DOCTOR OF PHILOSOPHY

Water soluble linear and hyperbranched P_{DL}LA-PEG-P_{DL}LA polymers for biodegradable hydrogels: synthesis, characterization and property evaluations

Hughes, Alan

Award date:
2014

Awarding institution:
Bangor University

Water Soluble Linear and Hyperbranched $P_{DL}LA$-PEG - $P_{DL}LA$ Polymers for Biodegradable Hydrogels: Synthesis, Characterization and Property Evaluations

Alan Hughes BSc. (Hons.)

Thesis submitted to Bangor University for

the Degree of Doctor of Philosophy

September 2014

Abstract

The aim of this PhD research project was to synthesise and characterize water soluble linear and hyperbranched biodegradable polymers based on bio-compatible (macro)molecules of poly(ethylene glycol) (PEG) and (D,L)-lactide using modern polymer synthesis approaches. The targeted $P_{DL}LA$-PEG-$P_{DL}LA$ polymers are aimed for use in the preparation of biodegradable hydrogels for tissue engineering and drug delivery. By changing the composition, topology and molecular weights of the copolymers, hydrogels with tailored biodegradability, swelling and mechanical properties can be achieved.

In the course of this project, a series of $P_{DL}LA$-PEG-$P_{DL}LA$ linear copolymers from poly(ethylene glycol) (PEG) (M_w = 1000 g/mol) and (D,L)-lactide (LA) were prepared with various LA chain lengths by ring opening polymerisation (ROP) of lactide and then functionalised with diacrylate groups. These reactions have been studied systematically in order to find optimised reaction conditions to obtain $P_{DL}LA$- PEG- $P_{DL}LA$ copolymers and $P_{DL}LA$-PEG-$P_{DL}LA$ diacrylate macromers with tailored composition and molecular weight. These linear block copolymers and macromers were fully characterised by Nuclear Magnetic Resonance (NMR), Fourier Transform Infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Thermogravimetric analysis (TGA) and Gel Permeation Chromatography (GPC).

Since diacrylated $P_{DL}LA$-PEG-$P_{DL}LA$ have photocrosslinkable and biodegradable properties, biodegradable hydrogels were prepared by directly photocrosslinking diacrylated $P_{DL}LA$-co-PEG-co-$P_{DL}LA$ copolymers alone and in conjugation with poly(ethylene glycol) methyl ethyl methacrylate (PEGMEMA) at different ratios. Hydrogels with tailored swelling and biodegradability were obtained and studied.

Diacrylated $P_{DL}LA$-PEG-$P_{DL}LA$ macromers were also used as branching agents to synthesize novel water soluble hyperbranched polymers with PEG-based monomers. Different copolymerisation approaches were used and these include Free Radical Polymerisation (FRP), Atom Transfer Radical Polymerisation (ATRP) and the Reversible Addition Fragmentation Chain Transfer (RAFT) Polymerisation. Challenges were found for the ATRP of $P_{DL}LA$-PEG-$P_{DL}LA$ macromers as it was found that acidic conditions interfere due to hydrolysis with the catalysis. RAFT approach has been found to be a better method for

the polymerisation of PEG-co-PLA macromers and the resulting novel hyperbranched polymers have also been characterised by NMR, FTIR, DSC, TGA and GPC.

The resulting hyperbranched polymers with multivinyl functional groups can be used to prepare biodegradable hydrogels and are envisioned to have more advanced properties compared to those obtained directly from photocrosslinking of diacrylated P_{DL}LA-PEG-P_{DL}LA macromers. Fine tuning of the P_{DL}LA-PEG-P_{DL}LA macromer constituents and their combination with co-monomers, for example PEGMEMA, can result in hydrogels with balanced hydrophobic and hydrophilic properties as well as tailored swelling and drug release profiles. These biodegradable hydrogels prepared by hyperbranched polymers have promising potentials for applications in tissue engineering and drug delivery.

The first chapter is an introduction to the topics covered in the project and a summary of state-of-art research and findings in the relevant areas. The second chapter is in three parts and concerns the materials used, the experimental procedures employed and the methods for characterising the products obtained. Chapter 3 presents a discussion driven by the results of the experimental work. The final chapter sums up the conclusions and how the work suggests possible future work.

Table of Contents

Acknowledgements

Firstly, I would like to thank my supervisor Dr. Hongyun Tai for all her help, support and guidance throughout the course of my PhD.

Secondly, thanks are due to the other members of Dr. Tai`s research group for all their good advice and moral support, in particular, to Alaa el-Betany, Anna Tochwin, and Stuart Bowers.

Thanks are also due to the technical staff in the Bangor School of Chemistry not only for their time and tuition but also for use of their facilities and chemicals.

Sincere thanks are extended to my collaborators who allowed me the use of their facilities, in addition to their generous advice, in particular Dr. Wenxin Wang and his group members.

I am grateful to Bangor University for the financial support by awarding me an EPSRC DTA scholarship.

Last, but not least, I wish to offer my thanks to my sister, Valerie, for her support.

Abbreviations

°C	Degrees Celsius
AIBN	2, 2`-azobis(isobutyronitrile)
ATRA	Atom Transfer Radical Addition
ATRP	Atom Transfer Radical Polymerisation
ATRP	Atom Transfer Radical Polymerisation
BPO	benzoyl peroxide
BSA	bovine serum albumin
CRP	Controlled Radical Polymerisation
DCC	Dicyclohexylcarbodiimide
DE-ATRP	deactivation enhanced ATRP
DMAP	4-(dimethylamino) pyridine
DMSO	DiMethylSulfOxide
DSC	Differential Scanning Calorimetry
EDC	1-ethyl-3-(dimethyl aminopropyl) carbodiimide
EDTA	ethylenediamine tetra acetic acid
EGDMA	ethylene glycol dimethacrylate
ESEM	Environmental Scanning Electron Microscopy
FDA	Food and Drugs Administration
FRP	Free Radical Polymerisation
FTIR	Fourier Transform Infrared spectroscopy
GMA	glycidylmethacrylate

GPC	Gel Permeation Chromatography
HEMA	2-hydroxyethyl methacrylate (HEMA)
HPLC	High Pressure Liquid Chromatography
LCST	Lower Critical Solution Temperature (LCST)
LCST	Lower Critical Solution Temperature
M_n	Number-average molecular weight
M_p	Peak molecular weight
M_w	Weight-average molecular weight
NHS	N-hydroxysuccinimide
NMP	Nitroxide-Mediated Polymerisation
NMR	Nuclear Magnetic Resonance Spectroscopy
PAA	poly (acrylic acid)
PDI	Polydispersity index
P_DLA	poly (l-lactide)
PEG	Poly(ethylene glycol)
PEGDA	Poly(ethylene glycol) diacrylate
PEGMEMA	Poly (ethylene glycol) methyl ether methylacrylate
PEGMEMA-MEO_2MA-EGDMA	poly (ethylene glycol) methyl ether methacrylate-co-2-(2-methoxyethoxy) ethyl methacrylate-co-ethylene glycol dimethacrylate
pHEMA	Poly (2-hydroxyethyl methacrylate)
PHPMA	Poly (*N*-(2-hydroxypropyl) methacrylamide)
PLA	Poly (lactic acid)
PLA–PEG–PLA	Poly (lactic acid)–poly (ethylene glycol)–poly (lactic acid)

PLGA	Poly (glycolic acid)
PLL	alginate–poly (L-lysine)
PMA	Poly (methacrylic acid)
PMDTA	1, 1, 4, 7, 7,-pentamethyl diethylenetriamine
PMMA	Poly (methyl methacrylate)
PNIPAAm	Poly(N-isopropyl acrylamide)
poly (DVB)	poly (divinylbenzene)
poly (EGDMA)	poly (ethylene glycol dimethacrylate)
PVA	poly (vinyl alcohol)
PVC	poly (vinyl chloride)
QT	Pentaerythritol tetrakis 3-merkaptopropionate
RAFT	Reversible Addition Fragmentation Chain Transfer
Rpm	Rotations per minute
SEC	Size exclusion chromatography
T_c	Critical temperature
TEMPO	2,2,6,6-tetramethylpiperidinyl-1-oxy
T_g	Glass transition temperature
TGA	Thermo-gravimetric Analysis
THF	Tetrahydrofuran
T_m	Melting temperature
UV	ultra-violet
wt%	Weight %
βCD	β-Cyclodextrins (βCD)

List of Figures

List of Schemes

List of Tables

Chapter 1: Introduction

1.1 Hydrogels

1.1.1 Introduction

Hydrogels are networks of hydrophilic polymers which have been cross-linked to render them insoluble.[1-3] They have the capacity to absorb and retain water but themselves remain insoluble with their three dimensional network intact. They can absorb many times their own weight of water. Hydrogels can be quite stable or they may degrade with disintegration of their structure and perhaps even dissolution of their breakdown products.[4] `Reversible` or 'physical' gels are networks that can form and be held together by entanglements between long molecular structures. 'Physical' hydrogels can also be made by utilising interactions such as ionic bonding, hydrogen bonding or hydrophobic associations.[5, 6] These reversible hydrogels are subject to in-homogeneities or localised domains, particularly, when formed from hydrophobic associations.

The permeation of nutrients into a gel and the diffusion of cellular products from the gel is affected by the bound water in a hydrogel.[7] On hydration, a dry hydrogel absorbs water; the water molecules will hydrate the most polar, hydrophilic domains, so becoming the primary bound water. The network swells as a consequence, exposing hydrophobic domains, which also bind with water, to form the secondary bound water. The primary and secondary bound water constitute the total bound water.[7, 8] The gel network will then absorb further water as a result of osmosis in an effort to attain an infinite dilution. The swelling effect is constrained by the network`s covalent or physical cross links which act as an elastic retraction force. Thus, an equilibrium swelling level is attained in the hydrogel. The water that is imbibed after saturation of the polar and hydrophobic domains with its bound water and which then leads to additional swelling is called bulk or free water and is assumed to occupy the spaces between chains within the network, and also fills the centres of large pores and other voids. If

the chains or cross-links within the network are degradable, the network will begin to disintegrate and dissolve as it swells. The rate will be dependent on its composition of the gel.

The hydrogel water content, i.e. the relative volume of the water fraction to that of the matrix and its free to bound water distribution 'character' will affect the absorption (partitioning) and diffusion of any solutes through the hydrogel.[7, 8] Pores are often a result of phase separation during the synthesis of the hydrogel, and smaller pores may also exist within the network. The pore size distribution, the interconnections between pores, and the average pore size of the hydrogel matrix are important but are often difficult to quantify. They are measured under the term called 'tortuosity'.[7]

The mechanical properties of a hydrogel and its drug release profile will be affected by the pore volume fraction, pore size distribution and the interconnections between the pores. The profile is also influenced by the size of the drug molecule and its interactions with the polymer chains of the hydrogel network. The pore-volume fraction, the pore sizes and the interconnections are in turn a consequence of the polymer chains and the crosslink density of the network. Drug molecule interaction with the polymer chains within the network will be determined by their compositions. Thus, designing a network for a particular release profile, it is necessary to 'match' the size and character of the drug molecule with the polymer`s composition and crosslink density.[7]

`Permanent` or `chemical' hydrogels are the result of covalently-crosslinked networks. These networks are made by covalent bonding between polymer chains and are generally more robust than hydrogels derived from physical cross-linking. Chemical hydrogels can be synthesised by crosslinking of water-soluble polymers. Poly(ethylene) glycol (PEG) is often used as a hydrophilic component of co-polymers and also has the advantage of being bio-compatible.[9]

Hydrogels are particularly useful in drug delivery.[9] Their porous structure can be controlled through fine tuning the density of cross-linking of the gel matrix. This porous structure allows loading of drugs and in part determines their subsequent release. In effect, the hydrogel is a depot system from which drugs can elute thus creating a local concentration of the drug in surrounding tissues. Depending on the release profile, the drug can be released over an extended period. Systemic delivery is also possible.

Hydrogels tend to be biocompatible as their high water content has a similarity to the soft matter of the extracellular matrix.[10] Biodegradability may be incorporated by design but makes hydrogels making them susceptible to hydrolysis, enzyme attack or other influences such as pH or temperature.[11]

Hydrogels, being deformable, adapt to the shape of the surface they are in contact with. They are, however, limited in their applications because of their low tensile strength. Load-bearing situations for instance lead to premature dissolution or the hydrogel may leak away from the intended local site.[12] Another problem may be the difficulty of incorporating hydrophobic drugs into the hydrophilic environment of the interior of the hydrogel. Amphiphilic materials contain hydrophilic and hydrophobic domains. They are able to swell in and interact with both aqueous and organic materials. They may be capable of absorbing hydrophobic molecules, which in turn can be released during biodegradation.[13]

Hydrogels can be produced from synthetic polymers e.g. PEG[14] or bio-polymers[15] (modified or not) and can consist of homo-polymers[16] or interpenetrating polymeric networks which are combinations of network polymers synthesised in juxtaposition.[17] Biodegradability can be featured into the polymer chain by incorporation of suitable building blocks by the synthesis of co-polymers with hydrolytically labile domains.[18] To produce hydrogel network structures from such a range of polymers, the ability of the polymers to be able to crosslink either physically or chemically is essential. Thus suitable chemical structures and functionality are required and a series of crosslinking and functionalisation methods have been used for the preparation of a wide range of hydrogels.[19] The following section will briefly discuss some important hydrogels based on the crossing methods used to prepare them.

1.1.2 Types of Hydrogels based on Methods of Cross-linking

1.1.2.1 Physically Crosslinked Gels

The physical cross-linking of polymers can be achieved by using non-covalent means, such as hydrophobic and ionic interactions, hydrogen bonding, host guest interactions or combination of the former. Hydrophobic interactions in an aqueous environment are strong interactions. They are used to prepare hydrogels from amphiphilic block copolymers for example 3- and 4-arm star polymers of the hydrophilic monomer N,N-dimethylacrylamide end capped with hydrophobic groups which eventually formed hydrogels.[20] Smart hydrogels, for example based on PEG and PLA, are often of this type.[21] Here, the biodegradable, thermo-sensitive, hydrogel is a copolymer composed of blocks of PEG and poly (L-lactic acid). Aqueous solutions of these copolymers undergo temperature-dependent reversible gel: sol transitions. These hydrogels can be loaded with drugs in the aqueous phase at a relatively high temperature of around 45°C forming a sol. On injection into subcutaneous tissue they undergo cooling to body temperature forming a gel that acts as a release matrix for the drugs

Physical hydrogels are not homogeneous, as molecular entanglements can be localised and hydrophobic and ion-bound domains can also form creating further in-homogeneities.[22] Free chain ends or chain loops also create defects in the network of physical gels.

Physically crosslinked gels have gained interest with the awareness that the use of crosslinking agents in the preparation of hydrogels can compromise the integrity of encapsulated substances such as proteins and cells.[4] To create physically crosslinked gels, a number of different methods have been investigated.

1.1.2.1.1 Ionic Interactions

When a polyelectrolyte, such as a polymer carrying multiple charges, is bound to multivalent cations such as Ca^{2+}, it may form a physical hydrogel. Such a hydrogel is called 'ionotropic'.[7] The polysaccharide polymer alginate consists of mannuronic and glucuronic acid residues (Figure 1) and crosslinking with calcium ions can be done at room temperature and physiological pH.[23] Alginate is a linear structure with polymer domains of (1-4)-linked β-D-mannuronate and α-L-guluronate residues, covalently linked in different sequences or blocks (see Figure 2) and calcium ions can induce gelation (Figure 3). Alginate gels are used for the encapsulation of living cells[24] and proteins.[25]

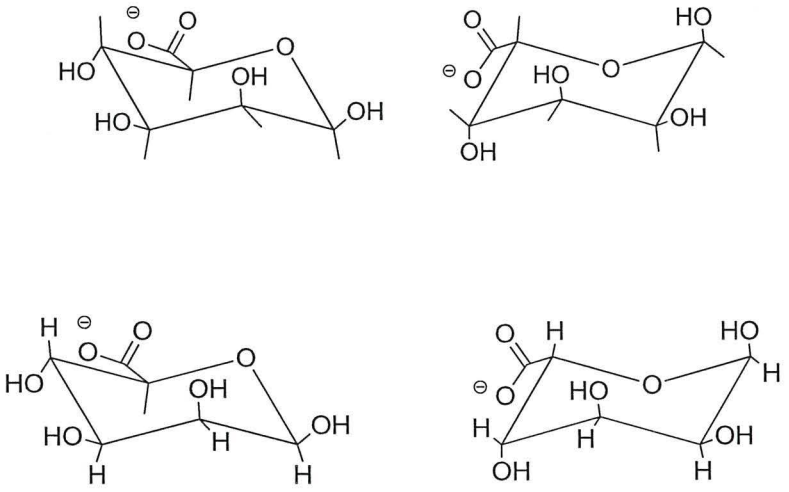


Figure 1: Monosaccharide constituents of alginate, D-mannuronate (left) and L-guluronate (right)

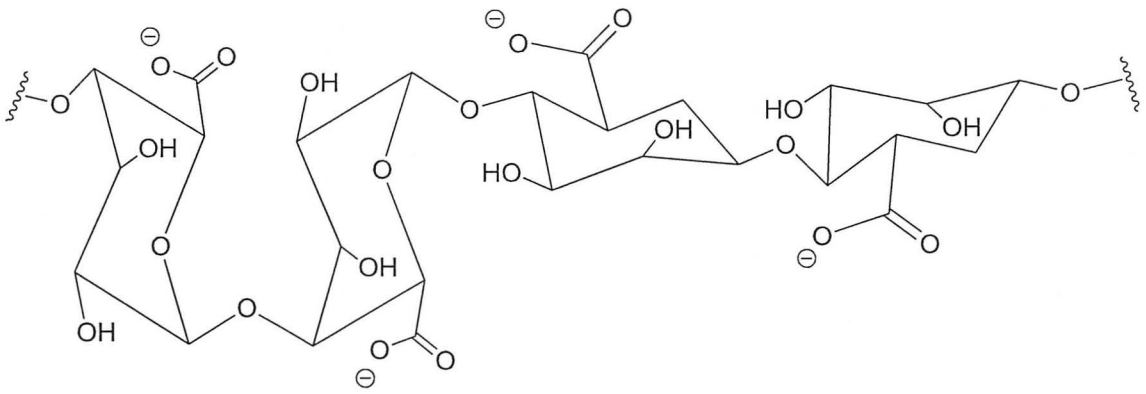


Figure 2: Polysaccharide of Alginate, on left two residues of L-guluroate bonded to two residues of L-mannuronate.

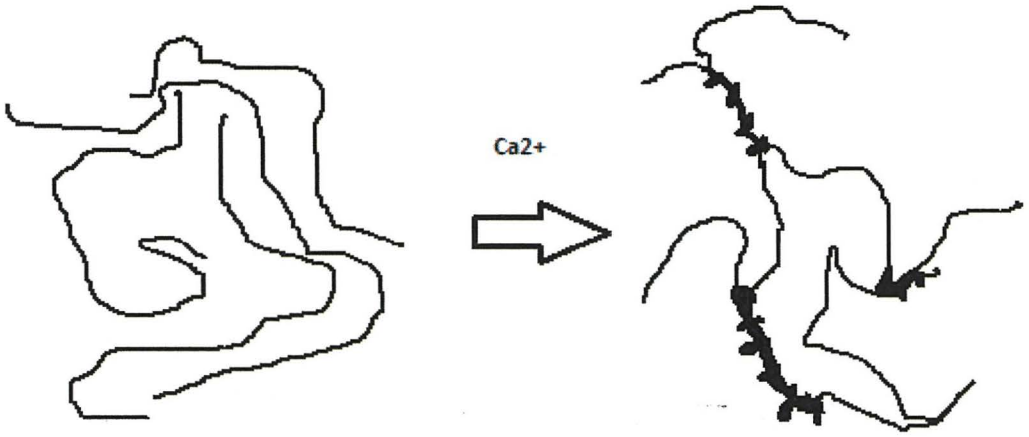


Figure 3: Calcium ions (black dots) induce gelation in poly L-guluronate domains [23]

These gels can be destabilized by removal of the Ca-ions using a chelating agent. Proteins can be released from alginate gels using cationic polymers, such as chitosan [26] and poly(lysine).[27]

When polyelectrolytes of opposite charges are added they may form crosslinked systems known as complex coacervates (also called poly-ion complexes or polyelectrolyte complexes).[7]The result is either a gel or a precipitate depending on the conditions.[19] The distinction between them is that a gel is a solid material of jelly-like appearance that can be soft and weak or hard and tough. They are cross-linked systems which do not flow when they

are subjected to deformation force. By mass gels are mostly liquid but behave like solids because of their 3D cross-linked network. Precipitation, on the other hand is the formation of a solid in a solution or in another solid during the course of a chemical reaction .If settling does not occur to aggregate the particles of the solid, the precipitate is held in suspension. A coacervate of alginate–poly(L-lysine) (PLL) ,for example has been prepared as early as 1980.[28]

1.1.2.1.2 Inclusion Complexes

β-Cyclodextrins (βCD), a type of cyclic oligosaccharide have an internal hydrophobic pocket. They and can bind with complementary low molecular weight guest molecules forming inclusion complexes[29], supramolecular self-assembly systems based on rapidly responsive hydrogels from polymeric hosts and low molecular weight guests.[30] Hydrogels were obtained after hydration of a mixture containing two components, one with an 8-arm poly(ethylene glycol) which has been terminnated with β-CD groups and the other component with 8-arm star-shaped PEG which has been terminated (or functionalised) with cholesterol moieties.[31,32]

The βCD/cholesterol inclusion complex formation by self-assembly is driven by hydrophobic and van der Waals interactions. Thermosensitive behaviour is exhibited by the hydrogels and is completely reversible upon cooling and heating. At low temperatures visco-elastic behaviour is apparent, whilst a viscous system is seen at higher temperatures resulting from faster chain relaxation processes as a reduced number of βCD/cholesterol complexes is obtained.[31] The distinction being that visco-elastic materials exhibit both viscous and elastic properties when subjected to defamation. Viscous material, such as honey, resists flow when subject to shear force and exhibit linear strain with time when stress is applied. Elastic materials strain under stretching and soon return to the original state when the stress is removed. Viscoelastic materials have the character of both, exhibiting time-dependent strain.

1.1.2.1.3 Stereo-complication

The co-crystallization of two enantiomers ("non-super-imposable" stereo-isomers) can be exploited in a synergistic interaction for cross-linking in a process called stereo-complexation. Thus, polymer or oligomer chains of poly (l-lactide) (P_LLA) and poly (d-lactide) (P_DLA) when combined *in situ* in a racemic mixture i.e. a mixture ratio of 1:1 formed stereo-complex crystals. [33]

Multi-armed PEG-P_LLA and PEG-P_DLA have been complexed to produce hydrogels. These were fine-tuned by varying the polymer concentration and the chain length of the PLA producing different cloud-points.[34] The advantage of this technique is that it can be performed under mild conditions. Also hydrophobic domains are avoided which might otherwise entrap any drug inclusions such as proteins which in turn might become denatured.[35] However, there is a relatively restricted range of enantiomers that can be employed for this method.

1.1.2.1.4 Peptide Interactions

Polypeptides are capable of forming a variety of conformations such as random coils, β-sheets and α-helixes. They have been used as building blocks with self-assembly properties and also incorporated with synthetic polymers to produce hybrid structures. Such bio-synthetic hybrid polymeric hydrogels have been made from fibrinogen and poly(ethylene glycol) and successfully used in tissue engineering.[36]

Some proteins can form coiled coils of left-handed super helices in which right-handed α-helices bind together two or more at a time.[4] These α-helices contain hydrophilic and hydrophobic domains due to repeating amino acid sequences with different polarities. Depending on temperature and pH, these proteins change their structural conformation. A polypeptide with a 'leucine zipper' motif attached to a hydrophilic central polypeptide domain forming an ABA type co-polymer, was made into a hydrogel.[37] The coiled-coil interactions allowed the solution to become more viscous as the temperature increased and when pH was 8 or above.

Proteins which exhibit coiled-coil interactions have been used as crosslinkers for poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA).[38] The crosslinkers were attached by histidine tags at one end of the protein to metal-chelating ligands attached to the polymer (Figure 4).[39] While hydrogels containing the natural protein kinesin exhibited a collapse near the melting temperature of the protein due to change in conformation from an elongated rod-like coiled-coil to random coils. Hydrogels made from engineered protein did not show this phase transition.[39]

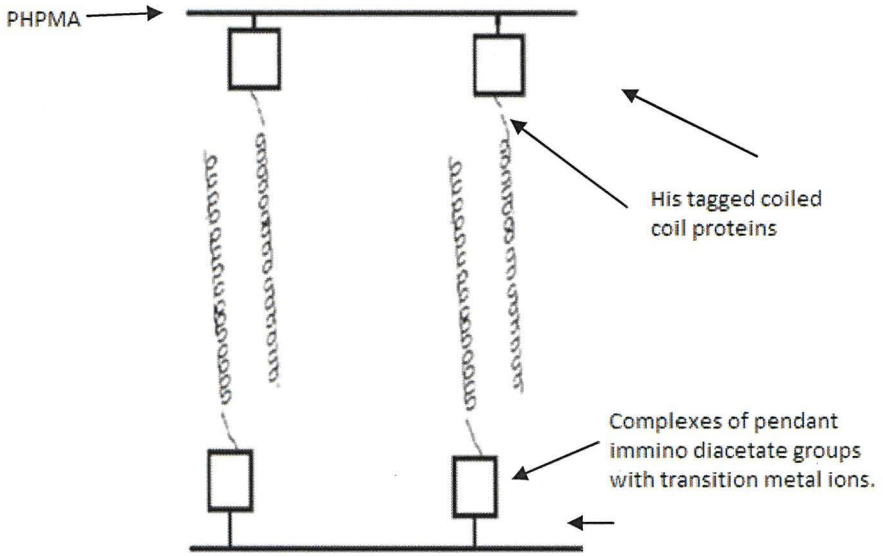


Figure 4: Hybrid hydrogel primary chains crosslinked with Histidine-tagged coiled-coil proteins.[38]

1.1.2.1.5 Amphiphilic block and graft copolymers

Self-assembly of amphiphilic block and graft copolymers can occur in aqueous solutions to form organized micelles and hydrogels. The hydrophobic domains of the polymers aggregate[40] multiblock or graft copolymers can be used to prepare physically crosslinked hydrogels. Water-soluble polymers, for example polysaccharides and polyethylene glycol (PEG), can be attached to hydrophobic units conferring both hydrophilic and hydrophobic domains in the molecular structure with the potential to self-assemble as micelles.

Block copolymers have been designed to incorporate hydrophobic and hydrophilic domains. Co-polymers of poly(ethylene glycol) (PEG) and poly(lactic acid) (PLA) or of PEG with poly(glycolic acid) (PLGA) have been synthesised and hydrogels made which have been evaluated for model drug release.[21] The drugs were released by passive diffusion and degradation of the hydrogel. At low concentrations micelles were formed in water and at higher concentrations thermoreversible hydrogels are formed. Triblock PEG polymers with hydrophobic PL (G) A domains in the middle have been prepared by the coupling of two PEG–PL (G) A di-block copolymers.[41, 42,43]

The composition and molecular weight of PEG–PLGA–PEG block copolymers affects the sol-to-gel transition temperature and the concentration of the co-polymer gives rise to a critical gel concentration at which hydrogels can form. Sufficiently high concentrations (typically higher than 17 wt.%) are required at which the co-polymer changes from a free flowing solution at room temperature to hydrogel at body temperature.[42]

PLA-PEG-PLA tri-block copolymers have been synthesised using the ring opening polymerisation of lactide.[44,45,46,47,48] Molina *et.al.*[49] prepared a series of PLA/PEO/PLA ABA type copolymers by ring opening polymerisation of racemic lactide The hydrogels were prepared by a phase separation method in which small amounts of water were added onto the co-polymer solution which was made up in tetraglycol, a biocompatible organic solvent. The hydrogels obtained were more hydrophilic than those formed by adding water to the dried copolymers. The hydrogels derived from the phase separation were then loaded with two proteins, bovine serum albumin (BSA) and fibrinogen. The proteins were added to the hydrogels before gelation and then entrapped in the forming gel. Circular dichroism analysis of the released BSA showed no damage to the protein. Different release profiles for the two

proteins were thought to be due to higher gel-protein compatibility for BSA for the fibrinogen.

PEG and PLGA multiblock copolymers have been synthesised by polycondensation of dicarboxylated PLA with PEG.[50,51] Small PLA blocks relative to the PEG render the copolymer soluble in water and a Lower Critical Solution Temperature (LCST) which is the phase transition temperature depending on the PLA domain molecular weight although all the polymers which are not soluble will swell in water.

1.1.2.1.6 Hydrogen Bonds

PEG can form complexes with poly (acrylic acid) (PAA) and with poly (methacrylic acid) (PMA) as a result of hydrogen bonding between the oxygen in the poly (ethylene glycol) residues and the carboxylic groups of the poly (methacrylic acid). The hydrophobic nature of poly(methacrylic acid) also helps the aggregation.[52,53, 54]

The protonation of the PMA carboxylic acid groups facilitates the formation of hydrogen bonds. Thus the swelling and synthesis of hydrogels is dependent on the pH. At low pH a poly (methacrylic acid)/poly (ethylene glycol) complex is soluble in ethanol. If the ethanol is removed from the mixture by diffusion, the system is transformed into a gel which in time gradually dissolves with dissociation of the complex.[55]

The DNA double helix is bound by hydrogen bonding between the bases of each strand. Nagahara et al. developed a hydrogel system by crosslinking hybridisation of oligodeoxyribonucleotides to the water-soluble polymer (poly(N,N-dimethylacrylamideco-N-acryolyloxysuccinimide).[56] Upon the addition of a complementary strand of oligodeoxyribonucleotides, which were either combined to the same water-soluble polymer or in its free form, an aqueous solution of the oligodeoxyribonucleotides-derivatized water-soluble copolymer was transformed into a hydrogel. These hydrogels were formed at room temperature, but dissolved at higher temperatures.[56]

1.1.2.2 Chemically Crosslinked Gels

Chemically cross-linked hydrogels are polymer networks formed by covalent bonds, thus resulting in more robust gels than physical hydrogels when subjected to mechanical forces.

1.1.2.2.1 Radical Polymerisation of Monomers using Crosslinking Agents. (Addition reactions)

The polymerisation of low molecular weight monomers such as 2-hydroxyethyl methacrylate (HEMA) with a suitable crosslinking agent such as ethylene glycol dimethacrylate (EGDMA) can produce the Poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel system.[57] Many hydrogels have been prepared by this approach.[58] Hydrogel characteristics, e.g. swelling, can be fine-tuned by varying the amount of the crosslinker. Stimuli sensitive materials can be obtained e.g. using methacrylic acid monomer yielded pH sensitive gels[59] or N-isopropylacrylamide monomer gave temperature-sensitive gels.[60] Water-soluble polymers can also be used to obtain chemically crosslinked hydrogels by radical polymerisation if they are first derivatized with polymerisable groups. Dextran can be used to make biodegradable hydrogels.[61] Dextran is a polysaccharide consisting of α-1, 6 linked D-glucopyranose residues. Edman et al. [61] derivatised dextran to produce a hydrogel by reacting a dextran solution in water with glycidylacrylate (Figure 5(a)) in order to introduce a double bond. Hydrogels were synthesised after the addition of a catalyst of N, N, N′N′-tetramethylene-diamine and an initiator of ammonium peroxydisulfate to an aqueous solution of acryl dextran also containing N, N,-methylenebisacrylamide as the crosslinker.

