperscript{-1}) = 3345, 3336, 2907, 2867, 2857, 2948, 1731, 1699, 1532, 1220, 1167, 1412, 1083, 1066, 697.

**PCL-1-Z-Thr-PU:** \textsuperscript{1}H-NMR (400MHz, DMSO-d\textsubscript{6}): \( \delta \text{(ppm)} = \delta 0.8 - 0.9 \) (-CH\textsubscript{3} in neopentyl glycol in PCL), 1.1 – 1.5 (-CH\textsubscript{2} in PCL, threonine chain extender), 2.2 (-CH\textsubscript{2} in PCL), 3.7-3.8 (-CH\textsubscript{2}- PCL, MDI), 4.0 -4.2 (-CH\textsubscript{2}-C=O PCL, threonine chain extender and -CH- \( \alpha \) proton of amino acid), 4.5 (-CH\textsubscript{2}- in side chain of threonine based chain extender), 5.03 (-CH\textsubscript{2} - in Z- group of amino acid), 7.2 (-C\textsubscript{6}H\textsubscript{4}- MDI), 7.4 (-C\textsubscript{6}H\textsubscript{4}- MDI, Z-group), 8.0 (-N-H- urethane link).

FTIR (neat, cm\textsuperscript{-1}) = 3345, 3336, 2907, 2867 2857, 2948, 1731 1699 1532, 1412, 1083, 1066, 697.

**PCL-1-BDO-PU:** \textsuperscript{1}H-NMR (400MHz, DMSO-d\textsubscript{6}): \( \delta \text{(ppm)} = \delta 0.8 - 0.9 \) (-CH\textsubscript{3} in neopentyl glycol in PCL, m-CH\textsubscript{2} - BDO), 1.1 – 1.5 (-CH\textsubscript{2} in PCL), 2.2 (-CH\textsubscript{2} in PCL), 3.7-3.8 (-CH\textsubscript{2}- PCL, MDI), 4.0-4.2 (-CH\textsubscript{2}-C=O PCL), 4.5 (-CH\textsubscript{2}-C=O PCL,-CH\textsubscript{2}-terminal BDO), 7.2 (-C\textsubscript{6}H\textsubscript{4}- MDI), 7.4 (-C\textsubscript{6}H\textsubscript{4}- MDI), 8.0 (-N-H- urethane link).

FTIR (neat, cm\textsuperscript{-1}) = 3339, 3330, 3191, 3125, 2924, 2854, 1735, 1597, 1530, 1448, 1365 1239, 1060 1067, 1311.
Series 1 PUU - $^1$H-NMR and FTIR Spectroscopy data

**PCL-1-Leu-PUU:** $^1$H-NMR (400MHz, DMSO-d$_6$): $\delta$(ppm) = $\delta$ 0.8 – 0.9 (-CH$_3$- in neopentyl glycol in PCL, d- CH$_3$ of leucine chain extender), 1.1 – 1.5 (-CH$_2$- in PCL, m,-CH$_2$, -CH- leucine chain extender), 2.2 (-CH$_2$- in PCL), 3.5-3.6 (-CH$_2$- PCL, MDI), 4.0 - 4.2 (-CH$_2$-C=O PCL, leucine chain extender and -CH- α proton of amino acid), 6.2 (N-H amino acid-urethane proton), 7.1 (-C$_6$H$_4$- MDI), 7.2 (-C$_6$H$_4$- MDI), 8.4 (-N-H- urethane link).

FTIR (neat, cm$^{-1}$) = 3339, 3330, 3191, 3125 2924, 2854 1735, 1645, 1597, 1530, 1448, 1365, 1239, 1060, 1067, 1311.

**PCL-1-Ileu-PUU:** $^1$H-NMR (400MHz, DMSO-d$_6$): $\delta$(ppm) = $\delta$ 0.8 – 0.9 (-CH$_3$- in neopentyl glycol in PCL, d- CH$_3$ of isoleucine chain extender), 1.2 – 1.6 (-CH$_2$- in PCL, m,-CH$_2$, -CH- isoleucine chain extender), 2.3 (-CH$_2$- in PCL), 3.7-3.8 (-CH$_2$- PCL, MDI), 4.0 -4.2 (-CH$_2$-C=O PCL, leucine chain extender and -CH- α proton of amino acid), 6.4 (N-H- amino acid-urethane proton), 7.0 (-C$_6$H$_4$- MDI), 7.3 (-C$_6$H$_4$- MDI), 8.5 (-N-H- urethane link).

FTIR (neat, cm$^{-1}$) = 3339, 3330, 3191, 3125 2924, 2854 1735 1645, 1597, 1530, 1239, 1060.

**PCL-1-Val-PUU**: $^1$H-NMR (400MHz, DMSO-d$_6$): $\delta$(ppm) = $\delta$ 0.8 – 0.9 (-CH$_3$- in neopentyl glycol in PCL, d- CH$_3$ of valine chain extender), 1.3 – 1.5 (-CH$_2$- in PCL, m,-CH$_2$, -CH- valine chain extender), 2.2 (-CH$_2$- in PCL), 3.7-3.8 (-CH$_2$- PCL, MDI), 4.0 - 4.2 (-CH$_2$-C=O PCL, valine chain extender and -CH- α proton of amino acid), 6.2 (N-H- amino acid-urethane proton), 7.0 (-C$_6$H$_4$- MDI), 7.3 (-C$_6$H$_4$- MDI), 8.4 (-N-H- urethane link).

FTIR (neat, cm$^{-1}$) = 3339, 3330, 3191, 3125, 2924, 2854, 1735, 1645, 1597, 1530, 1448, 1365, 1239, 1060, 1067, 1311.

**PCL-1-Tyr-PUU**: $^1$H-NMR (400MHz, DMSO-d$_6$): $\delta$(ppm) = $\delta$ 1.1 – 1.3 (-CH$_3$- in neopentyl glycol in PCL, d- CH$_2$ of tyrosine chain extender), 1.4 – 1.4 (-CH$_2$- in PCL, m,-CH$_2$, tyrosine chain extender), 2.2 (-CH$_2$- in PCL), 2.8 (-CH$_2$- benzyl proton tyrosine),
3.7-3.8 (-CH$_2$- PCL, MDI), 4.0 -4.2 (-CH$_2$-C=O PCL, tyrosine chain extender and -CH-α proton of amino acid), 6.2 (N-H- amino acid-urethane proton), 6.2-6.9 (-C$_6$H$_4$- Tyrosine), 7.0 (-C$_6$H$_4$- MDI), 7.3 (-C$_6$H$_4$- MDI), 8.3 (-N-H- urethane link).

FTIR (neat, cm$^{-1}$) = 3339, 3330, 3191, 3125, 2924, 2854, 1735, 1645, 1597, 1530, 1448, 1365, 1239, 1060 1067, 1311.