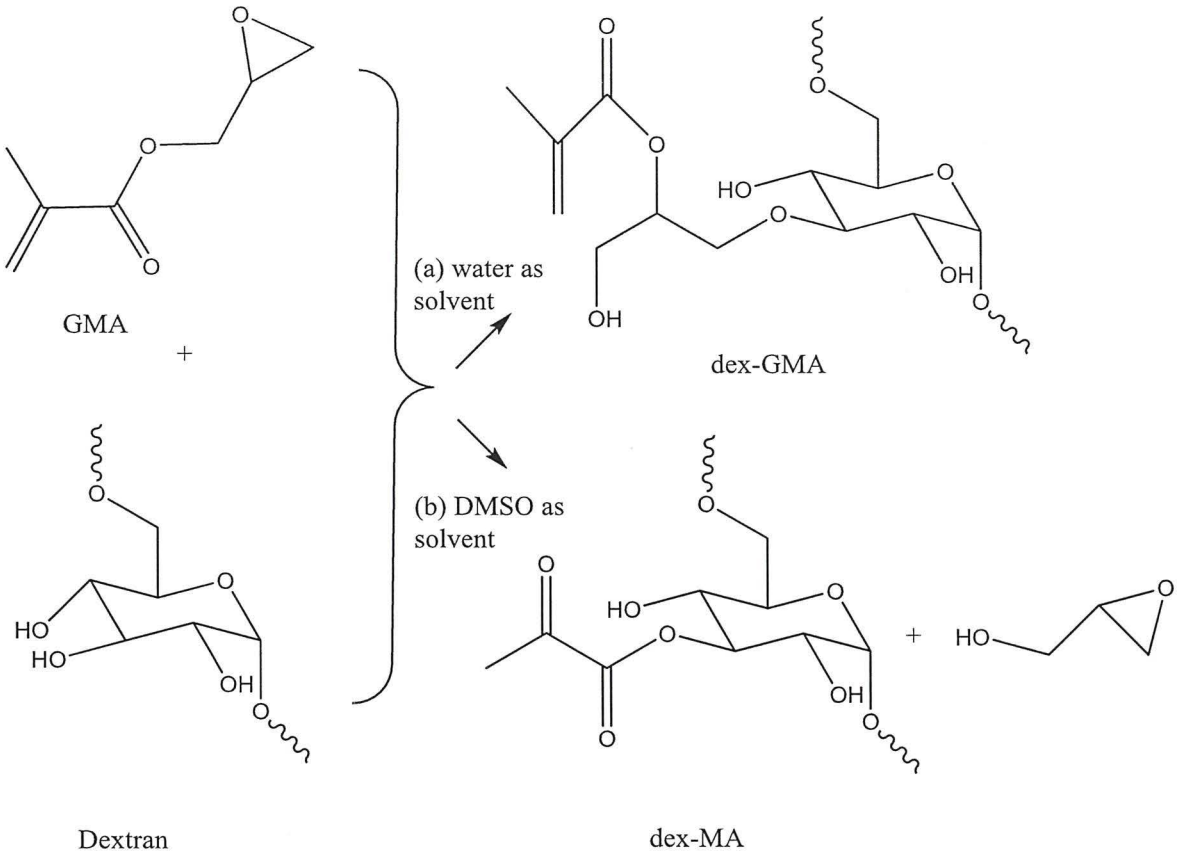


Figure 5: Principal Reaction of dextran with GMA.[4]

Hydrolysis of the glycidyl(meth)acrylate caused low yields, therefore, an alternative method to synthesize methacrylated dextran was obtained in which dextran was dissolved in the aprotic solvent DMSO and functionalised with glycidylmethacrylate (GMA), 4-(N,N-dimethylamino)pyridine (DMAP) was used as the catalyst for the esterification.[62] Under the given reaction conditions transesterification resulted in a dextran derivative with the methacrylate directly attached to the dextran chain in quantitative amounts (Figure 4(b)).

Other water-soluble polymers for instance albumin[63] and hyaluronic acid[64], have also been derivatized with (meth)/acrylic groups.

Other compounds were also methacrylated with similar procedures such as insulin[65] and sucrose.[66]

Methacrylate esters polymers are difficult to hydrolyse.[67,68] Methacrylate ester hydrogels are hydrolysed enzymically to break main chains when degradation is required under

physiological conditions. Chemically degradable gels have been created in which polymerisable groups and hydrophilic polymers are linked by means of hydrolysable moieties. Hubbell has synthesized macromers with a central domain of poly(ethylene glycol), terminated with poly-α-hydroxy acids and end capped with polymerisable acrylate groups.[18]Radical initiation of the acrylate groups induced polymerisation of the macromers which on addition of water formed a hydrogel. When UV light was applied to an aqueous solution of the macromer, radicals were generated from the photoinitiator (2, 2-dimethoxy-2-phenylacetophenone) which was added as a solution in the reactive solvent (N-vinylpyrrolidone). Degradation of the hydrogels yielded PEG, α-hydroxy acids e.g. lactic acid and oligo (acrylic acid). The time to degrade varied from 1 day to 4 months and can be fine-tuned by varying the bio-degradable domain.[18]

1.1.2.2.2 Covalent Linking of Complementary Groups (Condensation reactions)

Covalent linkages between polymer chains can be formed using complementary functional groups e.g. an amine with a carboxylic acid. Functional groups such as OH, COOH, NH_2 make polymer chains more hydrophilic. Other covalent linkages e.g. between isocyanate-OH/NH_2 can also be used.[4]

Polymer chains with hydroxyl groups such as poly(vinyl alcohol) (PVA) can be crosslinked with glutaraldehyde (Figure 6a)[69,70] but drastic conditions requiring low pH, a high temperature, and a quencher such as methanol PEG has OH groups terminating the linear molecule, thus is also an alcohol, and as such an *in situ* polymerisation under physiological conditions is not feasible in view of these drastic conditions.

Amine containing polymers such as albumin[19] and gelatin[71,72] and polysaccharide amines[73] can be crosslinked with glutaraldehyde under milder conditions forming Schiff bases.(Figure 6b). Glutaraldehyde is toxic, therefore, alternative strategies have been investigated such as the crosslinking of gelatin using polyaldehyde functional groups synthesised in the partial oxidation of dextran.[74]

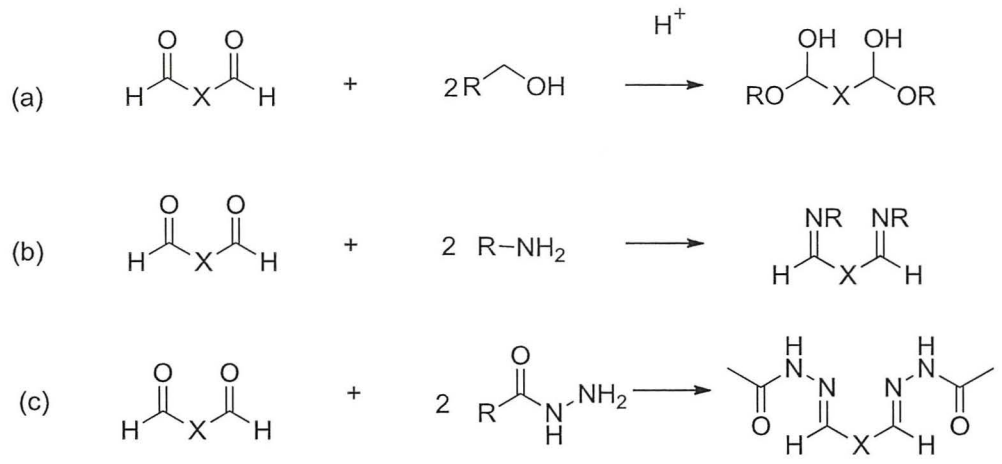


Figure 6 : Crosslinking polymers with aldehyde. X is a spacer. In Glutaraldehyde X=$(CH_2)_3$. (a) alcohol (b) amine and (c) hydrazide groups.

Cross-linking can be facilitated by means of addition reactions. Hydrogels can be prepared from water-soluble polymers with the use of bis-functional (or higher) crosslinking agents which react via addition reactions with groups on the polymer[75]. Thus, water-soluble polysaccharides can be converted into hydrogels with 1,6-hexanedibromide[76], divinylsulfone[77], 1,6-hexamethylenediisocyanate[76] and other reagents.[78] By varying the concentrations of reactants the network properties of the hydrogels can be fine-tuned. Organic solvents are generally required as the crosslinker is usually toxic and reacts with water, and therefore requiring an extraction step to remove the unreacted toxic reagents. To remove the solvent the hydrogel was purified exhaustively for 24 hours with acetone in a soxhelet and then dialysed at 4C against 1 M NaCl and then against distilled water before freeze drying and storage in a desiccator

The polymer linkages formed by this method are normally very stable, and degradation can only occur by means of enzymes under physiological conditions.[8]

Degradable hydrogels have also been synthesised by Hubbell via Michael Addition reaction of PEG-acrylates with PEG-dithiol[79] at physiological pH and room temperature (More discussions on Michael Addition Reactions can be found in section 1.1.2.2.5).

1.1.2.2.3 Condensation Reactions

Carboxylic acids or their derivatives can react with hydroxyl groups or amines in condensation reactions and can be useful for polymer synthesis to produce polyesters and polyamides. Similarly, such reactions can be used for the preparation of hydrogels.[75] Water-soluble polymers can be crosslinked with amide bonds by using N,N-(3-dimethylaminopropyl)-N-ethyl carbodiimide (EDC)[80] N-hydroxysuccinimide during the reaction to minimise any side-reactions and help to control the crosslink density in the gels.[75]

Alginate gel were designed with improved mechanical properties over ionically crosslinked gels by covalently crosslinking this polymer with PEG-diamines using EDC. The mechanical properties were controlled by adjusting the amount of PEG-diamine and the PEG molecular weight.[81] (Figure 7)

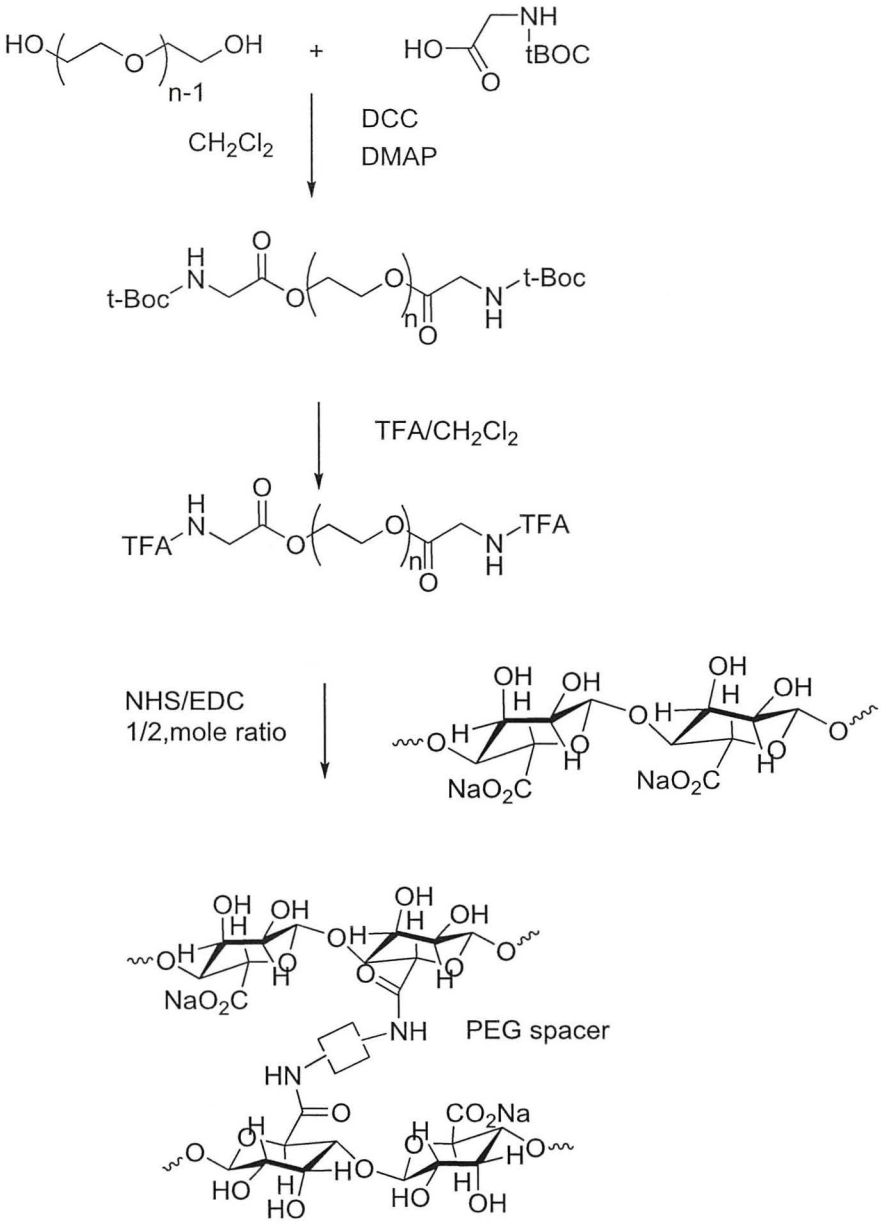


Figure 7: Synthetic scheme for PEG-diamine and its cross linking with Sodium Alginate[81]

1.1.2.2.4 Photo-polymerisation

Photo-polymerisation is a free radical polymerisation that can be conducted *in situ* by exposing the hydrogel precursors to UV/ visible light radiation of suitable wavelength. The

radiation activates photo-polymerisable groups such as carbon-carbon double bonds that are found in acrylate and methacrylate residues. Such residues can be attached to the polymer chain, thus rendering them photo-polymerisable.[82] The cross-linking reaction is initiated by a photo-initiator which is decomposed on exposure to appropriate radiation to form highly reactive free radicals. These free radicals then react with the carbon-carbon π-bonds of a monomer molecule to form a covalent link and a new free radical centre which can then go to react with another monomer molecule to form the propagating polymer species (see Section 1.2.2.2 Figure 18).

The advantage of this method is that it can be applied *in vivo* as a minimally invasive procedure by various clinical procedures such as trans-dermal illumination.[83] Generally, the curing process is completed in minutes and can be conducted at physiological temperatures with temporal and spatial control.[84,85]

Hubbell et al first investigated the use of photo-polymerisation in the synthesis of hydrogels and their use for drug release. He used Polyethylene (PEG) and co-polymerised this with Poly(lactic acid) and these co-polymers were functionalised with vinyl groups by reacting them with acryloyl chloride.[18]

Concern was raised whether the ultra-violet (UV) radiation and the free radicals generated might damage the loaded therapeutic. It was found, however, that a low intensity exposure of suitable wavelength maintains protein stability.[86]

1.1.2.2.5 Michael Addition

The Michael reaction is a very useful way of conducting an addition reaction as it can be carried out in aqueous conditions at room temperature and at physiological pH. Thus, the use of organic solvents and other toxic compounds is avoided. The Michael addition can be therefore well suited to synthesise injectable hydrogels.[87]

The reaction requires the use of a nucleophile to attack a β-carbon atom in a α-β carbon-carbon double bond which is itself conjugated to a carbonyl group.[87] From Figure 8 it can be seen that as a result of the nucleophile attack, an enolate intermediate is formed which the collapses with the addition of a hydrogen atom at the α-carbon atom.

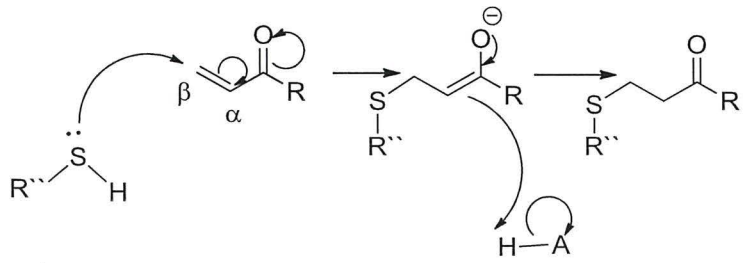


Figure 8: General reaction scheme for addition of thiol to an α, β conjugated carbonyl compound.

Thiols can be used as Michael donor species and have a higher selectivity for Michael acceptors compared to amines.[88] This is very apparent when the hydrogel precursor is loaded with peptide or proteins, when it is found that little cross reaction results with the vinyl groups present. Hubbell *et al* created an injectable matrix from a PEG-dithiol and PEG acrylate in aqueous conditions at room temperature and at physiological conditions.[18] The precursor gel was loaded with bovine serum albumin and, after the Michael Addition, which took approx. 15 mins; a hydrogel resulted with the albumin encapsulated. After swelling with water and subsequent degradation, the result of hydrolysis of the ester linkages, the albumin was released completely over 5-12 days. This experiment demonstrated the selective Michael addition of the acrylated PEG for the di-thiol PEG rather than for any di-sulfide bonds, free thiols or amine groups of the protein. It is thought the di-sulfide bonds and thiol groups were contained in inaccessible pockets within the protein thus preventing any cross-reactivity.[79]

Other Michael Addition donors have been used, such as pentaerythritol tetrakis 3`-mercaptoproprionate (QT) (Figure 10), with vinyl group acceptors including poly(ethylene) glycol diacrylate (Figure 9)[89, 90] This particular reaction was carried out in a phosphate buffer at physiological pH. Gels were obtained within 10 minutes when the two species were in a 1:1 molar ratio for the functional groups with complete conversion of the reactants. Side reactions, such as the formation of di-sulfide bonds were negligible within this time scale.[90]

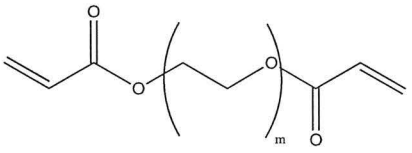


Figure 9: Polyethylene glycol diacrylate (PEGDA)

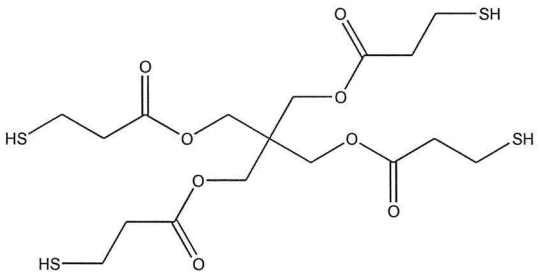


Figure 10: Pentaerythritol tetrakis 3-merkaptopropionate (QT)

Thermoresponsive hyperbranched co-polymers based on PEG have been synthesised with multi-acrylate functionality. Dong et al has synthesised such a polymer via an in situ deactivation enhanced Atom Transfer Radical Polymerisation.[91] A high level of vinyl groups was incorporated into the co-polymer making them suitable for Michael Addition polymerisation.

In addition, Dong incorporated into the system a semi-interpenetrated polymer network (semi-IPN).[91] An IPN consists of two or more cross-linked networks dispersed within each

other when viewed at the molecular level. A semi-IPN consists of one cross -linked polymer and the other is in the linear form. The linear molecule used was hyaluronic acid which is a component of the extra-cellular matrix (ECM).

Thus, Dong obtained *in-situ* gelling as a result of both thermoresponsive behaviour and also cross-linking by Michael Addition.

1.1.2.2.6 Click Chemistry

Polymers can be cross-linked by use of click chemistry. The most studied click reaction is based on the reaction between azides and acetylenes using Copper (i) ions as catalyst. The presence of copper in the final hydrogel is undesirable for many biomaterials, however, the level could be reduced to undetectable levels as measured by Environmental Scanning Electron Microscopy (ESEM) by washing with 0.1 M aqueous ethylenediamine tetraacetic acid (EDTA) solution.[92]

PEG based materials have been used. Figure 11outlines the synthesis of tetra-azide and di-acetylene functionalised PEG derivatives.[92]

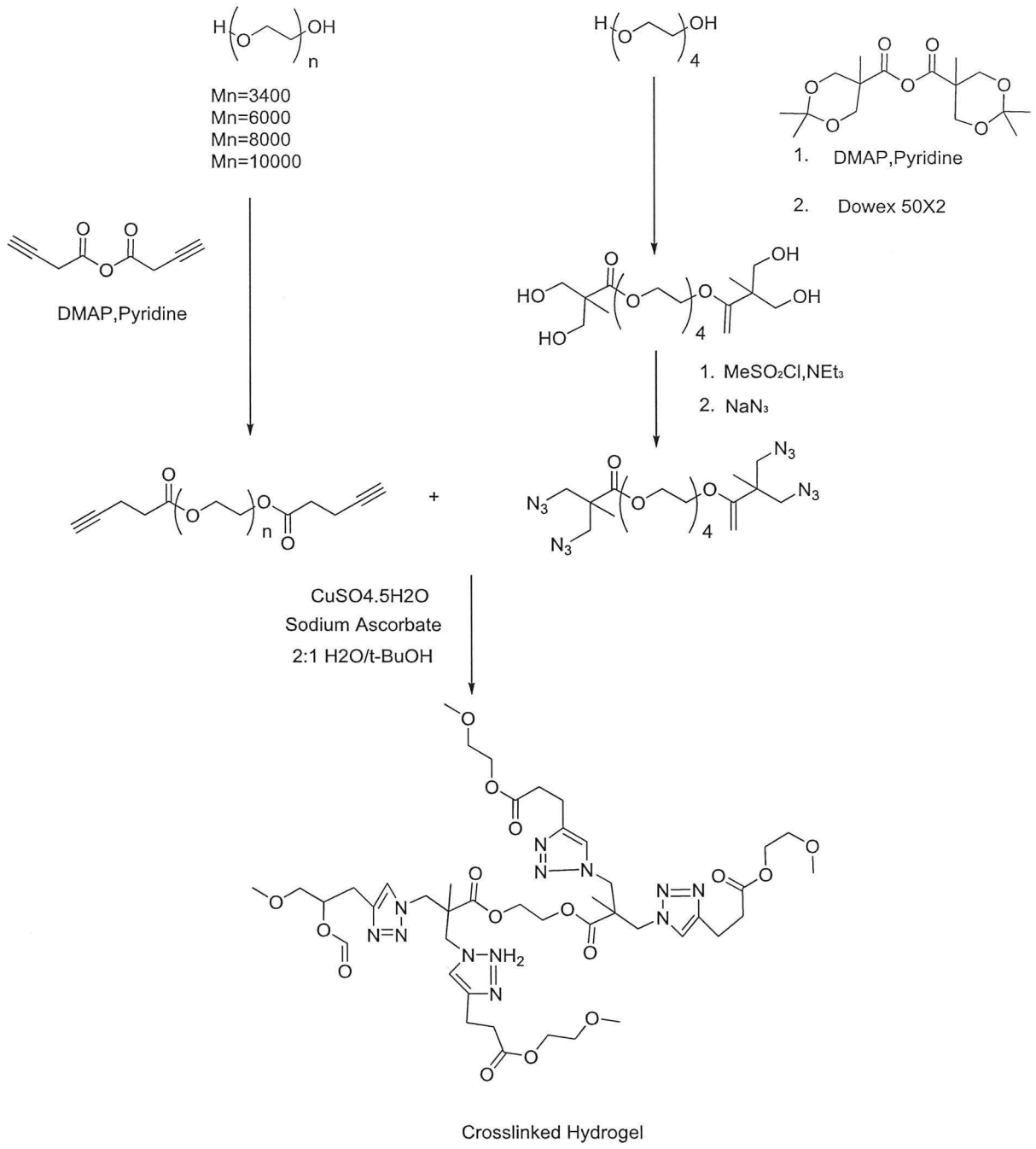


Figure 11: Modular approach for hydrogel synthesis using click chemistry and PEG based building blocks.

The reaction occurred within 30 minutes under physiological conditions giving rise to a controlled cross-linked network compared to photo-chemical or free radical methods. The major disadvantage is the rather toxic Cu(i) catalyst which needs to be removed before

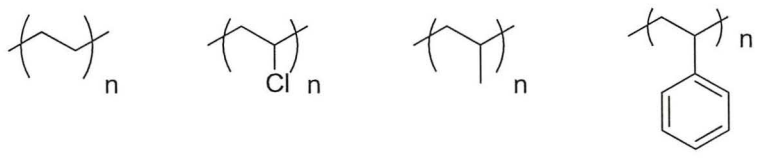
clinical use. Copper free click reactions are available but much slower and thus limiting possible clinical application.[93] Unreacted azide and acetylene can be usefully functionalised giving the potential for further chemical tailoring to produce novel cross-linked materials.

1.2 Polymers

1.2.1 Introduction

As discussed in section 1.1, hydrogels can be prepared from synthetic or natural polymers. Polymers have high molar mass and are composed of identical sub-units called monomers that are covalently bonded together in various arrangements. The type of monomers and how the repeating unit connects with each other play key roles in determinations of the properties of polymers thus their applications. Naturally occurring polymers include proteins, carbohydrates such as starch and cellulose as well as nucleic acids DNA and RNA. Many synthetic polymers for example polyethylene and polypropylene are produced on an industrial scale and have a wide range of useful properties and applications. The materials commonly known as plastics are synthetic polymers.[94] Synthetic polymers can be classified as addition polymers or condensation polymers.

Addition polymers e.g. polyethylene (1), poly(vinyl chloride) (PVC) (2), polypropylene (3), and polystyrene (4) are linear or branched with little or no cross-linking (Figure 12). In consequence, they are thermoplastic materials, which when heated flow easily and can be moulded into a variety of shapes by thermal polymer processing techniques.



1 2 3 4

Figure 12: Structure of addition polymers polyethylene (1) polyvinyl chloride (2) polypropylene (3) and polystyrene (4).

Condensation polymers on the other hand are formed via a condensation reaction when molecules combine losing small molecules such as water or methanol as a by-product in contrast to addition polymerisation which involves the reaction between unsaturated monomers. Condensation polymers include polyesters, polyamides and polyacetals (Figure 13).In the reaction the HCl is removed from the reaction mixture to prevent the reverse reaction.

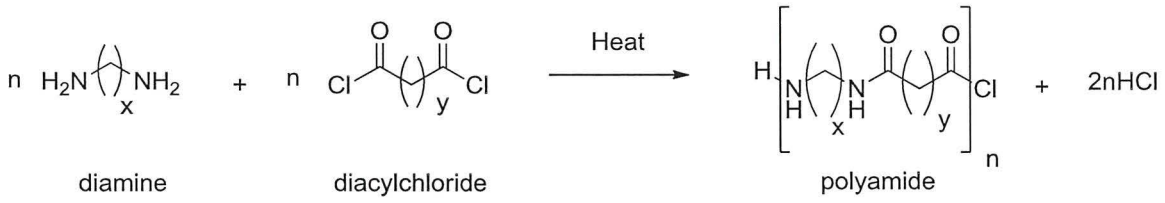


Figure 13: Condensation polymerisation in the synthesis of Polyamide.

1.2.2 Polymer Synthesis

There are three principle methods of polymer synthesis (a) step-growth polymerisation, (b) free radical addition or chain-growth polymerisation and (c) Ring Opening polymerisation.[94]

1.2.2.1 Step Growth Polymerisation

In a step-growth polymerisation, the polymer molecular weight builds up slowly through a single reaction type. This kind of reaction requires a monomer with two different functional groups within the monomer or equi-molar amounts of two different di-functional monomers to form a linear high-molecular weight polymer. Two monomers will react to form a dimer and the dimer may react with another dimer to form a tetramer, or may react with more

monomer to become a trimer. This process will continue with each reaction of the functional groups proceeding until a high molecular weight polymer is obtained (Figure 14).

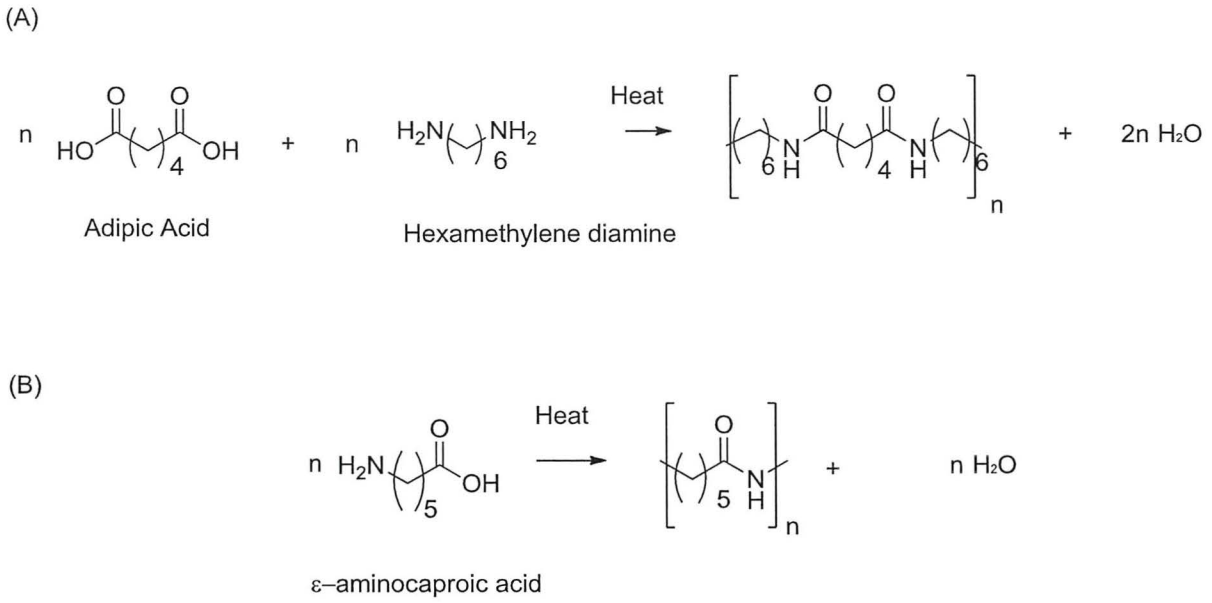


Figure 14: The step growth polymerisations of (A) adipic acid and hexamethylene diamine (the Nylon Process) (B) ϵ-aminocaproic acid

The reaction of adipic acid and hexamethylene diamine will produce a polymer of high molecular weight i.e. poly(hexamethvleneadipamide) i.e.nylon synthesis (Figure 14A). The amination requires a stoichiometric ratio of the reactants and proceeds via an intermediate ammonium salt. In the example of ϵ-aminocaproic acid which is a single di-functional monomer, a high molecular weight polyamide is obtained (Figure 14B).

1.2.2.2 Free Radical Addition or Chain Growth Polymerisation

Chain growth polymerisation occurs when monomer molecules add, one at a time, to the active site of a growing polymer chain. There are usually four steps in a chain-growth polymerisation:

Initiation: This step is primarily initiated either by thermal decomposition or by photolysis. In thermal decomposition the initiator is heated until a bond is homolytically broken, producing two free radicals. This is seen often in organic peroxides e.g. Benzoyl peroxide (BPO) (Figure 15) and azo compounds.[95]

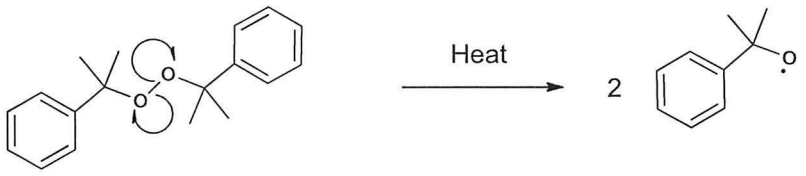


Figure 15: Thermal decomposition of Benzoyl peroxide.

In photolysis, electromagnetic radiation ruptures a bond homolytically producing **two free** radicals (Figure 15), for example azo compounds.[95] Figure 16 illustrates the photolysis of **α, α`-azobisisobutyronitrile (AIBN)** when irradiated with light of wavelength of 360 nm.

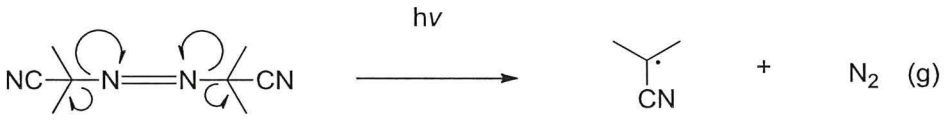


Figure 16: A bond ruptured homolytically producing two free radicals in α, α`-azobisisobutyronitrile (AIBN)

Photo-initiation also occurs by bi-molecular H-abstraction with the radical is in its lowest triplet excited state.[95] Thus, hydroperoxides such as cumyl hydroperoxide decomposes to alkoxy and hydroxy radicals, and with their active hydrogen atom, also decompose when initiated by a chain-end radical forming a peroxide radical by hydrogen abstraction. A suitable photoinitiator system absorbs in the 300-400 nm range.[96] Free radicals generated are

capable of reacting with the double bond of vinyl monomers. A suitable initiator should have an adequate solubility in the reaction mixture, promote a minimum of side reactions, and any by products resulting from its use should be non-toxic.

In redox reactions hydrogen peroxide is reduced by iron or other reductants such as Cr^{2+}, V^{2+}, Ti^{3+}, Co^{2+}, and Cu^{+} thus generating the free radical (Figure 17).[95]

Figure 17: Redox reaction of hydrogen peroxide and iron (II)

Persulfates can also be used as the initiating species (Figure 18). The dissociation of the persulfate occurs in the aqueous phase in an emulsion polymerisation in which the radical diffuses into the hydrophobic monomer-containing droplet.

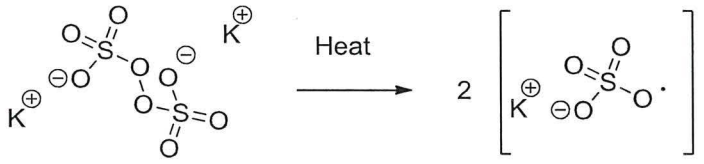


Figure 18: Thermal degradation of a persulfate.

Chain propagation: The initiator radical attacks a monomer (Figure 19). In an ethene monomer e.g. ethylene, one electron pair is held securely between the two carbons in a sigma bond. The other is more loosely held in a pi bond. The free radical uses one electron from the pi bond to form a more stable bond with the carbon atom. The other electron returns to the

second carbon atom, turning the whole molecule into another radical. This begins the polymer growing chain.[94]

Figure 19: Phenyl initiator from benzoyl peroxide (BPO) attacks a styrene molecule to start the polymer chain

Chain Transfer: In the transfer, the polymer growing chain is terminated but the active site is transferred to a new chain. This can occur with the solvent, monomer, or other polymer. This can occur by transfer of a hydrogen atom from a pendant group of another monomer (hydrogen abstraction).

Chain Termination: Polymer growing chain termination occurs either by combination (Figure 20) or disproportionation (Figure 21). Termination, in radical polymerisation, is when the free radicals combine and is the end of the polymerisation process. Chain termination will occur unless the reaction is completely free of contaminants. In this case, the polymerisation is considered to be a living polymerisation because propagation can continue if more monomer is added to the reaction. Living polymerisations are most common via ionic polymerisation approach, however, in recent years, living/controlled free radical polymerisations have been developed successfully and are discussed in section 1.2.2.2.1. Termination can occur by several different mechanisms. In chain combination, two chain ends simply couple together to form one long chain (Figure 20). One can determine if this mode of termination is occurring by monitoring the molecular weight of the propagating species: combination will result in doubling of molecular weight. Also, combination may result in a polymer that is C_2 symmetric about the point of the combination .[94]

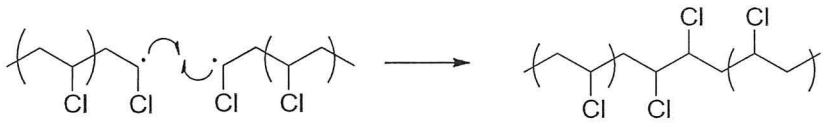


Figure 20: Termination by the combination of two poly(vinyl chloride) (PVC) polymers.

The other process is by radical disproportionation by hydrogen transfer in which a hydrogen atom from one chain end is abstracted to another, producing a polymer with a terminal unsaturated group and a polymer with a terminal saturated group (Figure 21).

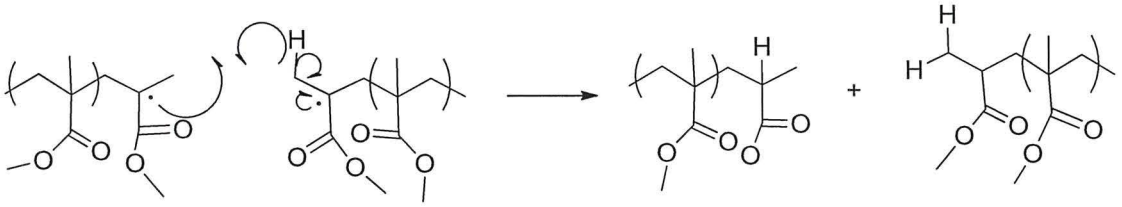


Figure 21: Termination by disproportionation of poly(methyl methacrylate)

Combination of an active chain end with an initiator radical will also lead to termination.(Figure 22).[94]

Figure 22: Termination of Polystyrene by reaction with radical initiator.

1.2.2.2.1 Controlled Radical Polymerisation (CRP)

Radical polymerisation is the major method used in industrial and academic laboratories for the preparation of vinyl polymers.[97] In free radical polymerisation a polymer forms by the successive addition of free radical species which are the building blocks of the polymer. Free radicals can be obtained via separate initiator molecules and are generated by a number of different mechanisms. Following its generation, in the initiating phase the free radical adds (nonradical) monomer units, thereby propagating the growing polymer chain. In conventional FRP, polymer growing chains will terminate uncontrolled (see the previous section). This leads to polymer chains with different chain length, thus a broad molecular weight distribution.

Recent developments on living/controlled free radical polymerisations enable the preparation of polymers with a very narrow molecular weight range and a predetermined degree of polymerisation based on the ratio of the concentration of monomer to the initiator and control of chain-end functionalities.[98] Controlled/ "living" radical polymerisations (CRP) have their origins in ionic polymerisations based on the equilibrium between active and dormant species and on the advances made in controlled organic radical reactions.[99] Most studied CRP approaches include Atom Transfer Radical Polymerisation (ATRP),[99-103] Reversible Addition-Fragmentation Chain Transfer Polymerisation (RAFT)[104] and Nitroxide Mediated Radical Polymerisation (NMP).[105] These CRP methods have be used for the preparation of a wide range of well-defined polymers, such as block and hyperbranched polymers with smart and biodegradable properties for tissue engineering and drug delivery applications[100, 106-108] which are the target polymer systems of this project.

The term controlled/"living" radical polymerisation is used to differentiate the polymerisation from a true living system in which chain growth proceeds without chain breaking reactions such as termination and chain transfer. True living radical polymerisation cannot occur because bimolecular termination between growing polymer radicals cannot be completely eliminated. Termination can, however, be significantly reduced resulting in block copolymers with predetermined molecular weights and low poly-dispersities and is thus essentially a controlled radical polymerisation.[98]

If the initiation is complete and exchange between competing reactive participants is fast, the final average molecular weight, i.e. when Monomer conversion is 100 %, can be designed by varying the initial monomer-to-initiator ratio (Equation 1).

$$DP_n = \Delta \frac{[M]}{[I]_0} \qquad \text{(Eq.1)}$$

where DP_n is the degree of polymerisation, M is the monomer and I is the initiator. A narrow molecular weight distribution is attainable with the polydispersity (M_w/M_n) between 1 and 1.5.[99] Further control is afforded by the availability of a range of initiators with a variety of different structures so that polymers can be synthesised with different end-functions and can also be co-polymerized with other monomers. This structure control results in polymers with diverse physical properties despite their synthesis from readily available low-cost monomers.[109]

1.2.2.2.2 Atom Transfer Radical Polymerisation (ATRP)

Atom Transfer Radical Addition (ATRP) polymerisation is based on the Atom Transfer Radical Addition (ATRA) reaction mechanism and normally requires the transfer of an atom (a Halide) from an organic halide to a metal- complex (with a transition metal centre) to generate the reacting radicals.[99, 107, 110] This is followed by transfer of the halide radical back from the transition metal to a product radical resulting in the formation of the final product.[111] In ATRA, the metal catalyst is a complex of a copper(I) halide with ligands such as 2,2-bipyridyl (bpy), and this undergoes a one-electron oxidation by abstraction of the halogen atom from an initiator- substrate (Figure 23).[99]

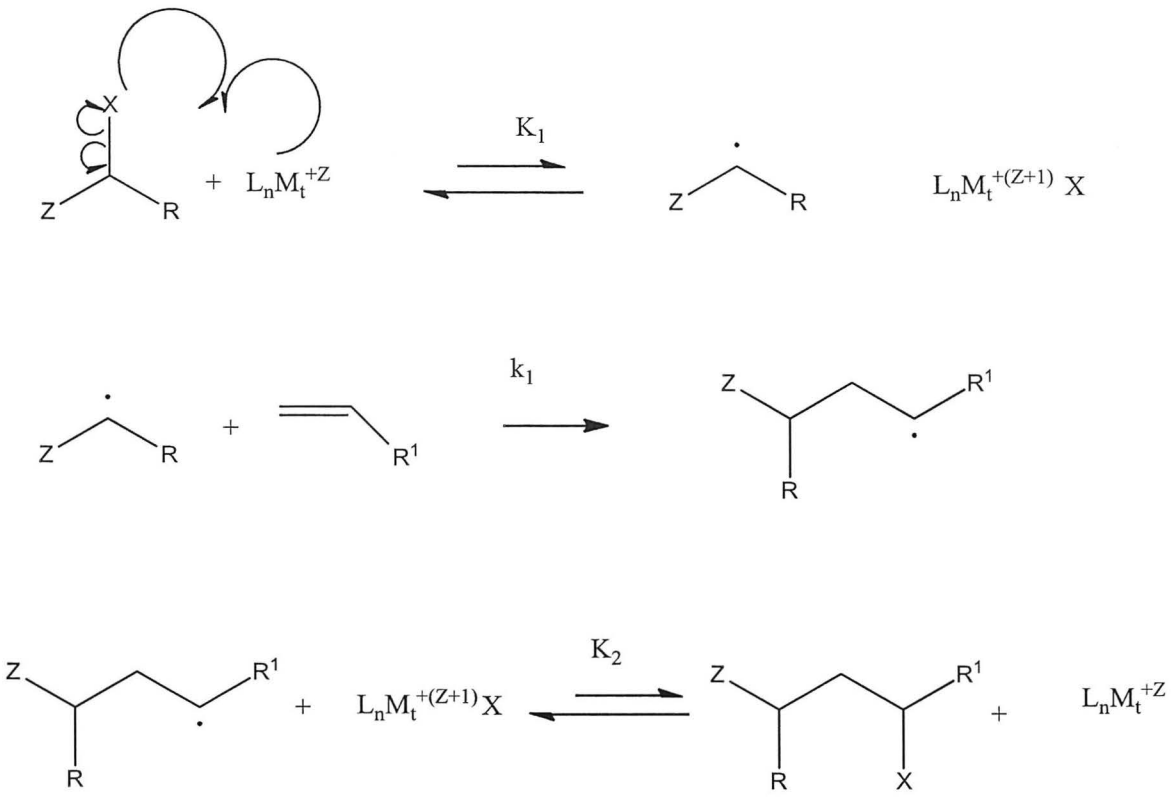


Figure 23: General scheme for Atom Transfer Radical Addition (ATRA) whereby the catalyst controls the concentration of the free radical

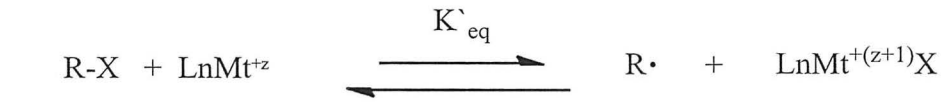
An organic radical is generated and also the copper(II) complex. For the reaction to occur substituents on the organic halide should be present to stabilize the resultant radical. It is not clear at present whether the intermediate radicals are free radicals or if they are formed in a solvent cage, or perhaps coordinated to the metal -centre.[99] The resulting organic radical can add to an unsaturated compound such as an acrylic moiety in an inter/intramolecular reaction. Alternatively, the halide atom is abstracted from the copper(ii) complex to form the dormant organic halide species. The catalytic cycle is completed when reduction to the copper(I) state occurs with the re-establishment of the copper(I) complex. Alternatively the radicals may react but as the concentration of propagating radicals is small termination reactions are minimised. The substrates for this type of reaction are chosen such that if addition occurs the newly formed radical is relatively less stabilized compared to the initial radical and will react irreversibly with the copper(II) to form an inactive product i.e. the alkyl halide (K2 >> K1 in

Figure 23). Thus in ATRA only one addition step occurs. This technique has been adapted in Atom Transfer Radical Polymerisation (ATRP) by modifying the conditions so that more than one addition step takes place.[99] Thus, if the propagating radical species before and after the addition of the unsaturated monomer has comparable stability an activation/addition/deactivation cycle will result and be repeated until the unsaturated monomer is exhausted. This result is a chain-growth polymerisation.

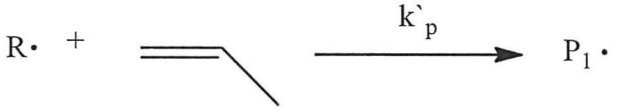
Copper-based ATRP have been successfully used in the controlled (living) polymerisation of acrylates, and methacrylates amongst other monomers[99] The monomer to be polymerized determines the nature of the other components that can be used. The initiator generally should be structurally homologous to the reacting polymer group. The halogen atom in the initiator and the catalyst metal complex should also correspond. In copper-based ATRP, usually two equivalents of a bidentate ligand are used per copper centre. Effective ligands for ATRP are, for example, derivatives of 2,2-bipyridine (bipy) and chelating nitrogen-based ligands N,N,N',N',N'-pentamethyldiethylenetriamine (PDMTA).[112] As copper(I)-bpy complexes with halides are usually insoluble in non-polar media of bulk polymerisations, long alkyl chain additions at the 4,4-positions of the bipyridine are used to increase the solubility of the copper complex.[99]

The mechanism of ATRP (see Figure 24) involve initiation and propagation processes in which an atom is transferred in equilibrium conditions and an intermediate radical is added to a monomer.[112] Termination occurs by radical coupling and disproportionation with a relatively significantly decreased rate constant. Intermediate radicals can be inferred in the mechanism as indicated in experimental data from trapping experiments, and is an insertion process. In copper based ATRP, the higher oxidation state copper(II) complex is formed during atom transfer and also an intermediate.

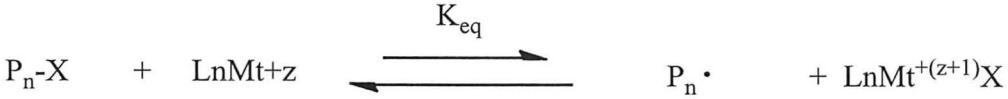
Initiation

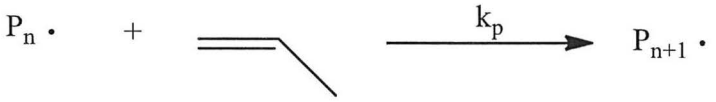


[X=Cl,Br]



Propagation





Termination

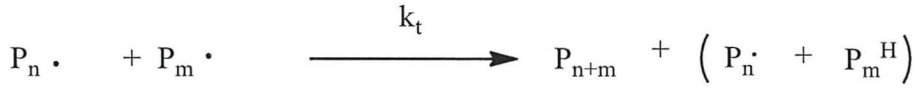


Figure 24: General Mechanism of Atom Transfer Radical Polymerisation (ATRP)[99] M is monomer; R· is a radical that can initiate polymerisation; P*n*· and P*m*· are polymer growing chains.

The rate of polymerisation R_p is given as Eq1 below in which termination is assumed not to occur as it is insignificant as a consequence of the persistent radical effect and rapid equilibrium is quickly attained.[99]

$$R_p = k_{app}[M] = k_p[P*][M] = k_p K_{eq}[ln]\left(\frac{[CuI]}{[CuII]}\right)[M] \qquad (Eq.2)$$

$$N.B. \qquad K_{eq} = \frac{k_{act}}{k_{deact}} = [P*]\frac{[\mathrm{CuIIX}]}{[\mathrm{CuI}][\mathrm{PX}]} \qquad (Eq.3)$$

In ATRP, reactants have to be appropriate to each monomer class with a particular combination of initiator, ligands, deactivator, metal, and the conditions of temperature, time, and solvent employed to suit the needs of the ATRP.[99] The alkyl halide (RX) concentration determines the number of initiated chains. The rate of polymerisation is first order for alkyl halide concentration, and the molecular weight is inversely proportional to the initial initiator concentration.[110] The halide group, X, shuttles rapidly and selectively between the polymer chain and the metal centre. Bromide and Chloride ions have proven to provide optimal control of molecular weight.[113]

A good initiator should have a structure of the alkyl group (R) which should be homologous to the dormant polymer structure with α-halopropionates being approximately similar to dormant acrylate end groups. This holds true for secondary radicals but tertiary radicals may differ somewhat. Thus isobutyrates are not the optimal initiators for Methyl Methacrylate (MMA), probably because of a β-strain effect[99] in which the presence of a third alkyl group sets up a condition of steric strain and reduces the effectiveness of the halogen as a good leaving group.

The rate of polymerisation is usually first order for the catalyst concentration, and the molecular weight is not related to this concentration.

A suitable ATRP catalyst should be a transition metal complex with an accessible redox couple that differs by one electron. However, as ATRP is an atom transfer process with one electron oxidation, the co-ordination number should increase by one to accommodate the new ligand. A good catalyst is selective for atom transfer, possessing little affinity for alkyl radicals and their hydrogen atoms, avoiding transfer reactions such as β-H elimination and the production of organometallic derivatives. These reactions would, if they occur, reduce selectivity in the propagation step and therefore the control of the polymerisation. [107] The metal centre should not be a strong Lewis acid, to avoid the possible ionization of initiators/end groups to carbocations. The most important system variable in selecting a good ATRP catalyst is the position of the atom transfer equilibrium and therefore exchange

dynamics between the dormant and active species. The equilibrium position is dependent upon the metal and ligands used.

Generally electron donating ligands stabilize the metal`s higher oxidation state accelerating the polymerisation. The dynamics of exchange between dormant and active species is very important for a controlled/living polymerisation.[102, 107, 112] The deactivation step should be very fast, to avoid poor control over molecular weight and yield high polydispersity values for the polymerisation.

In non-polar monomers such as styrene and methyl methacrylate, it may be necessary for the catalyst to be complexed with ligands with long alkyl chains to increase the complex's solubility.[110] Thus, when the copper halide is complexed with Bpy, it may be sparingly soluble in the polymerisation medium, the polymerisation being heterogeneous. The copper halides can be then be completely solubilised with Bipyridyl ligands derivatives with long alkyl groups, such as dNbipy, in which the groups are at the 4,4 carbon positions.[114] However, ATRP can be achieved despite the catalyst being relatively insoluble in the polymerisation medium as the catalyst is not attached to the growing chain. Somewhat higher poly-dispersities are observed in heterogeneous copper-based ATRP as a consequence of the lower concentration of the Cu (II) complex in the polymer phase.

ATRP has been successfully performed in bulk, organic solvents, aqueous solution, and mini-emulsion by homogenous or heterogeneous reactions.[102] Solution polymerisation is slower when using the same quantities of reactants comparing to bulk polymerisation. The solvents used are usually non-polar, but some polar solvents have been used. However, with polar solvents chain transfer is possible depending on the transfer constant. A drawback may be solvent interaction with the catalyst complex or with the halogen ligand or even displacement of spectator ligands.

ATRP polymerisation is also oxygen sensitive, although ATRP will tolerate a small amount of oxygen because the oxygen will be scavenged by the catalyst which is normally designed to be present in excess of the propagating radicals. Oxidation of the catalyst, however, reduces the effective concentration of the metal complex which acts as the deactivator, and may reduce the rate of polymerisation. The presence of a small amount of water and other polar compounds such as alcohols has little effect upon copper-based ATRP.[110] Amine and phosphine ligands in copper-based ATRP can inhibit polymerisation,

possibly by saturation of the coordination sphere of the copper(I) complex or by formation of copper(I) complexes not responsive to atom transfer.

It is observed in ATRP that the rate of polymerisation increases with temperature due to an increase in the rate constant for propagation and also the halogen atom transfer equilibrium constant. The activation energy for propagation is significantly higher than for termination for radical combination and for disproportionation. Therefore at higher temperatures the ratio k_P compared to k_t will be higher and will result in greater control of polymerisation control.

In recent years, deactivation-enhanced ATRP method have been developed and used for the polymerisation of multifunctional vinyl monomers resulting in soluble hyperbranched polymers rather than crosslinked gels.[115-117] (see further discussions in section 1.2.4). The control over the polymerisation is attributed to the slow growth of polymer chains in the presence of a high concentration of Cu (II) species, thus avoiding cross linking between chains and so delaying the onset of gelling. In-situ deactivation enhanced ATRP is further adopted for the preparation of hyperbranched polymers, in which instead of a Cu^{1+} species, a Cu^{2+} species is used to form Cu (I) *in situ* using a reducing agent L-ascorbic acid. The reduced form of copper is generated and functions as the catalyst in the ATRP process by being itself oxidised by the shuttling halogen radical which is released when a polymer radical is generated. and available to moderate the activation/deactivation equilibrium of ATRP.[117] (See Figure 25).

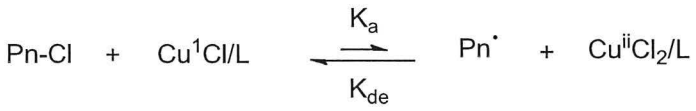


Figure 25: De-activation enhanced ATRP [117]

1.2.2.2.3 Reversible Addition Fragmentation Chain Transfer (RAFT)

Radical polymerisations with a living character offer benefits compared to conventional free radical methods.[98] Apart from ATRP another method for achieving a living radical polymerisation is by the process of Reversible Addition-Fragmentation chain transfer

(RAFT).[104,118,102, 119] The RAFT method utilizes dithio compounds as chain transfer agents which in their dormant state are responsible for the reversible trapping of most of the polymer radicals, thus reducing the possibility for bimolecular termination to take place (Figure 27). For a dithio compound to be an effective RAFT agent, the rate of addition and the rate of fragmentation must be fast when compared to the rate of propagation and also the ejected radical must be capable of reinitiating a polymerisation. These requirements ensure the rapid uptake of the RAFT agent and the fast equilibration of both the dormant and the active species while ensuring the continuation of the chain building process. The general structure of the dithio compounds is given Figure 26.

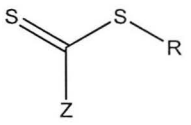


Figure 26: RAFT agent where Z is an aryl, alkyl, SR`, OR`, NR_2`, and R is a homolytic leaving group.

The RAFT agent modifies the rate of the polymerisation because both the monomer and the growing chains can combine with the RAFT agent. The RAFT complex can then fragment in two ways giving back radical species.

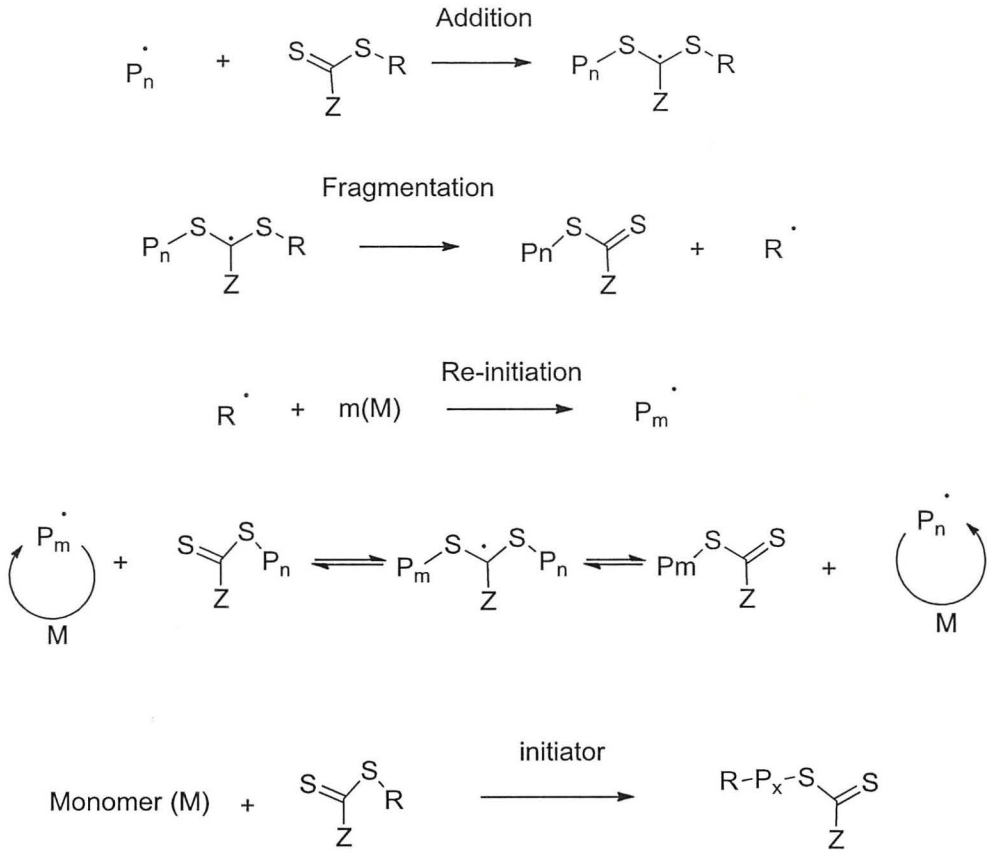


Figure 27: The RAFT process. M is monomer; R· is a radical that can initiate polymerisation; P*n*·, P*m*·, and P*x*· are polymer growing chains. [94]

The RAFT polymerisation has a number of advantages. The RAFT agent is simply added to a free radical polymerisation in which an initiator and monomers are present perhaps with a suitable solvent. Chain growth commences at the onset of the polymerisation and continues until the monomer is exhausted. Termination reactions are minimised as the effective concentration of radical species is reduced by their capture by the chain transfer agent. The polymerisation is thus classified as controlled/living with the molecular weights increasing linearly with conversion. The Molecular weight is given by[120]

$$M_{n(calc)} \approx m_M([M]_0 - [M]_t)/[In]_0 \qquad \text{(Eq.4)}$$

$[M]_0$ - $[M]_t$ is the reduction in the monomer concentration at the end of time (t) and m_M is the molecular weight of the monomer.[I] is the initiator concentration.

Narrow molecular weight distributions (polydispersities) are attained and complex molecular architectures are possible such as star or block structures.

The RAFT reaction can be undertaken in a range of solvents including protic solvents such as water and alcohols.[121] Thus, this technique might be more suitable in mildly acidic conditions which might be toxic to ATRP catalysts.

1.2.2.2.4 Nitroxide Mediated Radical Polymerisation (NMP)

The NMP of styrene was first published by Georges in 1993 using benzoyl peroxide as the initiator mediated by the stable free radical TEMPO (2,2,6,6-tetramethyl-1-piperidynyl-N-oxy).[102, 105] Control in NMP is achieved with dynamic equilibration between dormant alkoxyamines and actively propagating radicals (Figure 28).

NMP involves a reversible termination mechanism between the growing propagating polymer radical species and nitroxide, acting as an activation/deactivation control agent which results in a polymer-alkoxyamine as the predominant species which represents a dormant state. The nitroxide and the propagating radical are regenerated by homolytic cleavage induced by an increase in temperature to establish equilibrium between the dormant and the active species... The activation–deactivation equilibrium $K=k_d/k_c$, where K is the equilibrium constant, and the persistent radical effect, determines the kinetics of the polymerisation.[122]

This system is basically a radical polymerisation with a thermal initiator, such as benzoyl peroxide (BPO) or 2,2-azobisisobutyronitrile (AIBN) in conjunction with 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO)

The $[\text{nitroxide}]_0/[\text{initiator}]_0$ ratio determines the polymerisation kinetics as the amount of excess free nitroxide after initiation step shifts the activation–deactivation towards the dormant species, reducing the polymerisation rate. Thermal initiators are difficult to use accurately as their efficiency to produce primary radicals by thermal decomposition is difficult to assess due to factors such as the cage effect and any induced decomposition. The

initiating group may cause many of the primary radicals to undergo rearrangement reactions leading to poorly reproducible kinetics in the polymerisation kinetics. [105]

Alternatively, a uni-molecular initiator (an alkoxyamine initiator) can be used that decomposes into both the nitroxide and the initiating radical.[123] On dissociation this leads to a 1:1 release of initiating radical and nitroxide. Uni-molecular initiators lead to better control over molecular weight and MMDs than bimolecular initiating systems. See Figure 28 below.

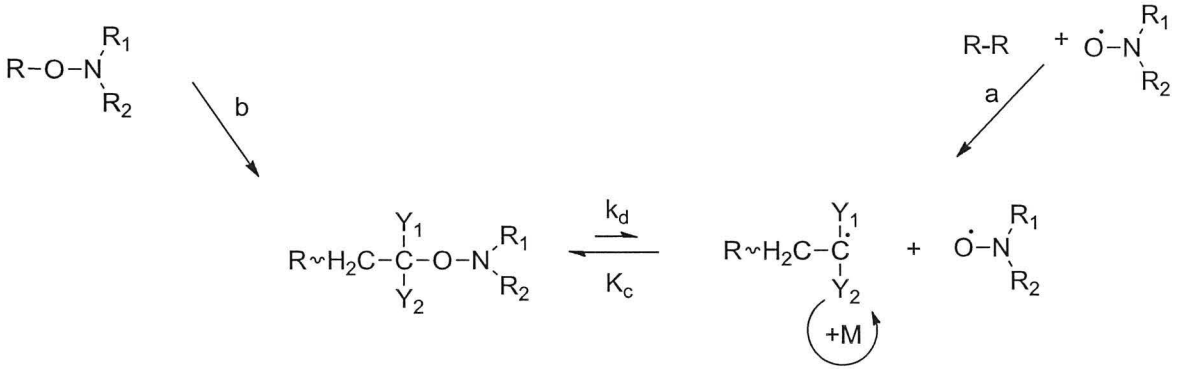


Figure 28: Nitroxide-mediated polymerisation showing activation–deactivation equilibrium. (a) Bi-component initiating system and (b) Mono-component initiating system.[105]

1.2.2.3 Ring Opening Polymerisation

Ring Opening Polymerisation (ROP) is well studied and widely used for the preparation of biodegradable polymers, for example polyesters and polyamide.[97, 124] Some ROP can be considered as a chain polymerisation (addition of monomer to a growing chain end), but many reactions are more complicated, therefore, ROP is regarded as the third polymerisation method alongside with chain growth and step-growth polymerisations. In most ROP, the driving force is the ring strain and associated steric considerations. Biodegradable PEG-co-PLA copolymers have been prepared by ROP of lactide in the presence of PEG[18] (see further

discussions on this type of polymers in section 1.2.3 and 1.2.5). The mechanism for the ring opening of lactones can be anionic, cationic and coordination-insertion.[171] Anionic ROP is the nucleophilic attack of the growing chain end on a heterocyclic monomer molecule (see Figure 29). The β route is the more common as the negative charge on the oxygen is more stabilised by the carbonyl carbon.

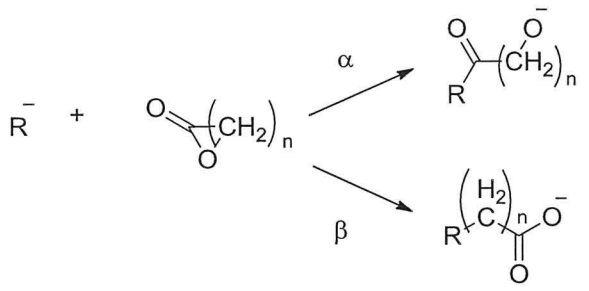


Figure 29: Anionic Ring Opening polymerisation of Lactones [122]

1.2.3 Poly(ethylene glycol) (PEG) based Polymers

Poly (ethylene glycol) (PEG) is a hydrophilic polymer consisting of repeating ethylene oxide units terminated by alcohol functional groups. The structural formula is: $HO\text{-}CH_2 – (CH_2\text{-}O\text{-}CH_2)n\text{-}CH_2\text{-}OH$. The polar but uncharged character gives rise to its hydrophilic properties. Also, PEG is a very flexible molecule providing an uneven, neutrally-charged surface prevents binding with proteins and confers its properties of minimal protein fouling when immobilized chemically[125] or physically[126], low toxicity, and negative immune response[127] and biocompatibility[128]. PEG is also an inhibitor of bacterial and animal cell adhesion.[129]

PEG is a versatile component for various co-polymers and can be used to tailor the physical and chemical properties of the co-polymer by changing chain length and structural geometries e.g. branched, star, or comb. A co-polymer of PEG is the tri-block copolymer poly(lactic acid)–poly(ethylene glycol)–poly(lactic acid) (PLA–PEG–PLA).[130] This is a

relatively novel material and was thought to offer some advantages over the poly(lactic-glycolic acid) co-polymer previously studied.

PLA–PEG–PLA is more hydrophilic than the PLGA co-polymer. It was found that the release of the cancer drug paclitaxel from PLGA micro/nano-spheres was extremely slow as both the drug and PLGA are highly hydrophobic.[131] The continuous release of the drug for one week to one month was required for effective treatment of the cancer. It was realized that incorporation of a hydrophilic domain such as poly(ethylene glycol) (PEG) into the hydrophobic poly(lactic acid) (PLA) chain, should greatly improve the drug release.

Biodegradation of PEG polymers in vivo can occur by a combination of mechanisms. Linkages in the polymer may hydrolyze either enzymatically or otherwise.In both cases the forming oligomeric degradation products may be water soluble, the products are excreted or metabolised. Water insoluble oligomers can be engulfed by phagocytosis whereupon they are further degraded within peroxisomes and other membrane-bound lysosomes. If the polymer is hydrophobic, degradation will occur at the surface of the material. If the polymer is hydrophilic and water has permeated the bulk of the material due to swelling, then degradation throughout the bulk of the material will occur. Biodegradable polymers have been much used in medicine for structural support e.g. sutures, etc. and for the controlled release of drugs[132,133]

PEG is approved by the FDA for several medical applications and is a commonly used as synthetic hydrogel polymer for tissue engineering. PEG can be made photocrosslinkable by modification of each end of the polymer with either methacrylates or acrylates[134 135 136]. Hydrogels are formed when the modified PEG is mixed with an appropriate photoinitiator and crosslinked using UV radiation.[135,137]

Thermally induced reversible hydrogels have been synthesised from block copolymers of PEG and poly(lactic acid) (PLLA) [50]. PEG hydrogels have been synthesised from block copolymers containing hydrolytically labile poly(lactic acid) (PLA)[138] and hydrolysable sequences of oligopeptides.[135,136]

PEG based hyperbranched polymers have been synthesized by deactivation enhanced ATRP method [116, 117, 139] (1.2.4 for more detail). These water soluble polymers have demonstrated thermal responsive and photocrosslinkable properties and have great potential to be used as the injectable hydrogels for tissue engineering and drug delivery applications.

1.2.4 Hyperbranched Polymers

Hyperbranched polymers are synthetic tree-like macromolecules. They are a subset of branching macromolecules called dendritic polymers.[140, 141] These are polymers with a densely branched structure and a profusion of end groups. Dendritic polymers include dendrimers which have regular branched star-like topology and include the hyperbranched polymers which have imperfectly branched irregular structure.[140] Both dendrimer and hyperbranched molecules are composed of repeating units originating at a central core. Functionality at the core is the means by which covalent bonding facilitates the connections through to the periphery at the surface of the molecule.

Through bonding to the core, linear units of monomers or chains are attached and these arms are terminated with functional groups. Larger molecules can be synthesised by adding extra layers of linear units to the terminal functional. If units are attached in a strictly regular fashion, a dendrimer results. However, the absence of regular addition of monomers, gives rise to a hyperbranched polymer structure.

A major physical property of hyperbranched polymers is their different viscosity compared to their analogue linear molecules.[141] This is a consequence of their architecture. Hyperbranched macromolecules in solution are characterised by a maximum intrinsic viscosity depending on their molecular weight as conformation changes to a compact globular structure. The relation between maximum intrinsic viscosity and molecular weight is still not clear. It is observed that for linear polymers, there is a linear dependence of melt viscosity increases to a critical molar mass when the viscosity dramatically increases as a result of entanglement of polymer chains. However, this critical viscosity increase is not found in dendrimers or hyperbranched polymers indicating that little entanglement of the branched chains occurs.[142] X-ray and small-angle neutron scattering techniques reveal that dendrimers have highly spherical conformations; hyperbranched polymers however, have a more irregular globular structure.

Dendrimers and hyperbranched polymers have potential applications ranging from drug delivery through to nano-building blocks. Dendrimers can only be prepared through multi-

step syntheses, while hyperbranched polymers, with their less well-defined structure, are more easily synthesised.[141]

The addition of a small amount of a multifunctional vinyl monomer as a branching agent to a mix of monomers can lead to the production of a cross-linked network.[143,115, 144] It is possible to control this conventional free radical polymerisation by using a chain transfer agent (a thio), as in a RAFT polymerisation, thus delaying the gelation.[143] However, these methods cannot control the molecular weight and branching structure because of their non-living character. As discussed in section 1.2.2, in living radical polymerisations, chain transfer and termination are suppressed by the low propagating radical concentration.

It is possible that the preparation of highly branched polymer architectures can be achieved through a reversible activation/deactivation controlled polymerisation using multifunctional vinyl monomers.[117,115] Thus, in ATRP, the addition of Cu(II) species to the system slows down propagation by keeping the concentration of the polymer radical species and thus reducing the probability of chain termination. This approach was first demonstrated in the polymerisation of highly branched poly(divinylbenzene) (poly(DVB)) and poly(ethylene glycol dimethacrylate) (poly(EGDMA)) both with a surplus of functionalities such as vinyl and halogen groups.[139] This approach is designed to allow only a slow growth of each independent hyperbranched molecule that minimises cross-linking between each independent growing polymer chain.[115] Using a stategy of reversible activation (Figure 29) it is possible to control the competition between chain growth and the reversible chain termination. As the polymers grow, branching is introduced by means of the multifunctional vinyl monomer and crosslinking is prevented, leading to the formation of a hyperbranched polymer. Figure 30 outlines the basic concept:

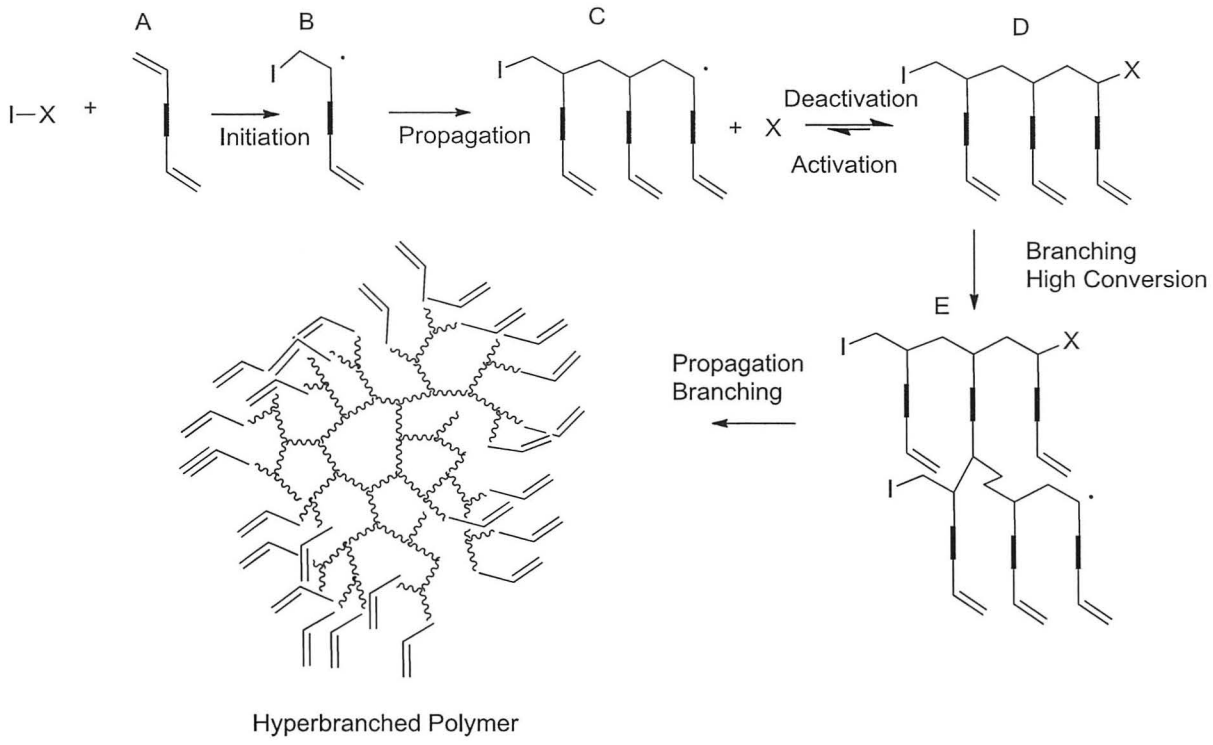


Figure 30: Strategy for Reversible Activation (or Deactivation) Controlled Hyperbranched Polymerisation Process[115]

(A) is the multifunctional vinyl monomer and is used with an initiator (I* + X) where I* is the radical which initiates the polymerisation of the vinyl monomer to produce a multi-vinyl polymer chain (C). The catalyst activates/deactivates the vinyl polymer species (C) to achieve equilibrium between active and dormant chains (D). The active species (C) can propagate either by linear chain growth with the addition of monomer residues to the chain, or can form a branched structure by attachment of multi-vinyl macro-monomer onto chain (C) and thereby form the growing chain. In either case equilibrium between the active and dormant species allows for a slow, controlled growth to minimise rapid cross-linking between the molecules.[115] The result is efficient suppression of crosslinking. Linear polymer chains, with a low degree of branching, are the probable outcome at low monomer conversion. However, highly branched structures are likely to be formed at higher monomer conversion due to the increased concentration of multi-vinyl macromonomers as the reaction proceeds. To avoid the production of insoluble gels, the overall conversion of monomer to polymer is reported to be about 60% of the monomer feed.[115]

In the ATRP synthesis of hyperbranched Poly(DVB), the rate of polymerisation of the divinyl monomer is first order for the concentration of monomer, initiator, and the Cu(I) complex, and inversely proportional for the Cu(II) concentration (Figure 31).[110 114]

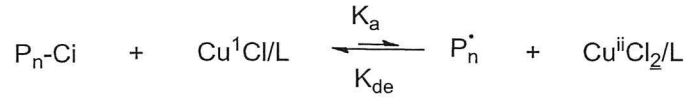


Figure 31: Illustration of kinetics of Activation and de-activation in ATRP

Therefore, to control the polymerisation rate, the feed ratio of Cu(I)/Cu(II) should be adjusted. Thus, increasing the concentration of Cu(II) relative to Cu(I) directs the equilibrium towards the deactivated state with the consequence that fewer monomer units add on to the active centre resulting in slower growth of the polymer chain.

For homo-polymerisation of divinylbenzene (DVB) in toluene, the absence of Cu(II) species leads to more rapid polymerisation due to the lack of deactivation and the system quickly results in insoluble gels. Under standard ATRP conditions, the polymerisation progresses are initially comparable to the deactivation enhanced method. At low conversions the conventional ATRP reaction results in hyperbranched polymers. As the reaction proceeds and yields above 20-25% are attained, gelling results and makes sampling impossible. The addition of Cu(II) increases the deactivation, reducing the polymerisation rate and increasing the yield of soluble hyperbranched polymer.[115]

PEG has been used as a multifunctional vinyl monomer (branching agent) in the form of ethylene glycol dimethacrylate and in an amount of up to 30% molar feed ratio obtaining a hyperbranched copolymer without causing macro-gelation.[145,116,117] In these reports, water soluble hyperbranched copolymers PEGMEMA-co-PPGMA-HB-EGDMA with thermal-responsive and photo-crosslinkable properties have been successfully achieved via controlled/living radical polymerisation techniques, including one-pot deactivation enhanced ATRP [116,145] (Figure 31) and *in-situ* RAFT (Figure 32).[146] These hyperbranched copolymers have the lower critical solution temperatures (LCST) ranging from 20 to 44 °C and high levels of branching (30-50 mol %) and vinyl functionality (5-25 mol %). The photo-crosslinking property of the materials has been investigated using UV curing system attached to the rheometer which is used to evaluate their mechanical properties such as the storage modulus which measures elasticity. Cytotoxicity assessments (Live/Dead staining and the

Alamar Blue assay) for these hyperbranched copolymers using mouse C2C12 myoblast cell line and mouse stem cells confirmed their cytocompatibility in vitro. Novel 'smart' dendritic multifunctional polymers in combination with a ECM biopolymer (Hyaluronic acid) as in-situ formed hydrogel systems via 'Click chemistry' was also developed.[91,147] The injectable and in situ crosslinking hybrid hydrogel system offers great promise as a new class of hybrid biomaterials for tissue engineering.

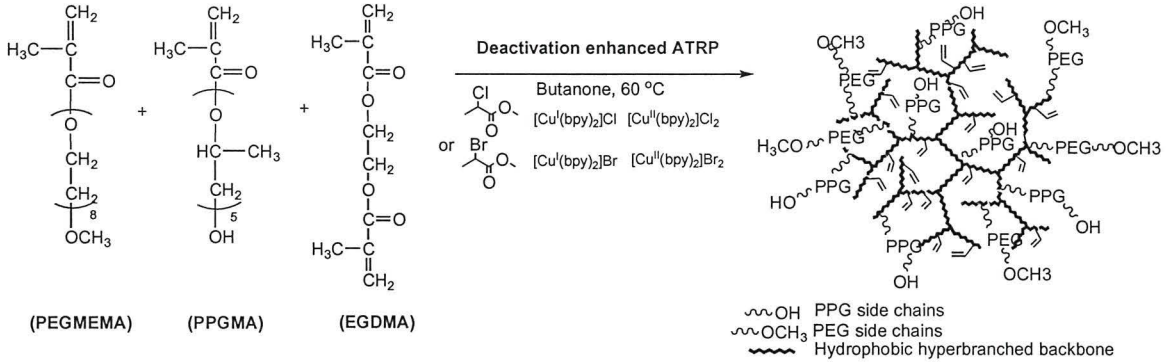


Figure 32: Synthesis of thermoresponsive and photocrosslinkable hyperbranched polymers by deactivation enhanced ATRP of monovinyl monomers (PEGMEMA and PPGMA) and multivinyl monomer (EGDMA)[116]

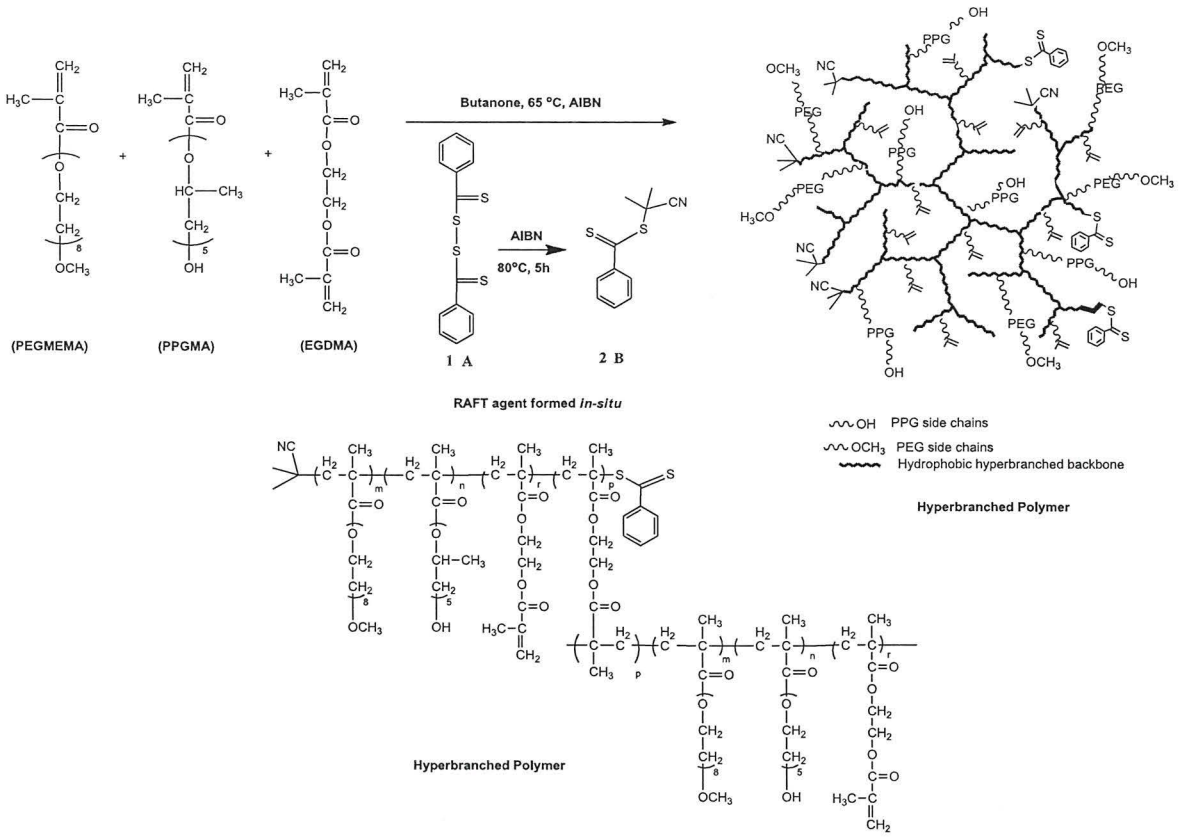


Figure 33: Synthesis of thermoresponsive and photocrosslinkable hyperbranched polymers by in-situ RAFT of monovinyl monomers (PEGMEMA and PPGMA) and multivinyl monomer (EGDMA)[146]

In the work of Dong et.al.[117] an situ a one-step deactivation enhanced ATRP (DE-ATRP) approach (Figure 34) was used to prepare hyperbranched polymers using the monomers poly(ethylene glycol) methyl ether methacrylate-co-2-(2-methoxyethoxy) ethyl methacrylate-co-ethylene glycol dimethacrylate (PEGMEMA-MEO_2MA-EGDMA). Adjusting the length of the PEG chain allows allowed to control hydrophilicity and the Lower Critical Solution Temperature LCST value to near body temperature. The PEG based structure was also considered to be nontoxic, non-immunogenic and bio-compatible.[148,149]

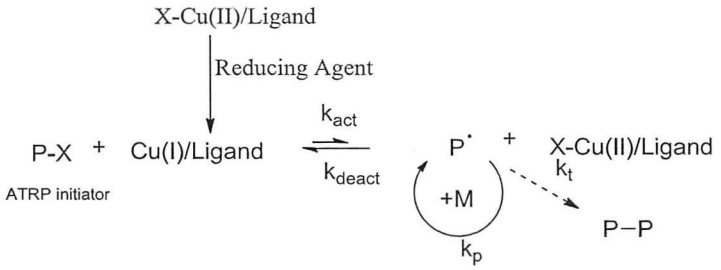


Figure 34: Possible Mechanism for the AGET process in ATRP

1.2.5 Biodegradable Polymers

Recent years have seen the development of a new generation of synthetic biodegradable polymers and derivatives of natural polymers specifically for biomedical use. Particularly the need for bio-degradable matrixes with the advent of biomedical advances in gene therapy, controlled drug delivery, regenerative medicine and tissue engineering has spurred this endeavor.[150]

A biomaterial is generally defined as a material used to interface with biological tissues to evaluate, treat, augment or replace any tissue, organ or function of the body. An essential property of the biomaterial is biocompatibility, in which the material is able to elicit the natural host response when used in a specific situation. The response of a tissue to an implant depends on many factors including the biological/ chemical properties of the material and the implant shape and structure.[151]

The material should not cause a toxic or inflammatory response when implanted. The degradation rate of the material should synchronise with the healing and regeneration of the tissue. The mechanical properties of the material should be compatible with the healing and regeneration requirements.

The degradation products should not be toxic and ideally be metabolized and cleared by the body. Polymer degradation, in which polymer linkages can be broken, can occur either in a passive manner by hydrolysis or actively by enzymatic action.[152] Enzymatic action generally applies only for naturally occurring polymers such as polysaccharides, proteins[153] and poly(β-hydroxy acids). For most biodegradable polymers, especially synthetic polymers,

hydrolysis is the most important type of degradation. There are several factors that influence the rate of hydrolysis including: the kind of chemical bond, the pH, composition of the copolymer and swelling character are important. Chemical and physical changes result from the degradation of biodegradable polymers such as crystallization of oligomers[154] and monomers[155] or pH changes. The rate of degradation is affected by the feedback effect of some of these factors. Parameters for monitoring degradation include molecular weight and loss of mechanical strength. These though are related, not necessarily kinetically the same, e.g. the complete degradation of poly(L-lactic acid) (PLA) can take more time than the loss of tensile strength.[156]

Poly(α-hydroxy esters) such as poly-(dl-lactic acid) (PLA) and poly(glycolic acid) (PGA) are biodegradable polymers, that degrade into naturally occurring substances.[157] Other materials that possess hydrolysable bonds along the polymer backbone have been developed and include poly(anhydrides)[158] and poly- (orthophosphoesters)[159] whose degradation is further controlled using unsaturated macromeric material for cross-linking the polymers. For biodegradable hydrogel applications, linear PEG-PLA diacrylates have be prepared and crosslinked by UV polymerisations.[18, 160] The biodegradability of the photocrosslinked hydrogels from linear PEG-PLA diacrylates can be tailored by the composition, thus crosslinking density of the hydrogels. To our knowledge, the hyperbranched PEG and PLA based biodegradable polymers have not been successfully prepared by facile Controlled Radical Polymerisation (CRP) approaches. Based on the previous success in the development of hyperbranched photocrosslinkable polymers via CRP of PEGMEA, PPGMA and EGDMA[116, 117, 145, 146], a further advance is to introduce biodegradability to this hyperbranched polymeric system. This biodegradable hyperbranched polymer can be used as an injectable material for tissue engineering applications.

One of the approaches to introduce biodegradability to the hydrogels is to use biodegradable branching agent in instead of EGDMA. Therefore, a series of P_{DL}LA-co-PEG-co-P_{DL}LA co-polymers were prepared by ROP and acrylation at each end gave the required functionality for their use as branching/.crosslinking agents The PEG-PLA Macromers synthesised were designed to be nontoxic and soluble in water. There is the requirement for it to be photo-polymerizable, when in direct contact with tissues, by irradiation with long-wave ultraviolet/visible light to form biodegradable hydrogels. The degradation profile of these

hydrogels should be fine-tuned by a suitable choice of components for the Macromer synthesis.

For the purpose of the project macromers were designed to have three structural domains, a water-soluble central polymer domain with hydrolytically degradable polymer extensions at each end and terminated with photo-polymerizable groups.

On crosslinking, by means of polymerizable end groups, degradation should then occur though scission of the chain at the site of the two hydrolytic extensions. Poly(ethylene glycol) (PEG) was chosen for the central domain as it was water soluble with a known biocompatibility and available hydroxyl functional groups which are useful for esterification.

PEG is expelled quite easily from the body and does not evoke an immune reaction. The hydrolytic domains were composed of poly(lactic acid) (PLA) which on degradation give products which are found as metabolites and can be naturally be processed by the body.[18] The mole fraction of the hydrophobic PLA domain relative to that of the hydrophilic central domain was designed to be small enough that the central water-soluble PEG domain was the dominant feature. The polymerisable terminals were vinyl groups as they photo-polymerize very easily with ultra-violet light when used with a suitable initiator.

The PEG due to its hydrophilic nature when used as a component of Macromers has the potential to be used to synthesise hydrogels. The rate of degradation and physical properties of these hydrogels can be customised by choosing appropriate components and the synthetic procedure for the macromer.

When crosslinked by means of the polymerisable end groups, it potentially should degrade when one chain is broken at each of the two extensions.

1.2.6 Stimuli Responsive Polymers

Stimuli-responsive polymers are responsive to small changes in physical or chemical conditions with relatively large changes in phase or other properties.[161] Many stimuli including pH and temperature can initiate this behaviour. Stimuli responsive polymers are also referred to as smart, intelligent and environmentally-sensitive polymers. Numerous

biomedical uses have been proposed for such polymers, particularly in aqueous conditions and they may be chemically or physically cross-linked and used as hydrogels. These responsive polymers can be used with a variety of biomolecules including proteins, nucleic acids and small molecules e.g., steroids, when these substances then effectively become entrapped within the collapsed matrix of the polymer.

Poly(N-isopropyl acrylamide), (PNIPAAm) is a stimuli responsive polymer that has been well studied and has been found to be thermally sensitive.[162] PNIPAAm is subject to a sharp phase change and precipitates from aqueous solution when warmed to a critical temperature, the Lower Critical Solution Temperature (LCST), which is approx. 32 °C in water. The reverse will occur when the temperature is reduced, with the precipitate re-dissolving, although the kinetics on reversal can vary depending the composition of the particular polymer system. The transition kinetics of hydrogels depends on their structure. On heating above the LCST, the hydrogels shrink rapidly compared to conventionally crosslinked PNIPAAm hydrogels.[163]

Stimuli responsive polymers may be sensitive to more than one stimulus.[164] The co-polymer of NIPAAm with acrylic acid is responsive to pH in addition to temperature. Biodegradable aliphatic polyesters, e.g. PLA, PLGA, and poly(caprolactone) (PCL), have been co-polymerised with hydrophilic (PEG) segments to produce thermo-sensitive, AB type, di-block or ABA tri-block copolymers.[165,166] These thermo-sensitive copolymers are free flowing sol phase at room temperature and gel at physiological temperature. They have, therefore, the potential to be used as injectable hydrogels in drug delivery and for cell therapy.

However, a problem arises when injecting the hydrogels into deep sites within the body as the sol–gel transition of these thermo-sensitive copolymers means that premature gelation can occur inside the micro catheter used to deliver the hydrogel.[167]

On raising the temperature of a solution of PNIPAAm in water, it is observed that the phase separation is endothermic and is thermodynamically spontaneous. As the Lower Critical Solution Temperature (LCST) occurs as the temperature is raised, with a resulting precipitation, this must mean that the free energy drop must be the result of an entropy gain.[168] If, on the other hand, a solution of polyacrylamide is heated no phase separation is observed. Thus, the phase separation of PNIPAAm must be accompanied by an entropy gain

due to the presence of the isopropyl groups of PNIPAAm. The entropy gain can only be the result of the release of water molecules bound to the isopropyl groups which are relatively hydrophobic and which themselves aggregate together at the LCST. The reversal back to the hydrated state, when the temperature is lowered, is found to be slower than the forward reaction causing the original collapse. This is because the individual groups within the hydrophobic domains have to be rehydrated, and this is opposed thermodynamically by the reduction in entropy of the water molecules. The overall rehydration on cooling, below the LCST, is a consequence of the small positive exothermic change on hydration accompanied with an entropy increase due to the expansion of the polymer chains.[169]

Thermally-responsive polymers can be synthesised by linking hydrophilic polymers (e.g. poly(ethylene glycol) with hydrophobic oligomers (e.g. poly[lactic-co-glycolic acid]). The thermal response given by such a tri-block polymer is thermodynamically similar to PNIPAAm, i e, the release of hydrophobically bonded water from the PLGA domains which then aggregate.

1.3 Hypothesis and Aims of my Ph.D. Project

1.3.1Hypothesis and vision:

The topology and composition of polymers are key factors to be considered when designing the structure of polymers with desired properties. For tissue engineering and drug delivery applications, biodegradable hydrogels hold great promise as either a depot system or a nanostructure material to support cell growth and deliver therapeutic drugs. The biodegradable, mechanical and drug release properties of the hydrogels are important for such applications and can be tailored by tuning the topology (linear and hyperbranched) and composition of precursor polymers. Biodegradable and crosslinkable polymers can be prepared by living/controlling polymerisations such as ROP and CRP using selected monomers under suitable conditions.

The water soluble hyperbranched copolymers PEGMEMA-co-PPGMA-HB-EGDMA, with thermal-responsive and photo-crosslinkable properties, have been successfully achieved via controlled/living radical polymerisation techniques, including one-pot deactivation enhanced atom transfer radical polymerisation (ATRP) and (in-situ) reversible addition-fragmentation chain transfer polymerisation (RAFT). The use of biodegradable macromers as the branching agent can introduce biodegradability into this hyperbranched polymer system. This new design will lead to a new injectable, biodegradable hyperbranched system for potential use in tissue engineering and drug delivery applications.

Aims and tasks:

1. Synthesis and characterisation of multifunctional macromers, based on PEG and PLA, using ROP, including investigations on finding the optimum reaction conditions.

2. Synthesis and Characterisation of hyperbranched polymers from macromers prepared in the previous task, using living/controlled radical polymerisations, including kinetics studies.

3. Preparation and property evaluations of hydrogels from linear macromers and hyperbranched polymers synthesized in task 1 and task 2. Tailor swelling, release and biodegradable properties of hydrogels by changing the compositions and topology of the precursor polymers.

Chapter 2: Experimental and Methodologies

2.1 Introduction

This chapter describes all the experimental procedures in the preparation and characterisation of precursor linear and hyperbranched macromers as well as hydrogels. The hydrogels resulted from crosslinking of linear and hyperbranched PEG-PLA copolymers in which the monomer (PEGMEMA) was used as a hydrophilic co-monomer. The branching agent consisted of a central PEG domain with terminal lactoyl residues designed to confer biodegradability to the structure. To give this co-polymer the potential for free radical polymerisation, terminal vinyl groups were added to yield resulting Macromer branching agent.

The first stage in the preparation of macromers involves the Ring Opening Polymerisation polyethylene glycol (PEG) and lactide (LA). The co-polymer was then further functionalized with vinyl groups at the terminal ends of the molecule. The Macromer was synthesized with a view to incorporate particular design features in the linear molecule. These include a central hydrophilic domain composed of PEG. The water soluble nature of the PEG content was conducive to it's the potential to form hydrogels. The hydrolytically labile extensions, composed of lactoyl residues (poly(α-hydroxy acid)) forming poly(lactic acid) (PLA) domains, are known to be biodegradable.

The end capping of the PEG-lactoyl co-polymers with vinyl groups provides the means whereby the resultant Macromers can be polymerised using UV radiation when accompanied with a suitable initiator.[82]

Co-polymerisation of the Macromer as the branching agent with the monomer PEGMEMA was accomplished using the techniques of Free Racal polymerisation (FRP), Atom Transfer Radical polymerisation (ATRP) and Reversible Addition Fragmentation chain Transfer (RAFT). The resulting hydrophilic hyperbranched co-polymers can be converted into hydrogels by physically or chemically crosslinking methods, such as by changing temperature, photo-polymerisation or Michael Addition reaction

2.2 Materials

The special chemicals used in this project are listed below. General laboratory chemicals and solvents used in this work were purchased from Aldrich and used as received. Poly(ethylene glycol) (PEG) (average molecular weight (M_n) = 1000) was obtained from Polysciences Inc., DL-lactide, (3,6-dimethyl-1,4-dioxane-2,5-dione, stannous 2-ethylhexanoate, triethylamine and acryoyl chloride, poly(ethylene glycol) methyl ether methylacrylate (PEGMEMA, M_n = 475) (containing inhibitors 100 ppm MEHQ and 200 ppm BHT) and ascorbic acid were obtained from Aldrich and used as received. The initiator used for the ATRP was Ethyl α-bromoisobutyrate (97%) was purchased from Fluka. The initiators used for the free radical polymerisations were 1, 1` azobis(cyanocyclohexane carbonitrile), 4,4` azobis (4-cyano valeric acid), 2,2`-azobis (2-methylpropio nitrate) and 2-hydrox-4`-(2-hydoxy-ethoxy)-2-methyl-propriophenone were also purchased from Aldrich. The free radical initiators were used as received. Copper(II) chloride ($CuCl_2$), 1,1,4,7,7,-pentamethyl diethylenetriamine (PMDTA, 99+%), bipyridine, lithium bromide and DMSO-d6 was purchased from Aldrich and used without further purification.

2.3 Synthesis and Polymerisation Procedures

2.3.1 Schlenk Line

The Schlenk line is useful for manipulating air sensitive compounds particularly during polymer synthesis. It consists of a dual manifold with several ports (Figure 34). One manifold is connected to a source of an inert gas such as nitrogen while the other is connected to a vacuum pump. The inert gas line is connected to an oil bubbler while solvent vapours and gaseous reaction products are prevented from entering the vacuum pump through the use of a liquid nitrogen cold trap. Special two-way stopcocks on taps allow vacuum or inert gas to be selected without the need for transferring the sample to a separate line. Schlenk lines are also

useful as they often have many ports and lines, and it is possible for several reactions to be run simultaneously.

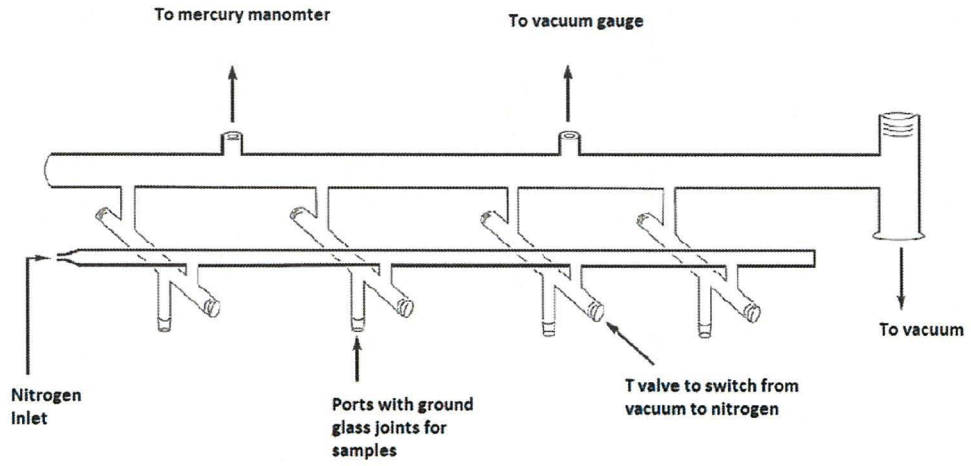


Figure 35: Schematic representation of a Schlenk line

The Schlenk line was used throughout this project for the preparation of PEG-co-PLA copolymers, acrylation of copolymers to synthesize macromers, homo- and co-polymerisations of macromers to prepare hyperbranched polymers via free radical polymerisations (including FRP, ATRP and RAFT) (See the detailed synthesis and polymerisation procedures in the following sections).

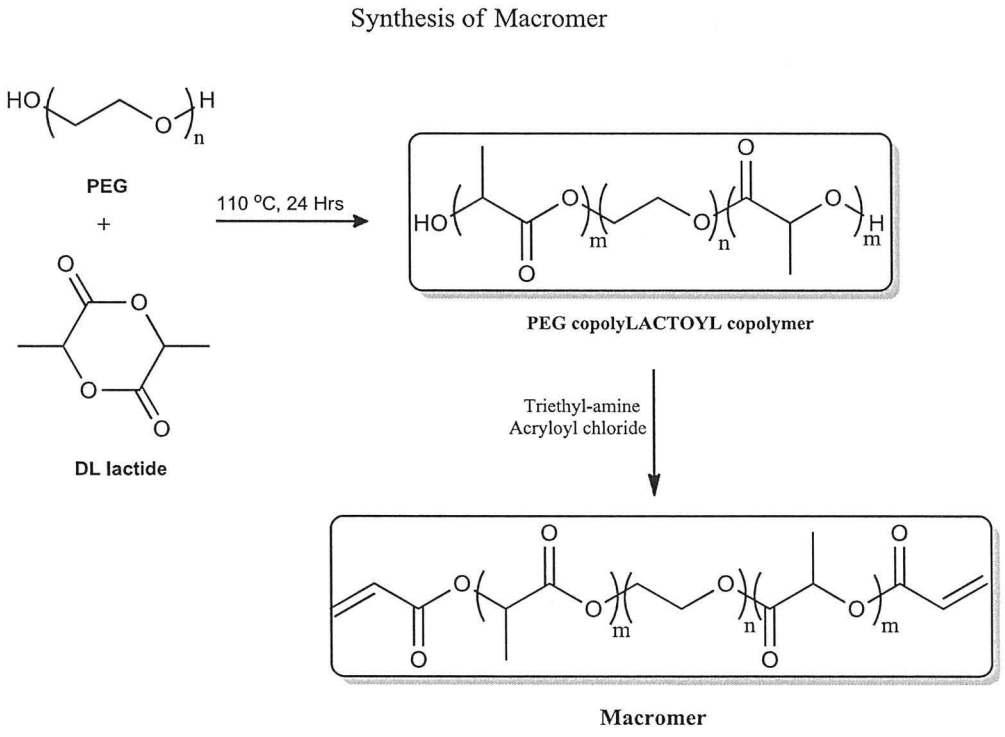
2.3.2 Synthesis and Purification of Poly(ethylene glycol)-co-poly(D,L-Lactide) Polymers ($P_{DL}LA$-co-PEG-co-$P_{DL}LA$)

The first stage in the preparation of the Macromer involves the co-polymerisation of poly(ethylene glycol) (PEG) and DL-lactide via ROP.

PEG and its moieties, as part of a co-polymer, are hydrophilic. The molecular weight of the PEG was chosen as 1,000 Daltons in this project, which is a value sufficiently large enough to allow it to confer net hydrophilic behaviour when co-polymerised with hydrophobic lactoyl terminal domains. The resultant co-polymer thus has the potential to form hydrogels, by virtue of its hydrophilic PEG domain, in the presence of water.

The compositions of the copolymer can be varied by changing the lactoyl content thus changing the physical and biodegradable properties of the co-polymer and of the Macromers formed from them.

The preparation of the Macromer was attempted in two steps. The first step in the synthesis was the co-polymerisation of PEG and D,L-lactide via ROP. This co-polymer was subsequently acrylated using acryloyl chloride in order to add vinyl functionality to the resulting Macromer. The scheme of the reaction is given below (Scheme 1).



Scheme 1: Synthesis of $P_{DL}LA$-co-PEG-co-$P_{DL}LA$ diacrylate Macromers

It is very important to eliminate any water present from the reaction mixture to prevent hydrolysis of the product.

Azeotropic distillation in benzene was initially attempted but was discontinued as generally best laboritory practice recommends that the carcinogenic benzene should be avoided.

Alternatively, it was dried by azeotropic distillation under vacuum with toluene. It was found, however, that a more convenient method involved drying the PEG by heating at 150°C under a stream of dry nitrogen gas for 3 hours while stirring at 400 rpm. DL-lactide was dried in a similar fashion but by means of adding it to the dried melt of the PEG. Upon addition to the reaction vessel, the lactide, along with the PEG, was heated at 150°C for 30 minutes, under a stream of nitrogen gas whilst stirring at 400rpm. The D,L-lactide has a melting point of 116-119°C.[170]

In another method to remove water present in the D,L-lactide recrystalisation from ethyl acetate was performed immediatelybefore use. Recrystallization is a purification technique in which both impurities and the compound are dissolved in an appropriate solvent and either the desired compound or impurities can be crystallised out of solution, leaving behind the other. The D,L-lactide was dissolved to give a saturated solution at 60°C in ethyl acetate or alternatively 70°C in toluene. On cooling the D,L-lactide precipitated out of solution and stored in a desiccator.

A typical procedure for the synthesis of the co-polymer is as follows. For the preparation of the Macromer 1KL3 a total of 30 g (30 mmol) of PEG was placed in a two necked flask and heated under a stream of nitrogen at 150°C for 3 hours with constant stirring to which was then added 12.97g (90mmol) of D,L-lactide and heating further continued for another 30 minutes under nitrogen. The stannous 2-ethylhexanoate (194μL (0.6mM)) was transferred to the reaction flask using an Eppendorf pipette. To prevent access of oxygen and moisture the reaction vessel was maintained under a counter flow of nitrogen and the temperature reduced to 130°C. The reaction was allowed to proceed for 22 hours. The reaction was terminated by turning off the heat and allowing to cool.

As the stannous 2-ethylhexanoate is a viscous liquid, a stock solution was prepared to enable accurate delivery of the correct amount into the reaction vessel. A mass of 0.02425 g was weighed into a volumetric flask and dissolved in 10mL of toluene of HPLC grade. Thus

a volume of 1ml. when withdrawn using an Eppendorf pippette will deliver a mass of 2.425mg. (0.6mM). This quantity is carefully added to the reaction flask which is maintained under a flow of nitrogen.

The resulting co-polymer was then dissolved in dichloromethane, precipitated in anhydrous ether, then dried in a vacuum oven at room temperature. The PEG with its α, ω-dihydroxy end groups acted as a ring opening reagent to initiate the polymerisation of the D,L-lactide.[171] Other co-polymers were prepared by varying the ratio of dl-lactide to PEG (see Table 2 in Chapter 3)

Extraction of the co-polymer from the reaction melt was attempted by completely dissolving the mixture in ice cold water (5-8°C), the solution was then heated to 80°C and this caused the co-polymer to precipitate out as a white solid leaving in solution the water soluble PEG and any unreacted monomers. The co-polymer was isolated by removing the supernatant liquid and drying under vacuum at room temperature.

Another method employed in co-polymer preparation involved a one pot two step procedure in which the co-polymer is synthesised as an intermediate in the first step and without extraction is used in the second step where it is acrylated.[172] The first part of the method follows the same procedure as given above for the preparation of the co-polymer.

2.3.3 Synthesis of Poly(ethylene glycol)-co-poly(D,L-Lactide) Diacrylate Macromers (P_{DL}LA-co-PEG-co-P_{DL}LA diacrylate)

The above PEG-co-polylactoyl co-polymers with their α, ω-dihydroxy end groups were end capped with acrylate moieties to introduce vinyl functionality into the co-polymer and thus form polymerisable macromers (Table 3 in Chapter 3). A typical synthesis is as follows.

A total of 12 g (8.38mmol) of co-polymer was dissolved in 100 mL of dichloromethane in a 250 ml two necked round bottomed reaction flask and cooled in an ice bath to 0°C. Triethylamine (2.8 mL, 20.11 mmol) was added to the mixture under constant stirring. Acryloyl chloride (3.39 mL, 41.90 mmol) of was then slowly added to minimise any local

evolution of heat during the exothermic reaction. The reaction was maintained at 0°C for several hours and then allowed to continue at room temperature for another 12 hours.The mixture was then filtered to remove the white precipitate of the salt triethylamine hydrochloride and the macromer precipitated out of solution pouring slowly into large excess of anhydrous diethyl ether. It was then redissolved in dichloromethane and re-precipitated out of a large excess of dry hexane and dried under vacuum at room temperature.

An alterative method involves the one pot two stage procedure outlined above in which the co-polymer is formed as an intermediate. Thus, without separating out the co-polymer from the reaction mixture the second step involves acrylation to yield the Macromer.

2.3.4 Synthesis of Biodegradable Hyperbranched Polymers from P_{DL}LA-co-PEG-co-P_{DL}LA Diacrylate Macromers

The Macromer synthesised above (see section 2.3.3.) has, by virtue of its vinyl functionality, the potential to polymerise with itself as in a homo-polymerisation or can be polymerised with other monomers where it functions as a branching agent.

2.3.4.1 Free Radical Polymerisation (FRP)

Hyperbranched polymers were prepared by homo-polymerisation of Macromer and co-polymerisation of Macromer and PEGMEMA via conventional free radical polymerisation (Table 5 in Chapter 3). Typically for the homo-polymerisation of 1KL10, 1.64 g (0.714 mmol) of macromer was dissolved in 2 mL of DMF to which was then added 0.004g of the initiator 1, 1' Azobis(cyanocyclohexane carbonitrile). The mixture was purged with nitrogen for 15 minutes to remove any dissolved oxygen which inhibit the reaction. The mixture is then heated to 65°C and GPC samples taken at suitable intervals. For the co-polymerisation of the Macromer and PEGMEMA, within the total monomer concentration the molar ratio of Macromer to PEGMEMA was varied). Samples were withdrawn at suitable intervals for GPC analysis.

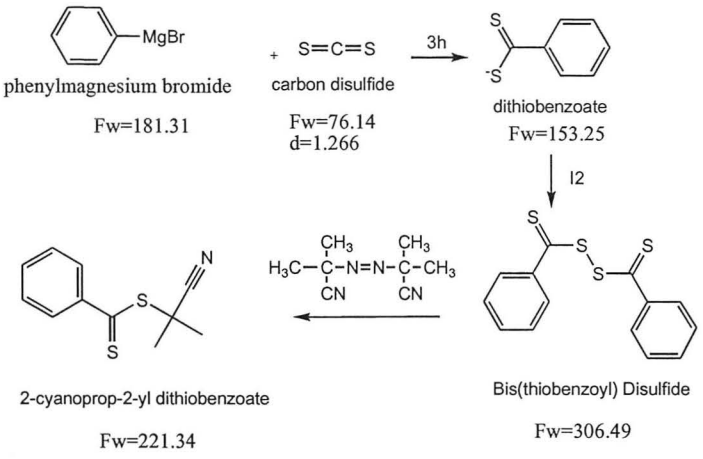
2.3.4.2 Atom Transfer Radical Polymerisation (ATRP) and Deactivation enhanced Atom Transfer Radical Polymerisation (de-ATRP)

Hyperbranched polymers were also prepared by homo-polymerisation of Macromer and co-polymerisation of Macromer / PEGMEMA via ATRP and de-ATRP (Table 6 and Table 7 in Chapter 3). For the homo-polymerisation of the Macromer a conventional ATRP polymerisation was undertaken. The Macromer 1KL10 (M_W = 2513) was dissolved in of butanone and the catalyst copper (I)) chloride was used with the initiator ethyl α-bromoisobutyrate. For the co-polymerisation of Macromer 1KL10 (M_W = 2513) and PEGMEMA by de-ATRP the method generally involves varying the molar ratio of Macromer to PEGMEMA. The quantity of initiator used is in the proportion of 1% of the total monomer concentration. Total monomers were taken to be 1 equivalent (equiv.). The molar ratio of Macromer to PEGMEMA is in the proportion of 1: 9. The initiator (I) ethyl α-bromoisobutyrate is used in a concentration of 1% i.e. [I]: [Monomer]=1 : 100. L-ascorbic acid (FW 176.12) and the catalyst copper (II) chloride were used in a molar ratio of 1 to five i.e. [LAA]: [Cu Cl_2] = 20%. The initiator (I), the catalyst copper[II] chloride and the ligand PMDTA were used in the molar ratios of 1 to 0.25 to 0.25 i.e. [I]:[C]:[L]=1:0.25:0.25. The reaction was maintained at a temperature of 50°C and samples were taken under a nitrogen atmosphere. The heterogeneous conditions result from the low solubility of Macromer in THF and butanone and the reaction was observed to be slow.

A stock solution of ascorbic acid was made in water. Initiator was also made up as a stock solution.

Typically the method required monomers to be dissolved in solvent in the ratio of monomers to solvent of 1 to 2. The Macromer 1KL7 (M_W = 2231) 4.48g (0.0632 mmol) was dissolved in 2.5mL of butanone in a two necked round bottom flask. Copper chloride (0.0025 equiv.) and ethyl α-bromoisobutyrate (0.01 equiv.) were added and oxygen removed by bubbling with nitrogen for 20 minutes. The reaction was commenced with the addition of 1 mL L-ascorbic acid and then stirred at 400 rpm at 50°C. GPC samples were periodically withdrawn. The reaction was terminated upon opening the flask with the exposure of oxygen to the catalyst in the mixture.

2.3.4.3 Reversible Addition Fragmentation Chain Transfer (RAFT)



Scheme 2: Scheme for the synthesis of RAFT agent

2-cyanoprop-2-yl dithiobenzoate was used as the RAFT agent in the copolymerisation of Macromer and PEGMEMA for the preparation of hyperbranched polymers (Table 8 in Chapter 3). This RAFT agent was synthesised in house according to published method (Scheme 2).[173]

Briefly, 100 ml (100mmol) phenylmagnesium bromide were dissolved in THF contained in a 250 ml flask, to make a 1M strength solution to which was then added carbon disulfide (8.36 ml, 139 mmol) in a drop wise manner. The solution was stirred for further 3 hours under an inert nitrogen atmosphere and the solvent then removed under vacuum.

The resulting deep red viscous liquid was dissolved in a diluted potassium carbonate solution (ca. 8g in 200 ml), filtered and washed with ethyl ether (2 X100 ml), and then poured into a round-bottom flask equipped with a magnetic stirrer. The aqueous bottom phase was the collected.

An aqueous solution of iodine 1 N (30 ml, 10 mmol) was then added drop wise, the solution started to change color from dark red to pink as the disulfide precipitated. To eliminate the excess iodine a few crystals of sodium sulfite were added, and the disulfide was

extracted with methylene chloride and dried using sodium sulfate, filtered and evaporated, and then left to dry in the vacuum oven. The crude product was used for the subsequent reaction without any further purification. A crystalline red solid was obtained (yield 90%) which was the Bis(thiobenzoyl) Disulfide.

A solution of Bis(thiobenzoyl) Disulfide (0.429 g, 1.4 mmol) and the azo compound (AIBN) (0.266 g, 1.62 mmol) in ethyl acetate (120 mL) were degassed in a 250 ml round-bottom flask equipped with condenser and magnetic stirring and then refluxed in a nitrogen atmosphere for 16 hours with monitoring by TLC. The solvent was removed. Flash chromatography was used to purify the product using an eluent of hexane/ethyl ether 9:1.

For the RAFT co-polymerisation of Macromer 1KL11 (M_W = 2639) and PEGMEMA (FW 475), typically the molar ratio of Macromer to PEGMEMA is in the ratio of 1 to 9. The initiator (I) used is 1, 1' Azobis (cyanocyclohexane carbonitrile) and is used in the molar ratio of 1% i.e. [I]: [Monomer] =1: 100. The RAFT agent, 2-cyanoprop-2-yl dithiobenzoate, was in the molar ratio of 1%. The temperature of the reaction was maintained at 50°C and samples were taken under a nitrogen atmosphere. The heterogeneous reaction conditions were apparent. The method required monomers to be dissolved in solvent i.e. butanone in the ratio of monomers: solvent = 1:2. Total monomers comprise 1 equivalent (equiv.).

Typically, the Macromer 1KL11 (M_W = 2639) 5g (1.894 mmol) was dissolved in 2.5mL of THF in a two necked round bottom flask, 46.18 mg of 1, 1' Azobis(cyanocyclohexane carbonitrile) and 41.83 mg of RAFT agent were added and oxygen removed by bubbling with nitrogen for 20 minutes. The reaction commenced and then stirred at 400 rpm at 50°C. GPC samples were periodically withdrawn. The reaction was terminated upon opening the flask with the exposure of oxygen to the catalyst in the mixture.

2.3.5 Preparation of Hydrogels

Biodegradable hydrogels were prepared using linear $P_{DL}LA$-co-PEG-co-$P_{DL}LA$ diacrylate macromer in the presence and absence of PEGMEMA via photocrosslinking and Michael addition crosslinking methods.

2.3.5.1 Photocrosslinking Method

A 1% (w/v) stock solution of the photoinitiator Irgacure 2959 (2-Hydrox-4'-(2-hydoxy-ethoxy)-2-methyl-propriophenone) was prepared as follows. A round bottomed flask was taken to which was added 0.1004 g of the photo-initiator and then thoroughly dissolved in 10 mL of de-ionised water. Nitrogen was bubbled into the solution to remove any dissolved oxygen. The solution was sealed to remain airtight during storage until required. Macromer 1KL3 was used on its own by dissolving in the Irgacure stock solution to yield a 54% and a 30% solution. 1mL of each was placed in a small glass vial to a depth of 1 mm and exposed overnight to UV light of strength 2.3mW/cm^2 at room temperature. Photo-polymerisation of the macromer 1KL11 and PEGMEMA was undertaken using Irgacure 2959 as the initiator. The ratio of Macromer to the monomer PEGMEMA was varied as shown in Table 10 in Chapter 3.

2.3.5.2 Michael Addition Method

A chemically cross-linked hydrogel system was developed from the PEG lactoyl based Macromer via Michael-addition reaction between multi acrylate functionality, and also that of the vinyl group containing PEGMEMA, with thiol functional cross-linker of pentaerythritol tetrakis (QT) (3-mercaptopropionate) (Table 12 in Chapter 3). Hydrogels can also be prepared from hyperbranched polymers synthesized in section 2.3.4 because they afford multiacrylate functional groups. A typical synthesis involves the addition of 0.5 g (0.019 mmol) of Macromer (M_W = 2693.43) to vial containing 500 μL of phosphate buffered de-

ionised water (pH 7.4). To this was added 171.6 μL of QT in a stoichiometric molar ratio of vinyl group to thiol of 1 : 1. The base Triethylamine (TEA) 42.13 μL was then added and the vial and contents incubated at 37°C for 2 hours. Upon completion of the reaction a transparent gel was seen to have formed. Further studies were undertaken to achieve co-polymerisation with the monomer PEGMEMA, adjusting the ratio of Macromer to PEGMEMA.

2.3.6 Dialysis

Dialysis was used for the purification of the polymerisation products. This technique uses semi-permeable membrane tubing made from cellulose. The tubing contains the reaction mixture which is immersed in the solvent of choice. For the water soluble mixtures used in the following experiments water is used to immerse the tubing. The membrane allows the passage of small molecules from a high concentration to low concentration across the membrane as a result of diffusion. In water, osmosis occurs causing water to pass through a semi-permeable layer to reach equilibrium. The dialysis tubing will only allow molecules to pass through a semi-permeable membrane if the molecule is small enough to fit through the membrane or a membrane's pore. Larger molecules are unable to pass though the pores and are retained in the tubing.

The polymer reaction mixture is placed into the semi-permeable dialysis tubing and is sealed with plastic clips and then placed in a large container of water. The water will cross the semi-permeable membrane into a hypertonic solution (a solution that has a higher solute concentration than the solution it is referenced to) in an attempt to reach equilibrium (Figure 34).

The tubing used was a Spectra/Por® Regenerated cellulose membrane. It does not carry a fixed charge and does not absorb most solutes. It is hydrophilic, being capable of spontaneous wetting with very good wet strength. It is extremely chemically resistant and suitable for aqueous and organic media. Spectra/Por® grade 1 was selected as it has a Molecular Weight Cut Off point (MWCO) of 3500 Daltons.

The general dialysis procedure is as follows. The tubing was cut to allow for extra tubing length (about 10% of total sample volume) for a small head space. This will ensure that the tube will float and not be caught up in the rotating stirrer bar. The tubing and the stirrer bar are placed in a large beaker which was previously filled with water.

Typically, dialysis was run overnight. During dialysis the entire dialysate volume is changed for fresh dialysate solution. The solution is changed after the first hour, then 4, 10 and 24 hours later.

Finally, polymer materials were freeze-dried and fully characterised.

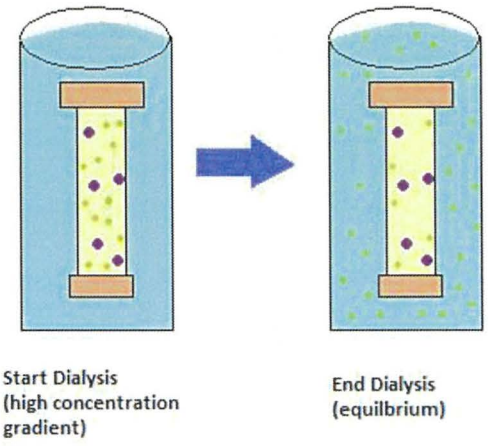


Figure 36: Illustration of the Principle of Dialysis

2.4 Methods of Characterisation and Analysis

2.4.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy (NMR) is the pre-eminent technique for determining structure including that of polymers.[174] Many isotopes possess a characteristic nuclear spin (I), for ^{1}H and ^{13}C this is equal to 1/2. Interaction with an external magnetic field (B_0), gives rise to two spin states, +1/2 and -1/2. The lower energy +1/2 state is aligned with the field, but the higher energy -1/2 spin state is aligned against the external field with

the energy difference being very small and dependent on the strength of the external field. Strong magnetic fields are therefore necessary for NMR spectroscopy. Commonly, this small energy difference is usually quoted as a frequency in units of MHz (10^6 Hz), and range from 20 to 900 MHz or higher. Irradiation of a sample with radio waves of specified frequency delivers energy corresponding exactly to the energy separation of the two spin states and will cause those nuclei in the +1/2 state to flip to the higher -1/2 spin state. On relaxation the higher energy state degenerates to the lower state and energy is emitted as electromagnetic radiation and it is this which is detected and analysed.

Since all ^{1}H and ^{13}C atoms have the same magnetic moment they might be expected to give resonance signals at the same frequency values. However, in covalent compounds and ions, as the electron(s) surrounding these isotopes are charged particles, in the external magnetic field they will generate a secondary field in opposition to the applied field and this secondary field shields the nucleus from the external field which therefore must be increased in order to achieve resonance.

The NMR resonance signal is dependent on both the strength of the applied magnetic field and the frequency of the radio waves. In any spectrum, it is necessary to assign the location of an NMR signal relative to a reference signal from a standard compound. Tetramethylsilane($(CH_3)_4Si$, TMS) is usually used as it is unreactive and easily removed from the sample.

Since the separation of NMR signals is dependent on the magnetic field and to correct their field dependent frequency differences, the difference between the resonance signal from the sample and that for the reference is divided by the spectrometer frequency. The resulting number is multiplied by a million giving the Chemical Shift (δ) and is measured in parts per million (ppm.)

All NMR spectra in this project including proton and ^{13}C NMR were recorded on a Brucker 500 MHz NMR and was analysed with MestReNova- NovaLite software which was transferred from Bruker WinNMR software. Samples were first dissolved in a deuterated solvent, for example Chloroform ($CDCl_3$) or DMSO. A concentration of 50 mg/mL was used for proton NMR and ^{13}C NMR. To facilitate accurate computation of integration values, all samples were scanned 304 times, a figure found to produce a smooth baseline. This ensured a

more even base line i.e. higher signal to noise ratio. Tetramethylsilane (TMS) was used as an internal standard.

2.4.2 Differential Scanning Calorimetry (DSC)/Thermo Gravimetric Analysis (TGA)

Differential Scanning Calorimetry (DSC) and Thermo Gravimetric Analysis (TGA) are techniques used to study thermal transitions.[174] As a polymer is heated/cooled in a specified temperature range it may undergo morphological and chemical changes with the evolution/absorption of heat. The heat flow in the sample will be determined by the specific heat capacity and the temperature applied.

In DSC/TGA both a sample and a reference substance are placed in separate pans made of inert material and heated in a stream of nitrogen. Sample sizes of approximately 8 mg were used.

In TGA, the thermal stability of the polymer sample is primarily determined. The sample is placed in a sensitive thermo-balance in an inert atmosphere inside a furnace and the temperature increased. This is called non-isothermal TGA. The thermogram is a record of weight against temperature. Weight loss may occur as a result of evaporation of any residual solvent or moisture and with higher temperature decomposition of the polymer may occur. During a transition e.g. a glass transition (Tg), the temperature of the sample will lag behind that of the reference if the transition is endothermic or will increase compared to the reference, if the transition is exothermic.

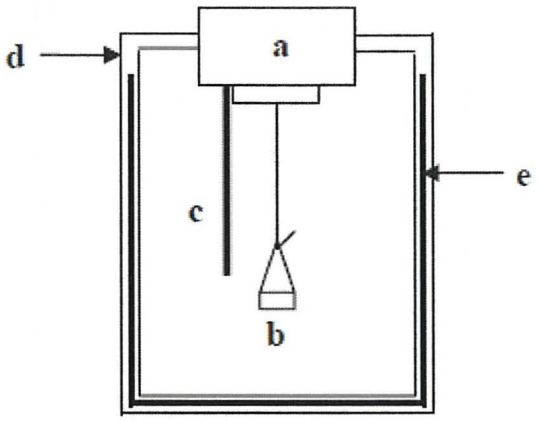


Figure 37: Schematic drawing of TGA (a) balance ; (b) sample pan and pan holder ; (c) thermocouple ; (d) furnace ; (e) heating element[175]

With DSC, the sample and the reference are heated by individual heaters and heat is supplied to restore the same temperature for both. The reference pan is normally empty and is positioned alongside the sample pan in the cell head. The instrument is flushed with dry nitrogen to remove any atmospheric moisture which may otherwise condense and contaminate the sample. The sample and reference are the cooled to well below any expected transitions.

As the temperature is increased, any thermal transitions become apparent. In an exothermic transition e.g. crystallisation, heat is evolved and is absorbed by the sample, which increases in temperature compared to the reference pan, and is recorded as a peak in the thermogram. In DSC, the change in the energy input is measured when a temperature change is detected. The area under the peak is proportional to the energy input and allows for quantitative measurement of the process provided the instrument has been calibrated. Calibration involves using a standard with a known enthalpy of melting and a sharply defined melting point.

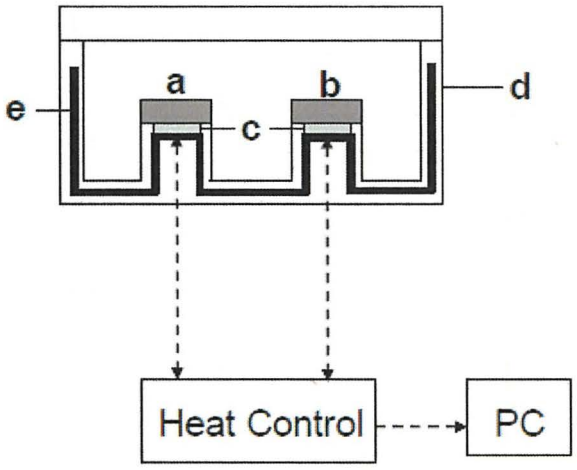


Figure 38: Principle of the Differential Scanning Calorimetry technique. (a) sample pan; (b) reference pan; (c) thermocouples; (d) cell head; (e) heating elements [175]

The DSC thermograms were performed on a Universal V3.7A TA instrument the heating rate 5°C per minute. The temperature range used is 20-200 °C.

2.4.3 Gel Permeation Chromatography/ Size Exclusion Chromatography (GPC/SEC)

The characterisation of polymers includes a determination of the molecular weights and their distribution within the substance as these are factors affecting the properties of a given polymer.[95, 174] The molecular weight of a polymer cannot be stated as one single value but will have a distribution which will depend on the way the polymer is synthesised. The value is usually reported as average molecular weight and this can be calculated in a number of ways but most commonly as Number Average Molecular Weight (Mn). For any synthesis, the polymer molecules come in different sizes, for instance, the chain lengths for linear polymers, so the average molecular weight depends on the statistical method used for averaging. The Number Average Molecular Weight (Mn) is the ordinary arithmetic average or mean of the molecular weights of individual molecules. It is determined by measuring the

molecular weight of *n* polymer molecules, summing the weights, and dividing by *n*. Mn is a useful parameter when we wish to consider a property which is sensitive to the number (concentration) of molecules present i.e. not by the size of any particle. Mn is, for instance, relevant to colligative properties of solutions such as boiling point elevation, freezing point depression, and osmotic pressure.[94] Mn is not a continuous function, as only discrete values of the monomer molecular weight (M) are measured, M_n being a multiple of the monomer molecular weight, Mo. However, in condensation reactions, in which by-product small molecules such as water are lost during the reaction, M_o, is the monomer molecular weight after loss of the by-product of condensation.

Each value of M in the range of numbers with discrete values is labelled Mi. If Ni is the number of polymers with molecular weight Mi then the total weight of all polymers is

$$Total\ Weight = \sum_{i=1}^{\infty} NiMi \qquad \text{(Eq.5)}$$

and the total number of polymer molecules is

$$Total\ Number = \sum_{i=1}^{\infty} Ni \qquad \text{(Eq.6)}$$

the number average molecular weight is

$$Mn = \frac{\sum_{i=1}^{\infty} NiMi}{\sum_{i=1}^{\infty} Ni} \qquad \text{(Eq.7)}$$

For some properties such as light scattering, rather than the number of polymer molecules but the size or weight of each polymer molecule is important, a more relevant measure is the weight average molecular weight. Thus the weight average molecular weight is given by

$$Mw = \frac{\sum_{i=1}^{\infty} NiM_i^2}{\sum_{i=1}^{\infty} NiMi} \qquad \text{(Eq.8)}$$

The Polydispersity Index (PDI) is the ratio of weight average molecular weight and number average molecular weight and measures the distribution of molecular weights in the sample.

$$PDI = \frac{Mw}{Mn} \qquad \text{(Eq.9)}$$

As the molecular weight distribution range becomes narrower the PDI tends to unity. Thus, for a PDI of 1 all the polymer molecule chains are essentially the same length. A PDI in the range of 1~1.5 denotes a controlled/living polymerisation.[101] PDI values greater than 1.5 are to be found in less controlled polymerisations as free radical polymerisation.

The most common method for measuring molecular weight of polymers is by Gel Permeation Chromatography (GPC) also known as Size Exclusion Chromatography (SEC) and a schematic diagram is given to illustrate the component parts (Figure 38).

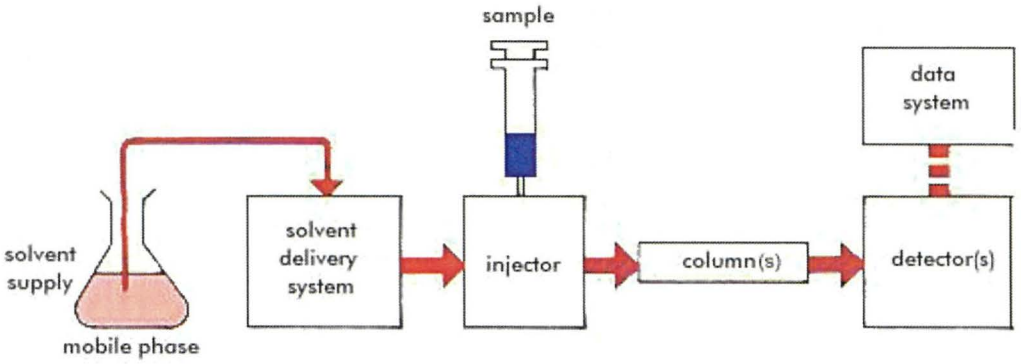


Figure 39: Schematic diagram of GPC components.

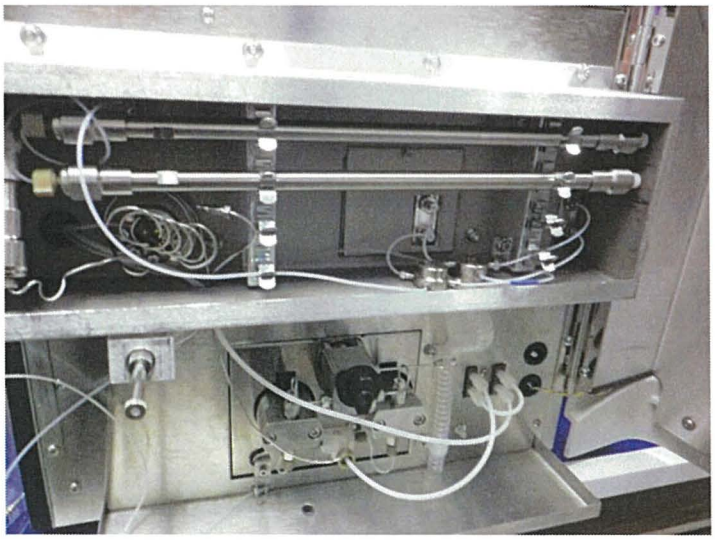


Figure 40: GPC-50 instrument showing separation columns within the oven.

GPC is a method based on separation by means of columns filled with porous beads in which there should be no interaction between the sample and the column packing. This method can be compared with conventional HPLC in which interactions such as adsorption, partition, etc. do occur.

The polymer under investigation is dissolved in the same solvent as used as eluent. The mode of separation is based on the hydrodynamic volume of the material being analysed in solution. Separation is achieved by using porous mechanically stable highly cross linked gels as beads packed in a column which by their sieving action can separate the sample into fractions which reflect their molecular volume. The pores in the packing retain the smaller molecules which will fit into most of the pores and be retained longer but the larger particles are not hindered as they will not fit into the smaller pores and pass straight through (Figure 40 illustrates this principle).

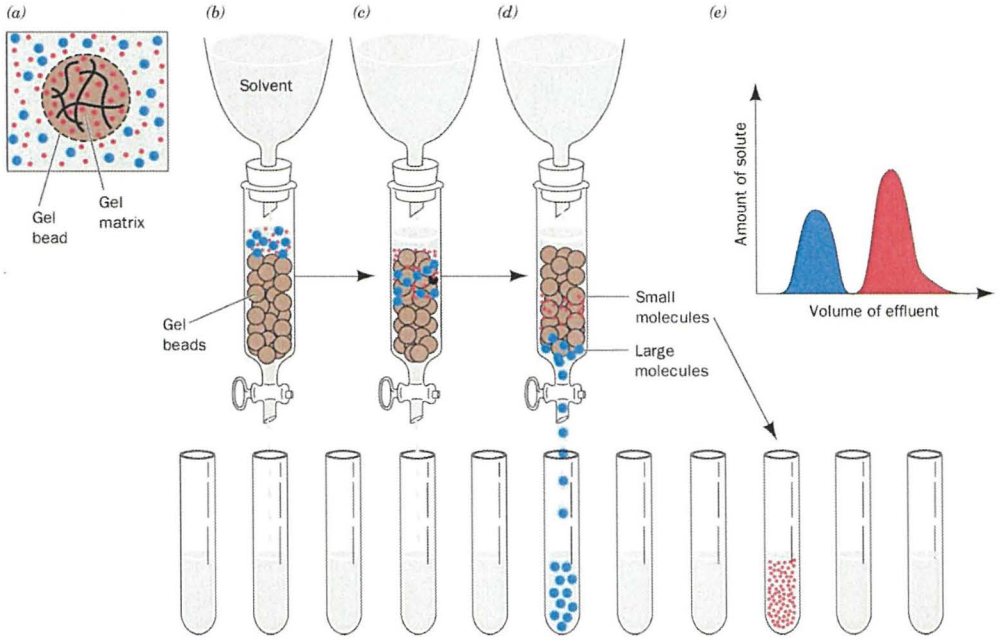


Figure 41: illustration of principle of separation by size exclusion (Courtesy of Dr Remzi Becer University of Warwick/Agilant technologies)

The concentration required for sample preparation depends on the molecular weight of the polymer but a concentration of 1mg/ml (w/v) for a polymer of molecular weight of

approximately 100,000 is typical. The dissolved sample is then introduced via an injection mechanism into the set of columns which are combined in series which act as a molecular filtration system.

Individual pore size columns are traditionally used to accommodate the range of molecular weights being sampled but are now replaced with Polymer Lab's Mixed gel columns which are packed with a mixture of individual pore size materials to allow for a broad range of molecular weights. A range of MIXED gel GPC columns are available commercially from Agilant Technologies.

Chromatograms were performed on a PL-GPC 50 Plus Integrated GPC/SEC System from Agilent Technologies.

The detection of solute components within the eluent can be obtained by various means including UV-VIS absorption, viscosity measurement and light scattering. The most widely used method is based on refractive index. This project used analysis based on detection by means of a differential refractometer.[176] This is a concentration sensitive detector measuring the difference in refractive index between the solvent and that of the sample. The refractive indices (n_D) for the solvents used in this project are: chloroform-1.446, THF-1.407, DMF-1.431, acetone-1.359 and water-1.333. The refractive indices for calibration standards used are: for PMMA-1.4914 and for polystyrene-1.6.

The project used PEG (M_w. 1000) as a component of the co-polymer making up a large fraction of the material and thus conferring a considerable hydrophilic character to the co-polymer. The refractive index (RI) of the PEG is 1.4580 which contrasts with THF of 1.4072, resulting in the greatest RI difference and, therefore, provides greater detection of the PEG based material.

The columns used were two PLgel MIXED-C columns with dimensions 300 × 7.5 mm packed with particle size 5μm and linear calibration range M_p 690 to 1.9×10^6 g/mol approx. and a PLgel guard column 50×7.5 mm with particles packing 5μm. The solvent used was THF at a temperature of 40°C and the rate of flow was 1.0 ml/minute.

GPC calculates the molecular weight of an unknown sample with reference to a set of polymer standards with narrow molecular weights in the form of a calibration curve (Figure

41) which relates the known molecular weight with its retention time (Table 1) under the particular conditions that apply for the given instrument.

Table 1: Typical calibration data for the GPC measurement (PMMA standards in THF solvent at 40°C at rate of 1.0 ml/minute)

Peaks	Retention Time (minutes)	Mp (Daltons)	Log Mp	Percentage error
1	11.06	1944000	6.29	1.46
2	11.80	790000	5.90	-11.42
3	12.37	467400	5.67	-3.26
4	12.92	271400	5.43	0.75
5	13.53	144000	5.16	2.73
6	14.08	79250	4.90	1.36
7	14.87	35300	4.55	3.50
8	15.83	13300	4.12	8.10
9	16.43	7100	3.85	8.89
10	17.62	1960	3.29	5.90
11	18.17	1020	3.01	-0.92
12	18.37	690	2.84	-20.69

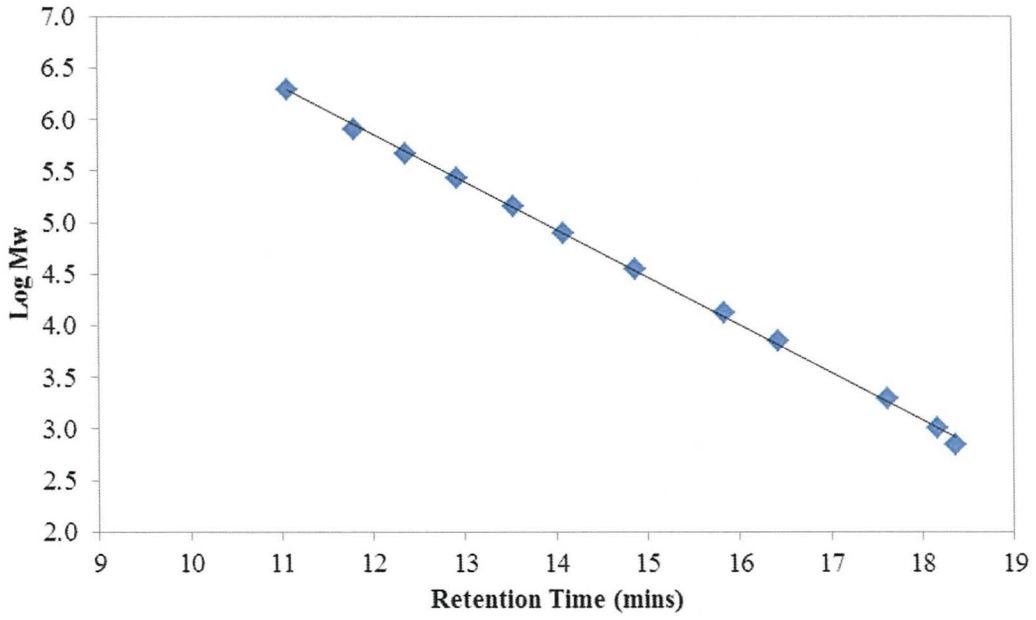


Figure 42: A typical calibration curve for GPC measurement.

2.4.4 Fourier Transform Infra-Red Spectroscopy (FTIR)

The Infra-Red (IR) region of the electromagnetic spectrum covers the range from 700 nm to 1 mm. In IR spectroscopy, wavelength is measured in wavenumbers which have the units cm^{-1}, i.e. the wavenumber is the reciprocal of the wavelength in centimetres. IR radiation, unlike the higher energy UV, cannot induce electronic transitions, and on absorption can only cause transitions in compounds with small energy differences between the possible vibrational and rotational states. For a molecule to be IR active and absorb the radiation, upon vibration or rotation a change in the dipole moment of the molecule must result. The oscillating electrical and magnetic field of the radiation interacts with the fluctuating dipole moment of the molecule. If the IR frequency resonates with the vibrational frequency of the molecule then the radiation will be absorbed resulting in a change in the amplitude of molecular vibration.

Rotational transitions are quantized and in gases give rise to line spectra upon IR absorption. These lines broaden in solids and liquids giving rise to a continuum as a result of molecular collisions and are not very useful.

The positions of atoms in a molecule are not rigid and are subject to a number of different kinds of vibrations mainly stretching and bending. Stretching is the change in the inter-atomic distance along bond axis. Bending is the change in angle between two bonds of which there are four kinds i.e. Rocking, Scissoring, Wagging and Twisting.

Thus, for a two-atom system, at a first approximation, can be considered to obey Hook's law and so the frequency of vibration will be proportional to the strength of the bond and inversely proportional to the masses of the atoms. In consequence, different bonds of the different groups within a molecule will vibrate at different frequencies and affords a means of identifying many organic function groups by their IR absorption properties.

FTIR is an abbreviation for Fourier Transform Infra-Red, a modern version of dispersive infrared spectroscopy. In infrared spectroscopy, infra-red(IR) radiation is passed through a sample which absorbs some of it whilst the remainder is passed through (transmitted). The detector records the resulting spectrum of the molecular absorption and transmission, creating a unique molecular fingerprint of the sample.

Infrared spectroscopy is useful as it can identify unknown materials and it can determine the quality or consistency of a sample and the amount of components in a mixture.

The basic layout of the instrument is given in the schematic below (Figure 42)

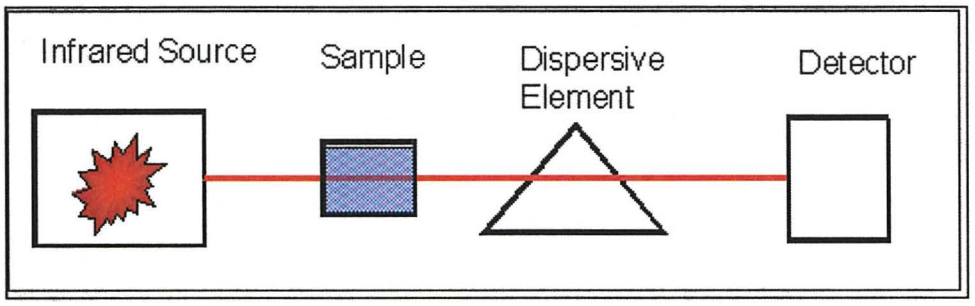


Figure 43: Schematic illustration of principle of IR

Transform Infra-Red Spectroscopy (FTIR) spectra were recorded on a PerkinElmer spectrum 100 using a scan range of 450 to 4000 cm^{-1}.

Samples of the various substances (co-polymers, Macromers and hyperbranched polymers) were prepared in chloroform by taking a small measure (20 mg approximately) in a small glass vial and adding a small quantity of chloroform (1 mL approximately).The solution was applied to a sodium chloride disc with a dropper and evenly spread out by covering with another disc and then applying pressure thus squeezing the discs together. The solvent readily evaporates and any IR signals due to -C-H absorptions due to the residual solvent present are subtracted as they are not functional groups of interest in this experiment. The sample was scanned 3 times.

For preparing samples of solids, KBr disks have the advantage of no overlapping signals from the matrix (KBr) although the disadvantage is that it may take practice to prepare good quality disks. The concentration of the sample in the KBr matrix should be in the range of 0.2% - 1%. The disk is much thicker than a liquid film, hence requiring a lower concentration of the sample (Beer's Law). If the concentration is too high this can cause difficulties in

obtaining clear pellets which can cause the IR beam to be absorbed completely, or scattered which results in very noisy spectra.

To prepare a sample for FTIR a small quantity (less than 300 mg) of dry IR-grade KBr was placed in an agate mortar. The amount of KBr was sufficient to cover an area of about 20 square millimeters to a depth of 1 millimeter. The KBr was ground in the mortar until there was no evidence of crystallinity. A normal lab mortar will introduce contamination into the pellet; therefore an agate mortar was used. A very small quantity of sample (about 1 to 2 mg) and representing approximately 1 to 2% sample was added to the KBr .The mixture was ground until it was uniformly distributed throughout the KBr. Ensure the press body and anvils are thoroughly dry... Care should be taken not to scratch the polished anvil surfaces. The sample/KBr pellet is placed between the anvils of the dye and pressure applied by means of wrenches to the bolt style anvils simultaneously while compressed air is removed under vacuum.

Apply pressure for about 1 minute, then remove bolts to eject disc which is then placed in a holder in the path of the IR beam. The sample is also scanned 3 times.

2.4.5 High Pressure Liquid Chromatography (HPLC)

HPLC is, in principle, a form of column chromatography using high pressure of up to 400 atmospheres rather than gravity to force eluent through the column. The packing material of the column consists of very much smaller particles than found in column chromatography giving a greater surface area on which interactions between stationary and mobile phases can occur. The components of the mixture can therefore be better separated.

There are two major kinds of HPLC.

In Normal-phase HPLC, which is not commonly used, the column is packed with very small silica particles and a non-polar solvent e.g. hexane, is used. A typical column has dimensions giving an internal diameter of 4.6 mm (sometimes even less) and a linear length of 150 - 250 mm. Polar components in the mixture, on passing through the column, will be

attracted for longer time on the polar silica than the non-polar compounds and will, therefore, pass more slowly through the column.

In Reversed-phase HPLC, the most commonly used form of HPLC, the column size is the same, but the packing is of a silica derivative which is non-polar made by attaching hydrocarbon chains to the silica surface. A polar eluent e.g. a mixture of water and methanol is used. Here, the polar solvent and the polar molecules in the mixture will attract each other strongly. On the other hand, the attraction between the non-polar stationary phase and the polar molecules in the solution will be minimal. Polar molecules in the solution will therefore, spend most of their time retained in the solvent. Non-polar compounds in solution will tend to be attracted to the silica, with its hydrocarbon groups, due to van der Waals binding. The non-polar molecules are less soluble in the polar solvent as hydrogen bonds have to be broken when they come in between the water and methanol molecules. These molecules, therefore, as they spend less time in the polar solvent and their passage will be slower through the column. Thus, the polar molecules that will travel through the column more quickly.

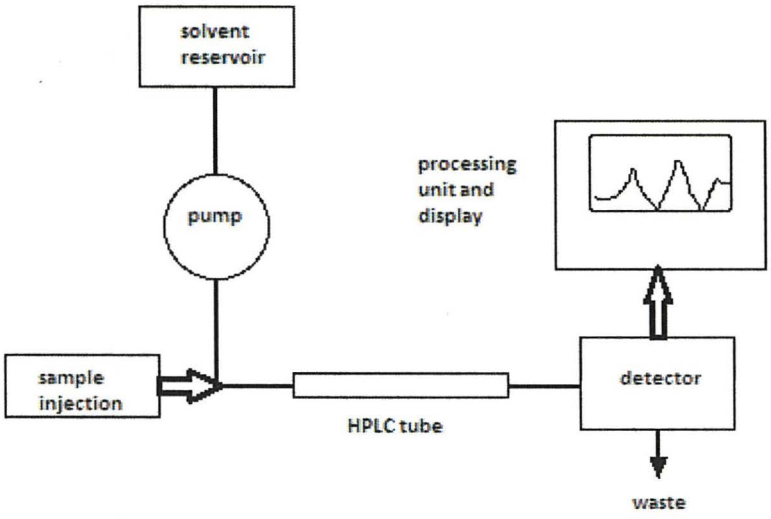


Figure 44: A flow scheme for HPLC

An advantage of HPLC is that the process including the injection can be fully automated. For any given compound, the retention time (RT) will depend on the pressure used as this

will affect the flow rate. The RT is also dependent on the material used for the stationary phase and its particle size and also on the solvent and the temperature of the column. This means that run conditions should to be carefully controlled if retention times are to be used as a means of identifying compounds. The detector commonly used is based on ultra-violet absorption. The sample under test must be UV active and must be distinguishable from the solvent. Methanol, for example, absorbs below 205 nm, and water absorbs below 190 nm. For a methanol-water mixture used as the solvent, a wavelength longer than 205 nm should be used.

When the detector is showing a peak, some of what is passing through the detector at that time can be diverted to a mass spectrometer. There it will give a fragmentation pattern which can be compared against a computer database of known patterns. That means that the identity of a huge range of compounds can be found without having to know their retention times.

2.4.7 UV/vis Spectroscopy

Ultraviolet-Visible Absorption Spectroscopy (UV/vis) refers to spectroscopy using wavelengths for ultraviolet and visible lights which range from 10 - 400 nm and 400 - 780 nm respectively. This energy absorbed by molecules can result in changes in the electronic nature of the molecule i.e. changes between ground state and excited states of electrons within the system.[177]

Electrons in ground state molecular orbitals can be promoted to anti-bonding molecular orbitals. The electrons in a molecule can be of one of three types: namely in sigma bonds (σ), pi (π) bonds, or non-bonding (n electrons including lone pairs). These electrons, when irradiated with electromagnetic radiation of suitable wavelength, are excited and jump from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) and the result in an excited or anti-bonding state. The following should be noted:

1. σ-bond electrons exist at the lowest energy level and are most stable, requiring a lot of energy to be elevated to a higher energy level. These electrons generally absorb light of a shorter wavelength of the ultraviolet spectrum and such transitions are rare.
2. π-bond electrons possess a much higher energy level when in the ground state. These electrons therefore, are relatively unstable and are excited more easily requiring less energy for excitation. These electrons, therefore, absorb energy in the ultraviolet and visible regions.
3. n-electrons (non-bonding) are generally electrons in lone pairs, are of higher energy levels than the π-electrons and excitation by both ultraviolet and visible light is possible.

Most of the absorption of energy in the ultraviolet-visible region is due to π-electron or n-electron transitions. The electronic states for a particular system are well defined i.e. the double bond of 2-butene has a particular energy level for the π-electrons and which can absorb a specific (or quantized) amount of energy to be excited to the π* energy level.

The concentration of an absorbing species is determined using the Beer-Lambert law (Equation 10):

$$A = log_{10}\left(\frac{I_0}{I}\right) = \epsilon.c.L \qquad \text{(Eq.10)}$$

where A is the absorbance in Absorbance Units (AU), I0 is the intensity of incident light of a given wavelength, I is the intensity of the transmitted light of the same wavelength, L is the path-length of the sample, and c the concentration of the sample. For a given absorbing species and particular wavelength, ϵ is the constant known as the molar extinction coefficient. This constant is specific to the species for a given solvent, temperature and pressure.

The absorbance and the extinction coefficient ε can be expressed in terms of natural logarithms rather than the base-10.

The Beer-Lambert Law is a useful but is not universal for all substances. Very large, complex molecules, for example, the organic dyes Xylenol Orange or Neutral red do not conform to the law.

2.4.8 Measurement of Lower Critical Solution Temperature (LCST) of Polymers

The solubility of macromolecules in solution depends on temperature. Generally, increasing the temperature increases solubility, but for certain synthetic polymers, the opposite is observed. The polymer molecules are thought to undergo drastic conformational changes. Thus, for example, polystyrene in cyclohexane undergoes a coil-globule transition. The thermal denaturation of proteins is another example of a conformational change.[178, 179]

The lower critical solution temperature (LCST) is the temperature below which the polymer and solvent are miscible. On raising the temperature above the LCST the polymer will fall out of solution. To determine this temperature, the aqueous solutions of PEG-PLA co-polymers, PEG-PLA diacrylate macromers and hyperbranched polymer products from PEG-PLA diacrylate macromers were subjected to UV/Vis Spectrophotometry under conditions of temperature control from 10 to 60 °C.

The Instrument used was a Cary 100 Double Beam UV/Vis Spectrophotometer with an attached temperature controller and samples were positioned in 6x6 multicell cuvette heating block.

Cary 100 Instrumentation setup and sample acquisition: The system was connected with the Thermal Analysis application as part of the Cary WinUV suite of software. The following parameters were inputted:

Heating rate: 1 °C/min
Data collection rate: 0.06 °C/point
Absorbance Wavelength: 550 nm
Temperature Range: 0-60 °C

A series of 2 blank runs with deionised water were performed using matched capped quartz cuvettes after zeroing the system and setting it up for blank correction in the reference beam. For the analysis, samples were made up to 0.1% concentration (w/v) in deionised water for the UV/Vis thermal analysis.

2.4.9 Swelling and Degradation Studies

For Macromer 1KL3 hydrogel, two samples were prepared at 54% and 30% concentration (Table 10 in Chapter 3) using the method described in section 2.3.5.

To study the swelling of the hydrogels, the dry hydrogels were weighted individually and immersed in 1ml deionized water (pH 7). At each time point, the surplus free water was removed and the gel re-weighed.

The percentage swelling ratio was calculated by the following equation.

$$\%\ Swelling = \frac{W_s - W_\emptyset}{W_\emptyset} \times 100 \qquad (Eq.11)$$

The experiments were done in triplicate.

Where W_s is the weight of hydrogel upon swelling and W_θ is the weight of dry hydrogel.

Readings were taken every 24 hours and results tabulated and presented as a plot of Swelling ratio v time in days.

Chapter 3: Results and Discussions

Macromer branching agents consisting of co-polymers of Poly(ethylene glycol) and poly(D,L-Lactide), with added terminal vinyl functionality, were synthesise. When further copolymerised with poly(ethylene glycol) methyl ether methacrylate (PEGMEMA) they were used to make biodegradable hyperbranched copolymers by means of conventional free radical polymerisation (FRP) or by living/controlled free radical polymerisation (ATRP and RAFT).

Hydrogels were then constructed using linear PEG-PLA diacrylate macromers and hyperbranched co-polymers, and these hydrogels were then studied for their swelling and degradation properties.

3.1 Synthesis and Characterisation of Poly(ethylene glycol)-co-poly(D,L-lactide) (P_{DL}LA-co-PEG-co-P_{DL}LA) co-polymers.

Telechelic co-polymers (polymer with both ends possessing the same functionality) of the form ABA with terminal hydroxy groups were synthesise with a central poly(ethylene glycol) (PEG) domain and end-capped with oligo(lactoyl) moieties. The central domain was derived from PEG which consists of repeating residues of "-O-CH_2-CH_2-" terminated with hydroxyl groups. Terminal domains of lactoyl oligomers were then added by means of the ring opening polymerisation of D,L-lactide which is a cyclic di-ester of lactic acid (2-hydroxypropionic acid). The ring opening is initiated by the α and ω hydroxyl terminal groups of the PEG and consists of a step-wise addition of the lactide to the linear PEG (Scheme 1,Chapter 2).[180]

The Ring opening (ROP) of lactide catalyzed by $Sn(Oct)_2$ involves many different steps, including initiation and propagation, to form low-molecular-weight species and ultimately to form the polymer.[181] The key step of initiation is thought to involve the OH-bearing molecules including water, alcohols, and carboxylic acids.

Currently there are two competing mechanisms for the initiation. Firstly, Kricheldorf et al [182] proposes a monomer activation in which a ternary complex is formed by the association of the monomer, a OH-bearing molecule and the catalyst. Tin atoms are not bonded to the growing chains. Kowalski et al[183, 184] proposed another mechanism which involves an alkoxide initiation. Here, stannous octoate ($Sn(Oct)_2$reacts with OH-bearing molecules to form an alkoxide that initiates the polymerisation. The $Sn(Oct)_2$ and OH species are initiator and co-initiator, respectively. This polymerisation mechanism is at present the most accepted.

A possible reaction scheme by Du[180] is illustrated in Figure 44 and is consistent with the latter mechanism. In step 1 the lactide/catalyst complex encounters a hydroxyl group of PEG which is then incorporated (Steps 2-4). In step 5 another lactide monomer coordinates to the Sn^{2+} atom forming a new complex. The PEG, being a primary alcohol initiator, is highly reactive. The reaction, therefore, proceeds through an intermolecular process with a resulting chain transfer, as the catalyst prefers to react with the initiator.

A co-initiator such as H_2O, an alkyl alcohol (ROH) or a primary amine functional group (RNH_2), is required to initiate the polyester chain growth. It is known from other studies that the ROP of the cyclic ester involves a metal alkoxide bond as an active intermediate during the monomer insertion.[180]

$$ML_y + ROH \rightleftharpoons RO\text{-}ML_{y-1} + LH$$

Ligand exchange at the metal atom provides the initiating and the propagating species.[184] The molecular weight is known to increase with polymerisation time.[185]

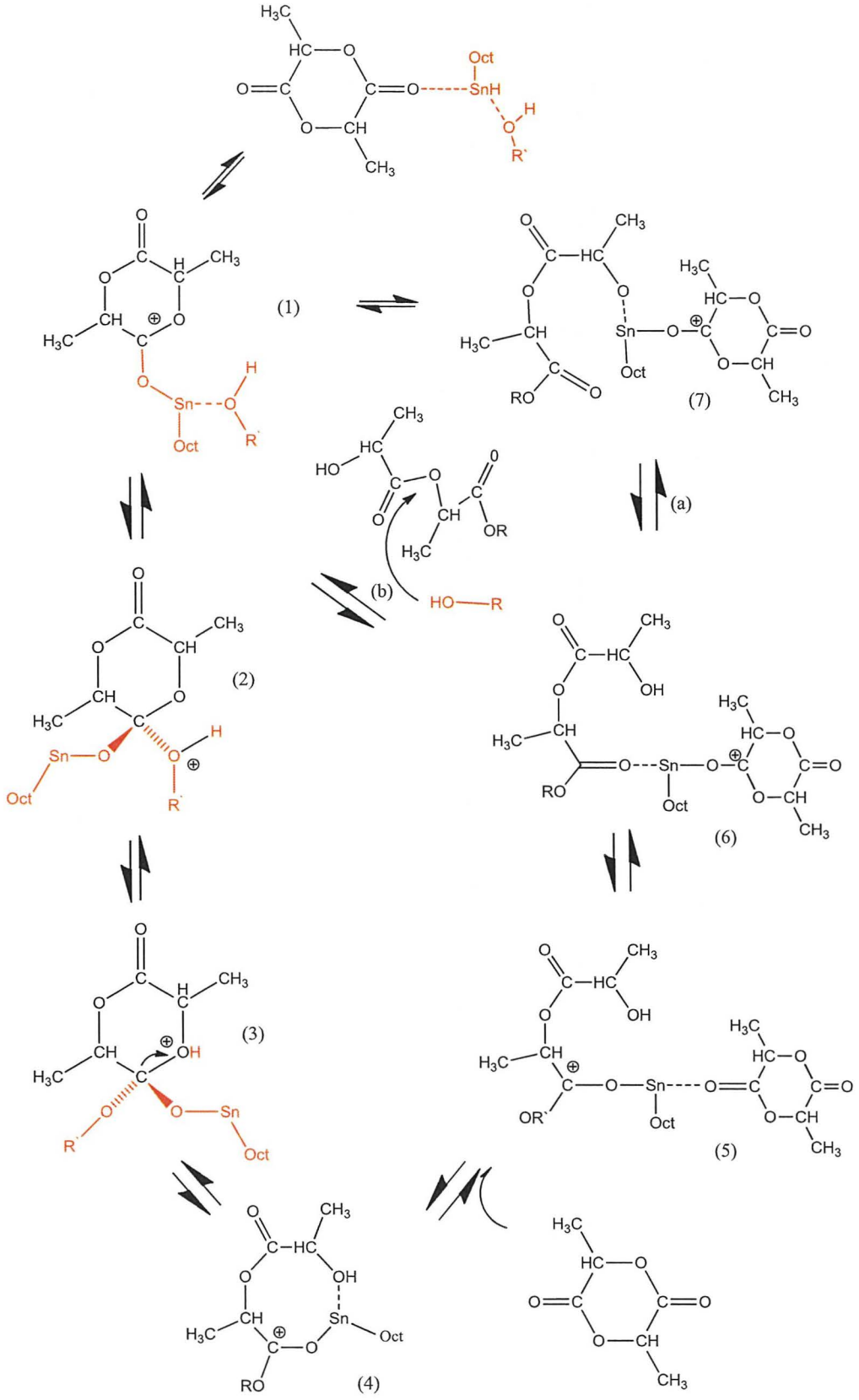


Figure 45: Proposed Reaction Mechanism of the Stannous octoate catalysed Ring Opening Polymerisation of Lactide with PEG[180]

The trans-esterification was performed with the aid of the catalyst Stannous octoate.[186] Stannous octoate has been approved by the Food and Drug Administration (FDA) when used in small catalytic amounts.[187] Calcium hydride (CaH_2) has been used as an alternatie to the tin catalyst and has been shown not to yield toxic products.[44]

The degree of polymerisation is dependent on the molar feed ratio of reactants i.e. the PEG and the lactide. P_{DL}LA-co-PEG-co-P_{DL}LA co-polymers were synthesised as given in section 2.3.2using PEG of molecular weight of 1000 and these are shown in Table 2 (section 3.1).

It is observed that the co-polymers 1KL3 (entries 8 and 9 in Table 2) were found to be readily soluble in water at room temperature. All the co-polymers listed were found to be soluble in water at the low temperature of 4°C. The solubility reflects its amphiphilic nature possessing both hydophilic and hydrophobic domains. The PLA portion of the molecule is hydrophobic while the PEG domain is hydrophilic. The work of Hubbell found that if the ratio of the degree of polymerisation (DP) of the PLA domain to the DP of the PEG domain is less that 0.45 the molecule will be soluble in water.[18] The co-polymers in Table 2 confirm the finding that with increasing PLA content, the the co-polymer becomes less soluble.

It was found that, other factors being equal, that increasing the reaction time gave a higher yield for the the same co-polymer. Thus, for co-polymer 1KL10, yields increased with increasing time (entries 2, 3 and 10 in Table 2). This was also found for 1KL 6 (entries 4 and 5 in Table 2).

The product yield obtained is, however, very much dependent on the separation procedure. After the reaction had run its course, the reaction mixture was dissolved in DCM and precipitated in anhydrous ether. It was then redissolved in DCM and precipitated out in hexane. Generally, a minimum quantity of DCM was used, sufficient to produce a non-viscous clear solution, and this was then slowly dropped into five times its own volume of the ether or the hexane as appropriate. Different co-polymers with their differential solubilities precipitated to slightly different extents.

An alternative extraction procedure was employed whereby co-polymer 1KL5 (entry 12 in Table 2) was precipitated out of warm water. The technique involved dissolving the co-

polymer mixture in ice-cold water and then gradually increasing its temperature. It was determined that at 52°C i.e. its cloud point, the co-polymer precipitated out of the solution. The yield was found to be 33.8%. The same co-polymer,1KL5, by the hexane method gave a yield of 41.3%. Thus, the water method appeared to be less satisfactory in this regard. An added disadvantage is water is more difficult to remove than hexane and increases the possibility of hydrolysis.

Table 2: $P_{DL}LA$-co-PEG-co-$P_{DL}LA$ Co-polymers synthesised and reaction conditions used

Entry	Feed ratio of PEG: Lactide	Co-polymer[a]	Temp (°C)	M_n (Da^a)	M_n (Da^b)	PDI[c]	Yield[d] %	R.T. (hr)	Water Sol [e]
1	1:8	1KL7.6	110	2095	2901	1.15	30.4	24	No
2	1:10	1KL10-1	130	2513	3786	1.1	38.1	17	No
3	1:10	1KL10-2	110	2480	2762	1.47	34.9	5	No
4	1:6	1KL6-1	130	2153	2162	1.5	97.0	16	No
5	1:6	1KL6-2	130	2009	2772	1.2	55.7	5	No
6	1:8	1KL8-1	130	2445	2570	1.2	12.0	20.5	No
7	1:10	1KL9-1	130	2297	2725	1.09	20.4	20	No
8	1:3	1KL3-1	130	1406	1529	1.31	30.0	11	Yes
9	1:3	1KL3-1	130	1490	1593	1.15	74.0	16	Yes
10	1:10	1KL10-3	130	2440	3520	1.17	41.7	22	No
11	1:11	1KL11	115	2527	3304	1.18	93.0	24	No
12[g]	1:5	1KL5-2	130	1720	2397	1.3	33.8	24	No
13	1:5	1KL5-1	130	1720	N.D.[f]	N.D.[f]	41.3	24	No

a) Molecular weight in g/mol determined by ^1H NMR. b) determined by GPC, using PMMA as the standard.

c) PDI- Polydispersity Index

d) The concentration was 200 mg in 1 ml of water. N.B. All the above co-polymers (apart from 1KL3) are insoluble in water at room temperature and all found to be soluble at 4°C . e) Yield is determined as weight of product as a percentage of theoretically possible yield

f) not determined. g) Water extracted.

A typical proton NMR spectrum for the PEG-lactoyl co-polymer in deuterated chloroform ($CDCl_3$) is given in Figure 45 with proton assignments as shown. PEG of molecular weight of 1,000 was used for the synthesis of the co-polymer.

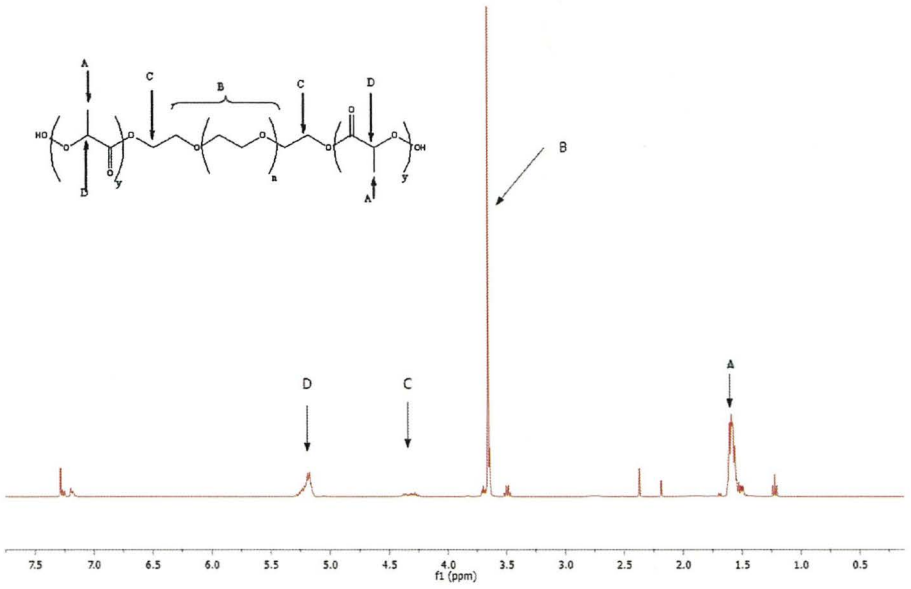


Figure 46: ^{1}H-NMR spectrum PEG-lactoyl co-polymer ($P_{DL}LA$-co-PEG-co-$P_{DL}LA$) 1KL10-1 measured in $CDCl_3$

Proton NMRs were performed for the starting materials in order to identify any unreacted materials present in the product material. The lactide ^{1}H NMR spectra was recorded in $CDCl_3$ (Figure 46) and the PEG ^{1}H NMR spectra are given in Figures 47 and 48 using the deuterated solvents $CDCl_3$ and DMSO respectively. This was to assist to identify the source of impurities in the co-polymer product. As DMSO, an aprotic solvent, does not exchange protons with the hydroxyl groups in the terminal positions of the linear PEG structure,[188] the hydroxyl proton gives a sharp peak at δ 4.58 ppm. This signal appears absent when deuterated $CDCl_3$ is used.[188]

The integration of the hydroxyl peak proton when normalised and compared with the signal for the methylene signal can be used to confirm the PEG molecular weight (M_n) which

is 1000 is as given by the supplier. The NMR for PEG in DMSO d_6 gives an integration value of 1 for -OH at δ4.58 and an integration value of 38.86 for (-CH_2-CH_2-O) at δ3.51, thus n, the number of repeating residues of (-CH_2-CH_2-O) in the PEG is given by

$$\frac{38.86\times2}{4} = 19.43\sim20$$

i.e. 20 residues which compares with 22.32 as calculated from the manufacturers given value for the molecular weight of 1,000 (see equations 12 and 13 in section 3.1)

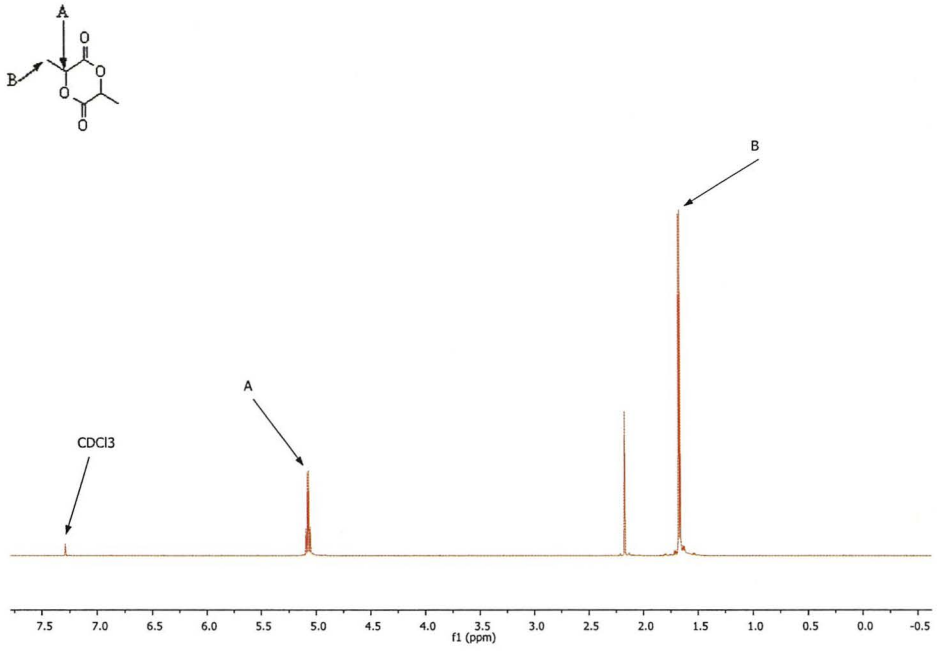


Figure 47: ^{1}H-NMR spectrum of Lactide in $CDCl_3$

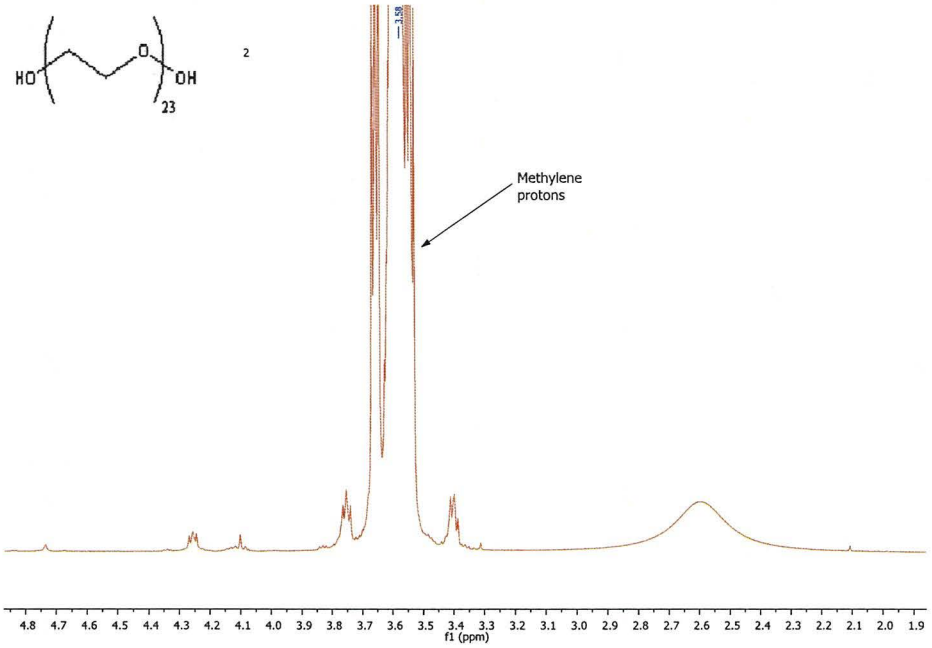


Figure 48: ^{1}H NMR spectrum of Poly(ethylene Glycol) in $CDCl_3$

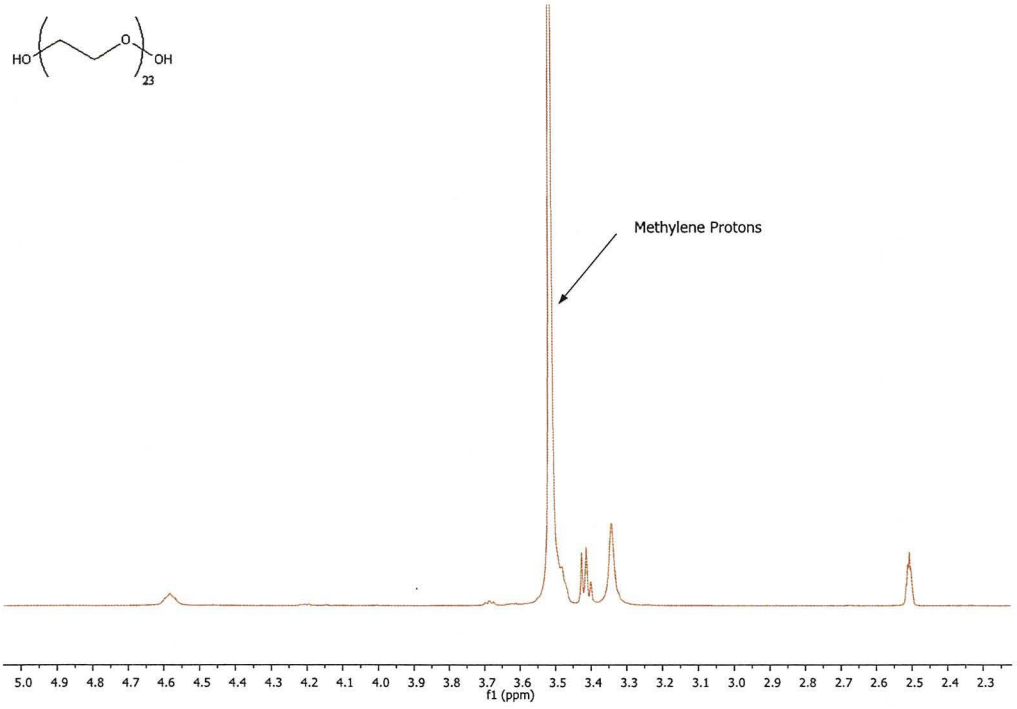


Figure 49: ^{1}H NMR spectrum of Poly(ethylene Glycol) in DMSO

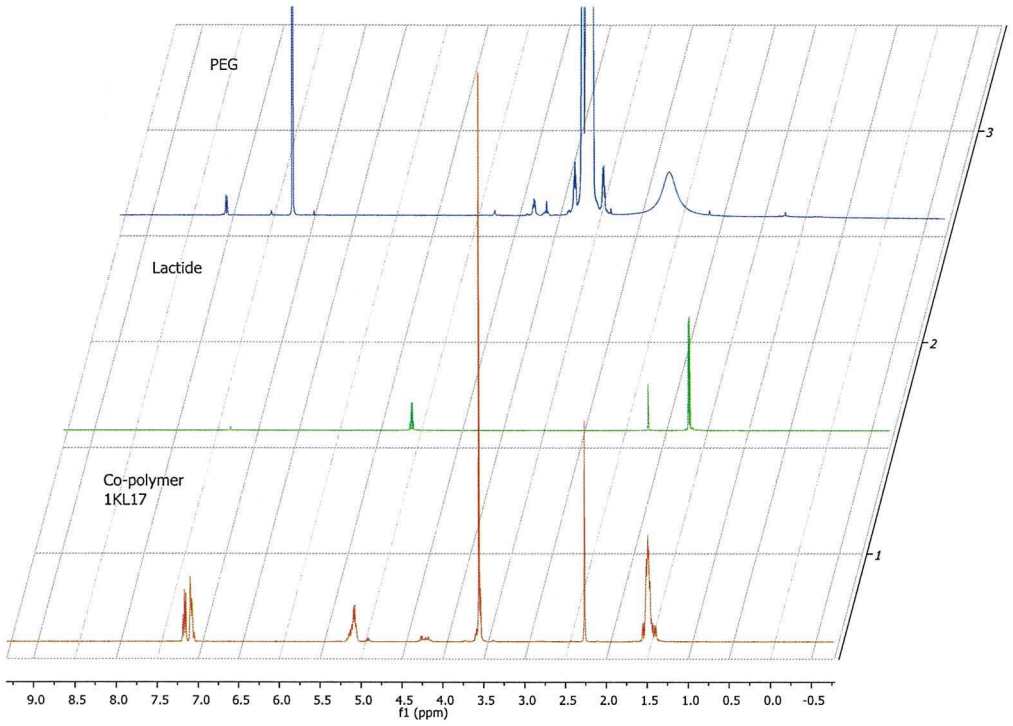


Figure 50: Overlay of ^{1}H-NMR spectrum P_{DL}LA-co-PEG-co-P_{DL}LA co-polymer 1KL17, Lactide Poly(ethylene Glycol) in $CDCl_3$

The overlay of the ^{1}H NMR spectra for the co-polymer product with the starting materials i.e. lactide and PEG shows clearly that all the free lactide has been consumed in the reaction or else eliminated during purification as the -CH3 group which gives a signal at δ1.67 in free lactide is now to be found at δ 1.49 in the product. An additional check could have been performed by adding the lactide starting material to the nmr sample to confirm that the free lactide signal can be distinguished from that of the co-polymer residue.

The number of repeating PEG moieties (-O-CH_2-CH_2-) with a molecular weight for each of 44 and allowing for the terminal -H and -OH is given by

$$\mathbf{44}\boldsymbol{n} + \mathbf{18} = \mathbf{1000} \qquad \textbf{(Eq. 12)}$$

$$n = \frac{1000-18}{44} = 22.32 \qquad \text{(Eq. 13)}$$

where the molecular weight of PEG used is 1000 and n is the number of PEG residues within the PEG structure.

The number of protons corresponding to the "B" signal of the NMR (Figure 44) is thus equal to

$$4 \times 22.32 - 4 = 85.27 \quad \text{protons}$$

Note, the 4 protons for both the terminal methylene groups of the poly(ethylene glycol) central domain are seen as a separate signal "C" in the assignments given in Figure 45 having a higher δ value by virtue of their attachment to carbon atoms adjoining highly electronegative oxygen in the ether linkage.

Proton NMR analysis has been used to determine the degree of polymerisation by others and was used to determine the lactoyl content of the co-polymer synthesized above.[189, 190]

Typically the proton NMR signal for the PEG moiety (–CH_2-CH_2-O-) is at δ=3.55 and the methyl signal for the poly(lactoyl) moiety is at δ=1.30 and typical integrations were of 2.75 and 5.17 respectively. Each lactoyl methyl group contains 3 protons and if we assume equi-molar addition at both ends of the PEG block the total number of protons for the lactoyl signal is equal to 6m where m is the number of lactoyl residues at each end of the linear co-polymer. Thus,

$$m = \frac{85.27 \times 2.75}{6 \times 5.17} = 7.56 \qquad \text{(Eq. 14)}$$

i.e. 7.56 lactoyl residues are to found at each end of the PEG domain. As it is not possible structurally to have a part residue it is more meaningful to round up this to m= 8.

Thus, from the calculation, the Co-polymer was characterised as 1KL8-1 i.e. eight lactoyl residues at each end of the central PEG domain. This gives a molecular weight of 2445 after rounding up (entry 6, Table 2).

The calculation of the degree of co-polymerisation of PEG and D,L-Lactide assumes that both terminal ends of the PEG react and that addition of lactoyl residues occurs in a symmetrical manner with equal addition at each end as the reaction proceeds. Such a co-polymer is termed an ABA type with monomer A added at each end of the linear B domain. Du[180] has confirmed that this symmetrical addition is in fact the case when L-Lactide was reacted with PEG M_W = 1000 and used in a feed molar ratio of 20:1. He proceeded to extract the reaction mixture using alcohol to remove un-reacted PEG and the subjected the extracted polymer to proton NMR confirming the co-polymerisation took place. The co-polymer was then methacrylated and proton NMR showed that the intensity at 4.3 ppm (the signal for the terminal methine proton of the lactoyl moiety) decreased by two thirds of the original value while the signal for the methylene protons in the PEG remains the same. This suggests that the methine proton shifts to lower field confirming an ABA type polymer and not an AB polymer which would give a reduction of only one third.[191]

The PEG/LA ratios of the copolymers was often found to be slightly lower than the feed ratio. This was in part due to loss of water from the pre-weighed PEG. Thus the actual weight of dehydrated PEG should be used for accurate work. Also the conversion of lactide was not complete as unreacted lactide vapourised and condensed on cooler parts of the reaction vessel and were effectively removed from the reaction mixture. Care was taken during purification to avoid re-introduction of condensed lactide (accumulated at the rim of the reaction vessel) into the product. If PEG of higher molecular weight is used the relative hydroxyl content will decline as there are two moles of hydroxyl groups per mole of linear PEG. We might expect some

difference in the behavior of a reaction involving low molecular weight PEG when compared to the higher molecular weight species. With increasing molar mass for a given weight in PEG, there are relatively fewer hydroxyl end-groups which function as initiation sites, with the consequent lowering of the rate of conversion of the lactide under the same reaction conditions.

It was found, generally, that a longer reaction time gave a higher yield and the product was of higher molecular weight as can be seen comparing entries 8 and 9 (see also comparisons between 4 and 5).

Furthermore, we can see (entries 1 and 6) that for the same reaction length that the higher temperature of gave a higher molecular weight.It would seem that the temperature of 130°C is nearer the optimum, at least under the reaction conditions considered here.

It can be seen that there is a dicrepancy between the molecular weight values determined by GPC and those determined by NMR with the latter being generally higher.

The solubility in water of the co-polymers was determined. The homo-polymer PLA is insoluble in water whereas the homo-polymer PEG is water soluble. As the mass fraction of the PLA domain in the co-polymer increases its water solubility should decrease. At room temperature (20-25 °C) the co-polymer 1KL3 (entry entries 8 and 9 in Table 2) was soluble in water whereas those of higher lactide content where insoluble. This confirms the findings of Sawhney[18] that generally when the ratio of the degree of polymerisation (DP) of the lactoyl residues is less than 0.45 this will confer water solubility. Thus, for the water soluble 1KL3 with its 6 lactoyl residues and its 22.32 PEG residues, the ratio is 6/22.32 = 0.269 ie. 26.9%. It was found that all the co-polymers synthesised were soluble in water at 4°C i.e. when left for 24 hours in a cold room under constant stirring. The water solubility of these copolymers also depends on the molar mass. Thus, a high PEG/PDLA ratio and a low molar mass confers water solubility compared to the same ratio in a high molecular mass compound.

After reactions two methods used for the separation and purification of the product. In the first method, the product was recovered by dissolution in dichloromethane and precipitated in anhydrous ether followed by drying under reduced pressure after filtration. In the second method crude product was dissolved in ice-cold water and then heated to 80°C to precipitate the polymer

thus removing water-soluble polymer and any un-reacted monomers. The supernatant was decanted and the precipitate re-dissolved in ice-cold water. The procedure was repeated once more and the purified polymer was obtained and then freeze dried to remove residual water to give the tri-block copolymer.

Entry 12 was precipitated out by the water method in which the material was first dissolved in cold water of 4°C and then the solution heated to the point at which it became insoluble i.e. its cloud point. It is seen from table 2 that the water method gives a lower yield.

The co-polymer was further characterised using DSC. Figures 50, 51 52 and 53 give the heating curves for the starting materials i.e. the PEG, Lactide, and the resulting co-polymer. The thermogram of PEG gave a T_m of 35.67°C (Figure 50) and the T_m of Lactide was determined as 127.07°C (Figure 51).

The T_m for PEG 1000 compares with a value of 42°C for PEG 1000 as given by Raskov.[44]

The presence of short PLA domains in the co-polymer leads to an amorphous polymer, thus PEG-PLA copolymer does not have a T_m (Figure 52). This copolymer shows a glass transition temperature (T_g) at about -40 °C. This is further evidence of the formation of the copolymer and that the co-polymerisation has proceeded to completion as any excess material would be expected to be in a localised form and give rise to a T_m.

The co-polymer thermogram (Figure 53) represents a cooling curve for another batch of 1KL5 copolymer (1KL5-2). This thermogram appears a little anomalous as a sharp exothermic peak was observed at 65°C which could be attributed to a crystallisation phase change (T_c). However, this transition is not observed in Figure 52 and may be an artefact due to an impurity. A second order transition can be seen at -45°C which can be attributed to a T_g for the PEG domains. An endothermic sharp peak was seen at 130°C which can be assigned to the T_m for PLA domains. However, this crystallisation peak was not observed in the both heating and cooling curves in Figure 52. Further investigation is needed to understand this difference. Figure 52, however, does show the presence of a Tg in the first and second heating curves at -45°C and at 130°C a gradual endothermal increase.

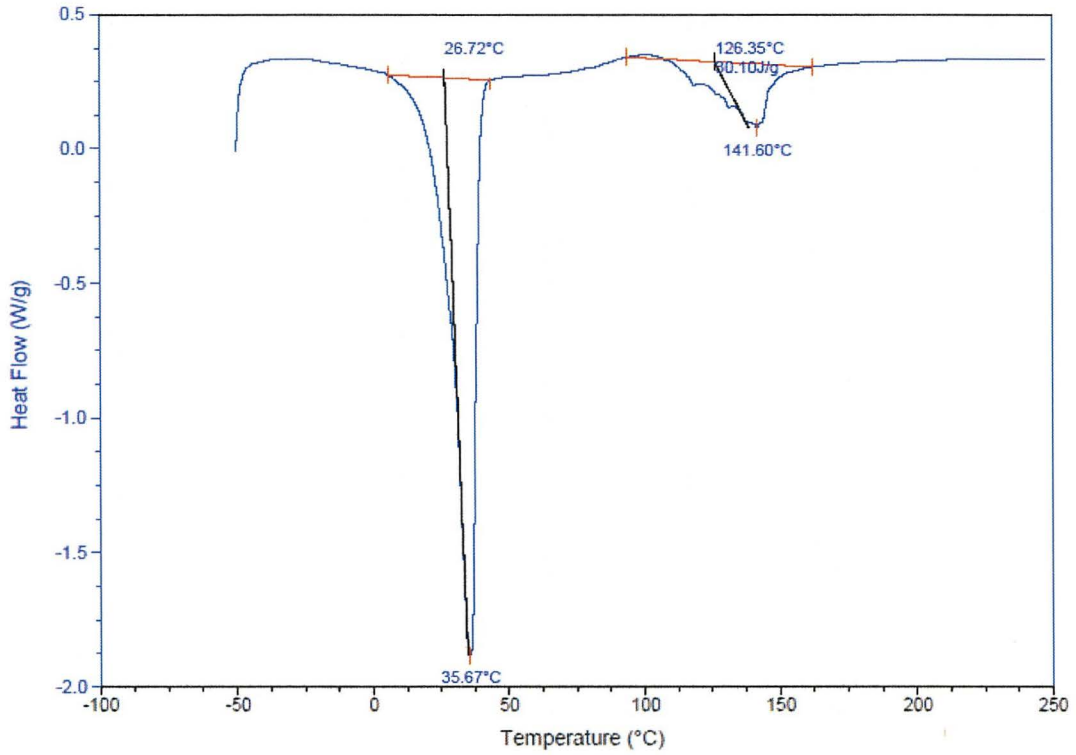


Figure 51: DSC thermogram for Polyethylene Glycol (M_W = 1000).T_m the melting point can be observed at 35.67°C

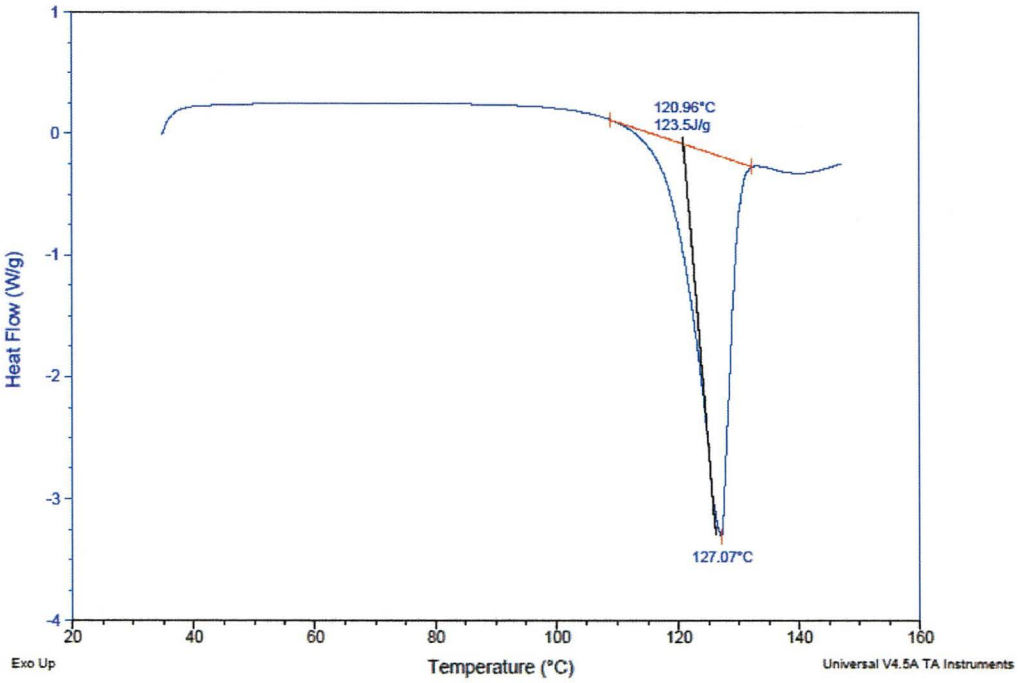


Figure 52: DSC thermogram for Lactide

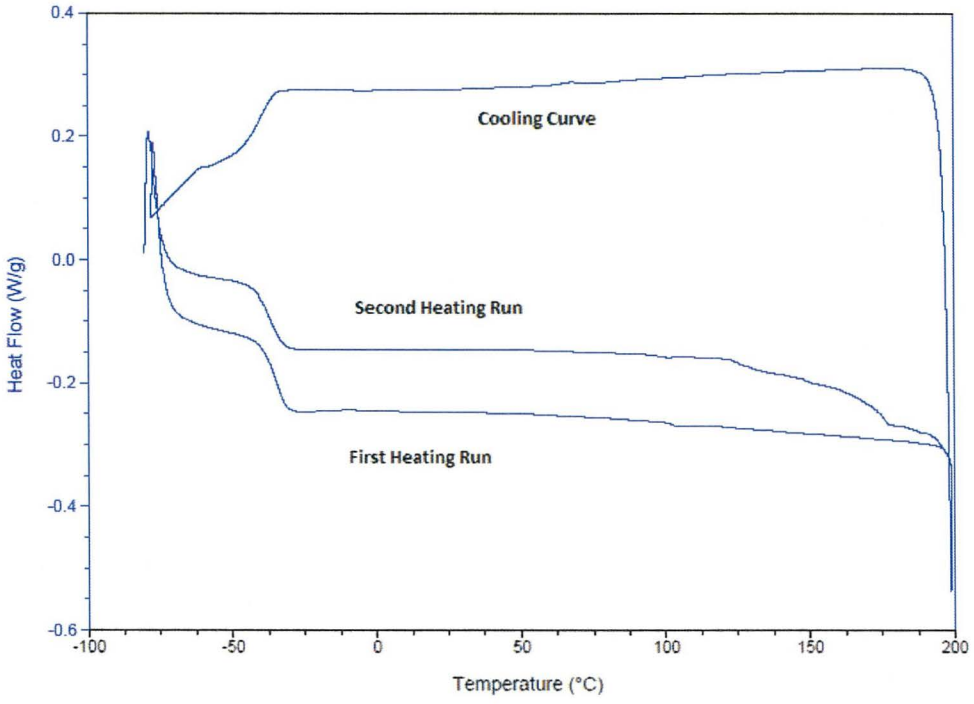


Figure 53: DSC for Co-polymer 1KL5-1.The $T_g$may be observed at -40°C

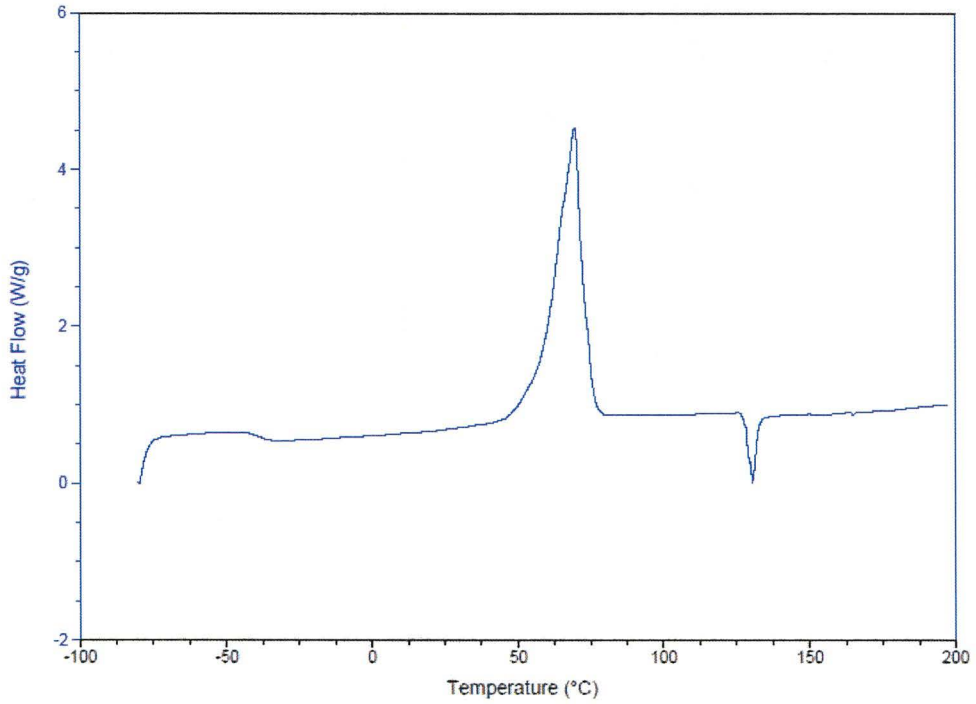


Figure 54: DSC of Co-polymer 1KL5-2

3.2 Synthesis and Characterisation of Poly(ethylene glycol)-co-poly(D,L-Lactide) ($P_{DL}LA$-co-PEG-co-$P_{DL}LA$) Diacrylate Macromers.

All P_{DL}LA-co-PEG-co-P_{DL}LA co-polymer prepared in section 3.1 with its α and ω hydroxyl end groups was both end-capped with acrylate groups i.e. to provide divinyl functionality with the capacity to react with other vinyl groups as a crosslinking agent or a branching agent. Many PEG derivatives have been synthesized which can be polymerised by free radical polymerisation from acrylates, methacrylates and fumarates, in this project P_{DL}LA-co-PEG-co-P_{DL}LA acrylates were prepared.

Introduction of the acryate groups onto the co-polymer chains allow the use of UV to crosslink the Macromer either to itself or to other vinyl functional reactants via a free radical reaction. The formation of the vinyl functional Macromer can be confirmed by FTIR and NMR.

The terminal hydroxyl groups of the PEG-PLA co-polymer was subsequently converted into acrylate functional groups by means of reaction with acryloyl chloride. The PEG-PLA copolymer has two terminal hydroxyl groups per molecule thus two acrylic groups are expected to be added to each molecule which would give a theoretical acrylation of 100%.

The degree of acrylation was determined by proton NMR. Vinyl group protons signals appear typically at δ = 5.9, 6.2 and 6.4 ppm. A typical proton NMR spectrum is given in Figure 54 and an integration value of 1.0 is given, the corresponding integration value for the methylene group of the poly(ethylene glycol) domain (i.e. the signal designated as B in Figure 54) has the value of 37.35. Ether was observed as an impurity in the spectrum and proved difficult to remove.

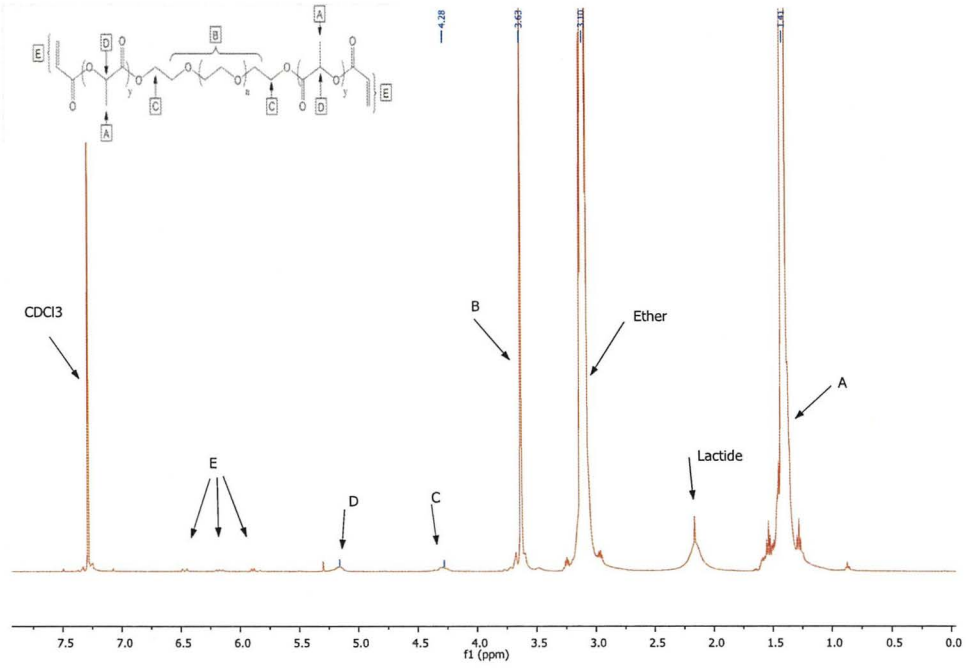


Figure 55: ^{1}H NMR of Macromer 1KL3-1 and general structure of Macromer (inset) (entry 11 in Table 3).

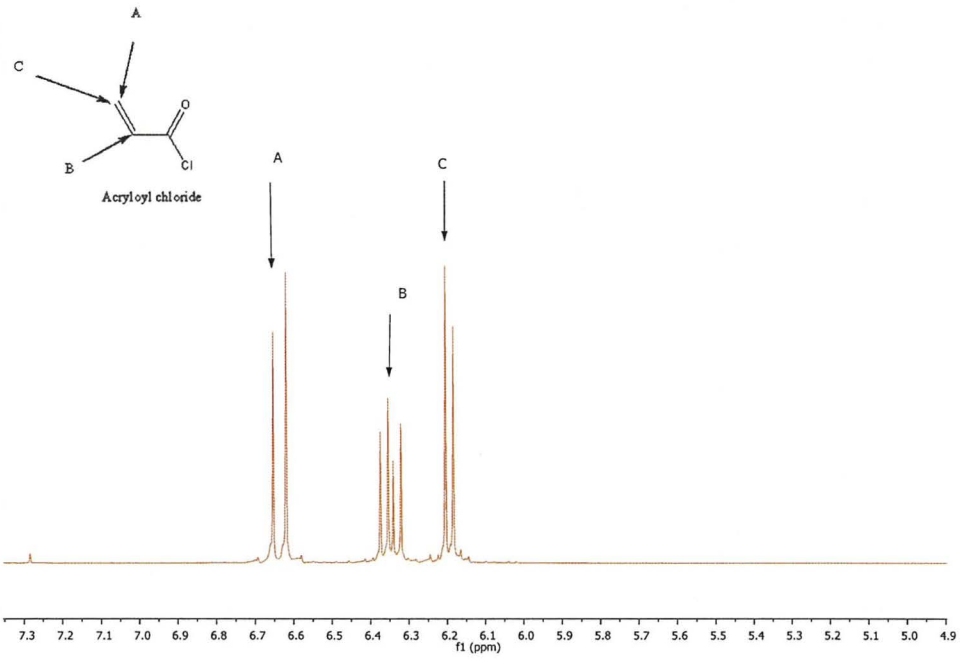


Figure 56: ^{1}H NMR of Acryloyl Chloride

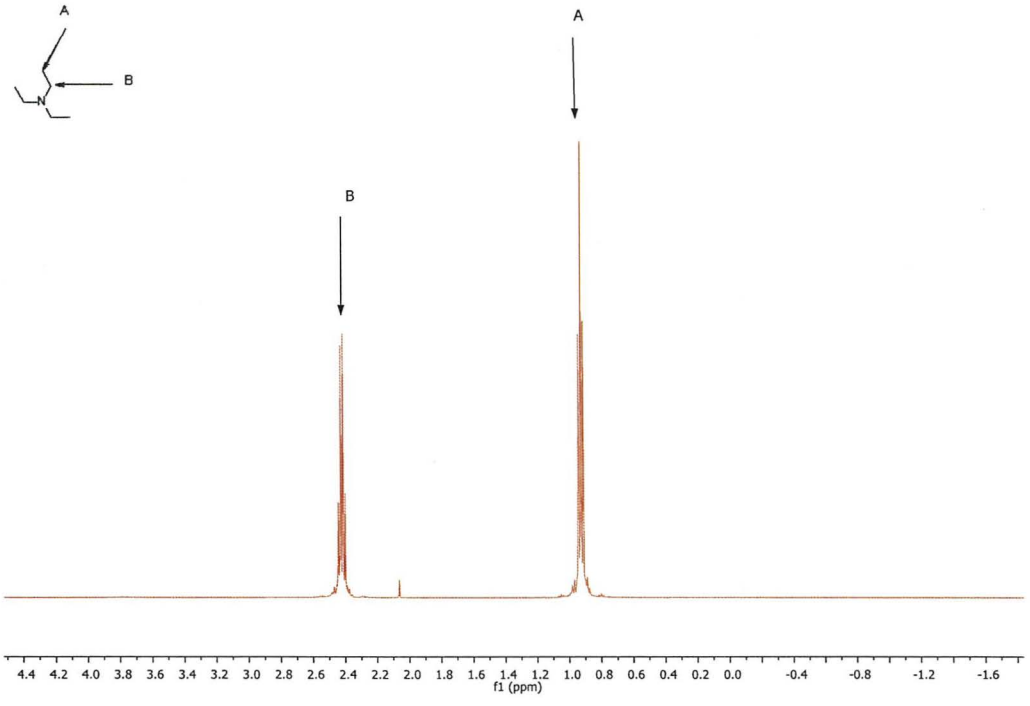


Figure 57: ^{1}H NMR of Triethylamine

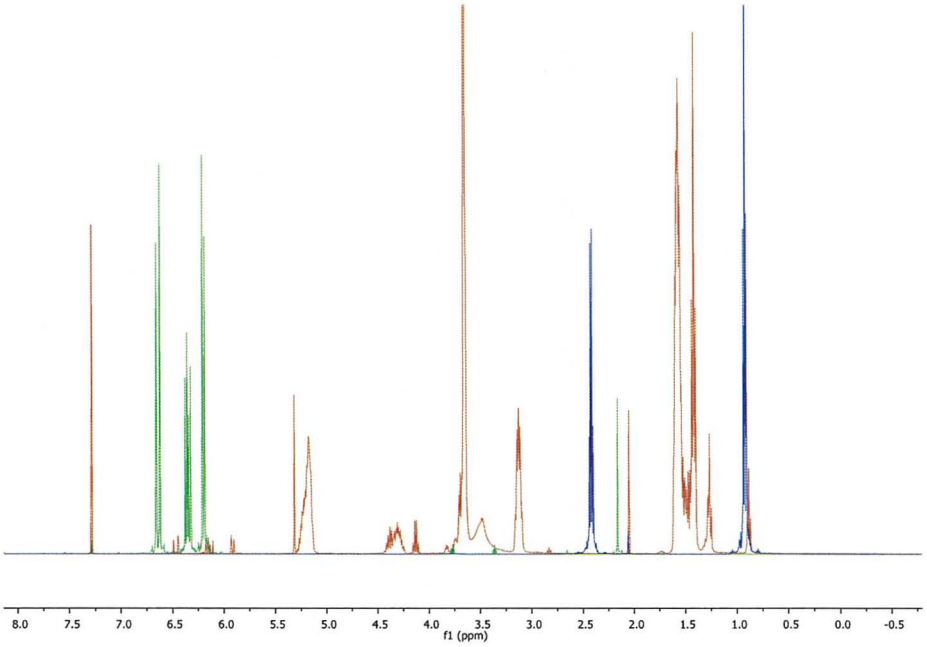


Figure 58: ^{1}H NMR Spectra of Acryloyl Chloride (green), Triethylamine (blue) and Macromer (red) superimposed and expanded to show signal for the vinyl group present in the Macromer.

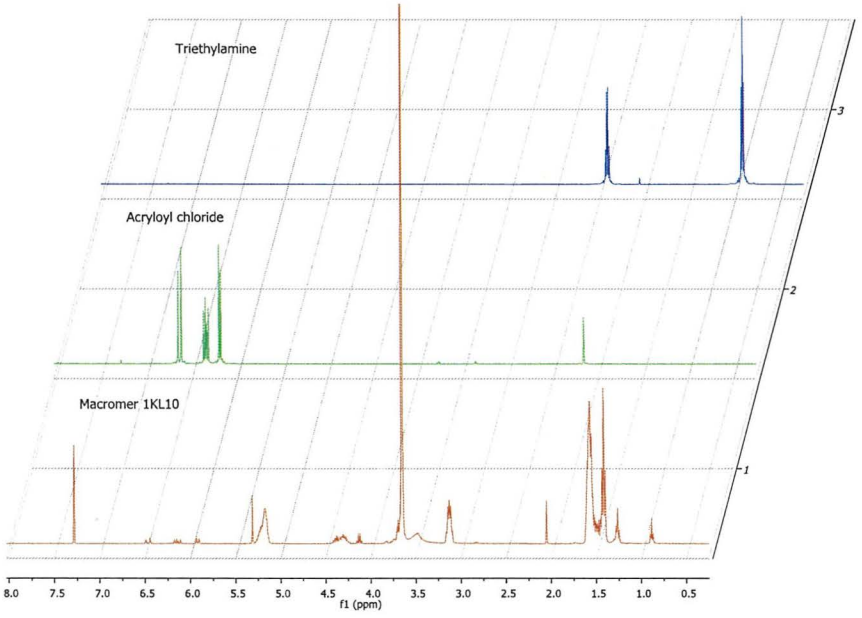


Figure 59: ^{1}H NMR Spectra of Acryloyl Chloride, Triethylamine and Macromer superimposed and expanded to show signal for the vinyl group present in the Macromer (red)

There are three vinyl protons attached to each carbon–carbon double bond for each terminal for every mole of macromer i.e. 6 protons per mole of Macromer when it is fully acrylated. Since the total number of protons in the PEG methyl groups is given by 85.27, the total number of protons in vinyl groups is therefore given by

$$x = \frac{1.0 \times 85.27}{37.35} = 2.28$$

As a maximum of 6 protons would be present if both terminals of all the macromer molecules were acrylated. Thus the degree of acrylation can be expressed as 2.28 divided by 6 which is equal to 0.38 or 38%. This is an average figure. All peak assignments were determined by comparison with the data and spectra of in the standard literature particularly the Spectral Data Base (SDBS).

The Macromer product obtained was also characterized by means of GPC (Figure 59).

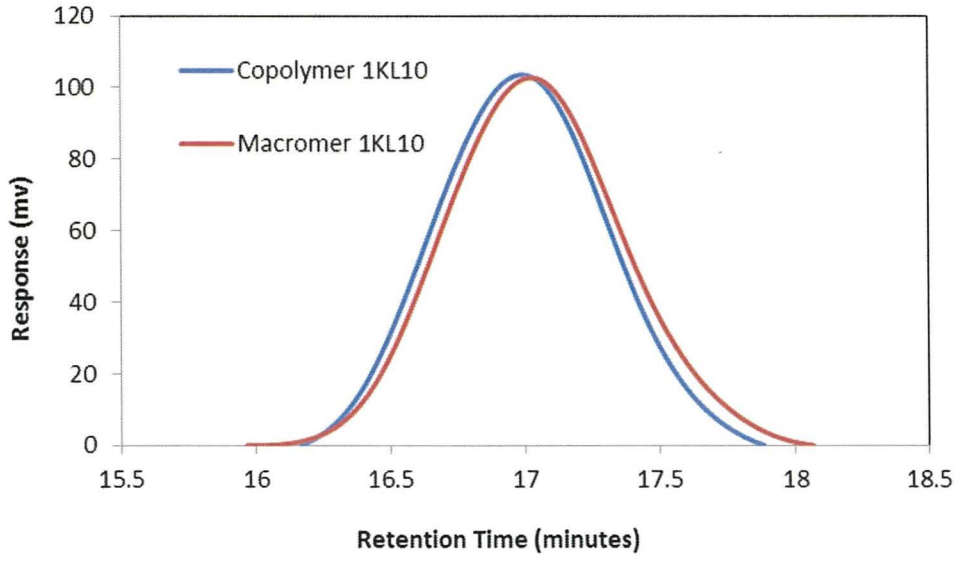


Figure 60: Overlay of GPC for Co-polymer 1KL10 and Macromer 1KL10

It can be seen that the peaks at retention time 17 minutes coincide for both the co-polymer 1KL10 and Macromer 1KL10. Although the macromer should theoretically have a slightly higher molecular weight due to the addition of acrylate groups to the terminals of the PEG lactoyl co-polymer, it was observed by GPC that the Macromer peak, in fact, appears to be lagging behind the co-polymer peak. This can be attributed to hydrolysis of the Macromer and the copolymer during acrylation reactions because they contain hydrolytically lactoyl labile groups.

A comparison of the FT-IR for co-polymer 1KL10 and Macromer 1KL10 confirms the presence of vinyl groups added to the co-polymer (Figure 60). At 1638 cm^{-1} an assignment of -C=C- of medium intensity can be made showing the addition of double bonds to the co-polymer. The other prominent signals for alkenes occurs at 3036 and at 810 cm^{-1} for =C-H.[89] The strong signal at 1700cm^{-1} was due to the carbonyl group.

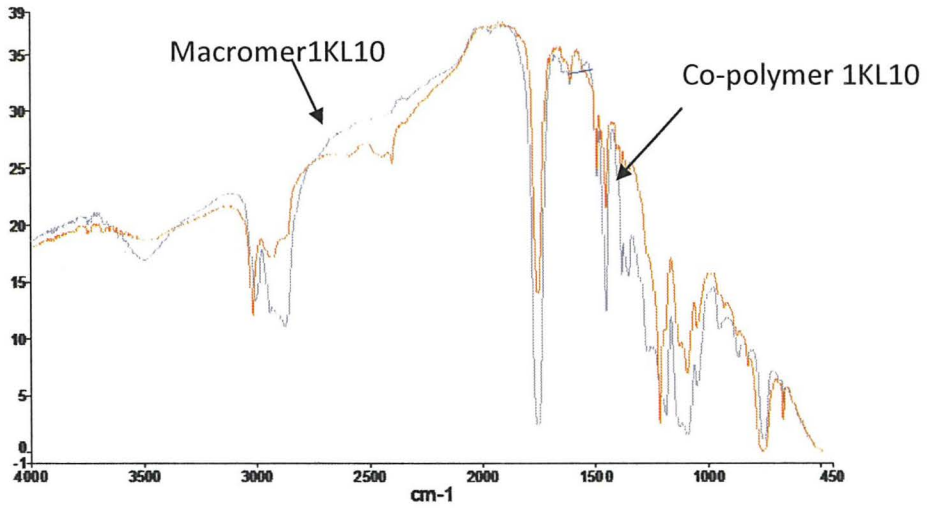


Figure 61: FT-IR of Co-polymer 1KL10 (red) and Macromer 1KL10 (blue)

It was found that the DCM solvent volume critical to the rate of acrylation. Too much DCM will redissolve the Triethylamine hydrochloride salt. It was noted that precipitate of the salt

correlated with high accrylation. It is presumed that if the solvent for the acrylation is in excess this reduces the rate of the acrylation reaction by reducing the concentration of the reactants. Therefore little of the salt is produced and readily dissolves in the solvent. Thus the presence of the precipitate gives some indication of acrylation.Thus a minimal amount of solvent is used, sufficient to dissolve the macromer.

From Table 3, the degree of acrylation,(for method of calculation see section 3.2) increases with increased molar ratios of acryloyl chloride and triethylamine. For instance entries 9 and 10 both had the highest acrylation 70% and 76% respectively, and corresponded to high molar ratios (OH:acrylaoyl chlorid:triethylamine=1:4:4). However, excessive ratios were avoided because of the possibility of causing cleavage of the polymer chain especially as the reaction is exothermic and the need to maintain the reaction at 0°C at least at the early stage.

To ensure that as high a conversion to acrylate was achieved the reaction was allowed to run for 24 hours in accordance with the procedure outlined by Zhang et.al..[192] The ice temperature affected the product quality and if not kept low a deep yellow colour results from the exothermic reaction between the acryloyl chloride and the diol groups of the co-polymer.

Solubilty of the Macromers in water was tested as this was regarded as important if the Macromer is to be used as a crosslinking agent in preparing hydrogels. It was found that a low lactoyl content was required to make the Macromer soluble in water confirming the work of Sawhney.[18] However,water as a solvent introduces the disadvantage of increasing the likelyhood of hydrolysis. As Macromers will be used as branching agents for the preparation of hyperbranched polymers via FRP or CRPs in this proejct, water is not a good solvent to be used for these reactions due to the posibility of hydrolysis. Therefore, their solubility in a range of common organic solvents are of interest and have been tested (Table 4). It can be seen that 1KL3 ,with its increasing hydrophobic lactoyl content, the less soluble it is in polar solvents such as DMF and THF. The reduced solubility or apparent insolubility of these amphilic block copolymers could be caused by their self-assembly behaviors in the solvents. The solubility in any solvent, including water, appears to be dependent on the relative proportions of the hydrophilic PEG and the hydrophobic lactoyl domains.

Entry	Co-polymer	Co-polymer structure	Mol.Wt. (g/mol)	Mole ratio[a]	Acrylation (%)	Macromer Conc. (g/ml)[b]	Reaction Time (Hrs)	Ppt. Formed
1	1KL6-3	$LA_6PEG_{22}LA_6$	1865	1:1.15:1.74	36	0.06	21	No
2	1KL7	$LA_7PEG_{22}LA_7$	2009	1:2.1:4.38	29	0.13	24	No
3	1KL10-1	$LA_{10}PEG_{22}LA_{10}$	2441	1:2.54:4.3	62	0.22	24	YES
4	1KL10-4	$LA_{10}PEG_{22}LA_{10}$	2441	1:2.54:4.3	79	0.30	24	YES
5	1KL8-1	$LA_8PEG_{22}LA_8$	2153	1:1.2:2.5	33	0.06	17	NO
6	1KL8-1	$LA_8PEG_{22}LA_8$	2153	1:1.2:2.5	8	0.06	24	NO
7	1KL8-2	$LA_8PEG_{22}LA_8$	2153	1:1.2:2.5	<1	0.05	24	NO
8	1KL9-2	$LA_9PEG_{22}LA_9$	2009	1:1.2:2.5	<1	0.05	24	YES
9	1KL8-3	$LA_8PEG_{22}LA_8$	2153	01:04:04	70	0.03	22	NO
10	1KL8-4	$LA_8PEG_{22}LA_8$	2297	01:04:04	76	0.1	24	YES
11	1KL3-1	$LA_3PEG_{22}LA_3$	1432	01:04:04	15	0.07	24	YES
12	1KL6-2	$LA_6PEG_{22}LA_6$	1865	1:1.2:2.5	54	0.06	24	YES
13	1KL2	$LA_2PEG_{22}LA_2$	1288	1:1.2:2.5	80	33.86	24	YES
14	1KL3-2	$LA_6PEG_{22}LA_6$	1432	1:1.2:2.5	80	0.08	24	YES
15	1KL10-3	$LA_{10}PEG_{22}LA_{10}$	2550	1:1.2:2.5	58	0.05	24	YES

Table 3: Synthesis of Macromers under various reaction conditions

(a) Mole ratio is the molar ratio of OH: Triethylamine: Acryloyl chloride. (b) No. of grams of Macromer in final volume of reaction mixture.(c) Triethylamine hydrochloride salt formed during acrylation.

Table 4: Physical appearance and solubility of Macromers (100mg/500µl of solvent at room temperature) for 1KL2 (Table 3 entry 13) and 1KL3 (Table 3 entry 14)

Macromers	1KL2	1KL3
Physical Appearance	White viscous liquid	Slight brown semi-solid
In THF	soluble	insoluble
In DMF	soluble	insoluble
In Butanone	v. slight suspension	insoluble
In DCM	fairly soluble	very soluble
In Chloroform	soluble	soluble

3.2.1 Characterisation Of the Macromer (P_{DL}LA-co-PEG-co-P_{DL}LA-diacrylate) using Differential Scanning Calorimetry

The macromer was semi-solid at room temperature, to determine T_g and/or T_m of the sample, DSC measurment was run from -80 °C to 200 °C. An endothermic transition was seen at 128.80°C (Figure 62). This corresponds almost exactly with the T_m of Lactide which was determined as 127.07°C (Figure 51). An inflection can be seen at -45°C which is a T_g point.

Figure 62 give a single heating cycle (heat, cool, heat) for the Macromer 1KL5. A T_g appears at minus 45°C.There appears to be little evidence of a Tm for the PEG and PLA phase and this is taken to mean that the material was homogeneous. This DSC traces are similar to the DSC traces obtained for PEG-PLA copolymer (Figure 52), indicating that the addition of acrylate functional groups has no significant impact on the T_g of the original copolymer.

Figure 63 is the DSC showing the 3 run cyclic trace for the Macromer. A endothermic peak at 23°C in the first run could be due to the T_c for the Macromer. A T_m can be seen in the third run which is assigned to melting of PLA domains. A further three endothermic peaks may be seen at 160°C, 178°C and 191°C which appear not to be first order transitions and would not be further

melting peaks for example. The exact nature remains to be further investigated, possibly decomposition has taken place.

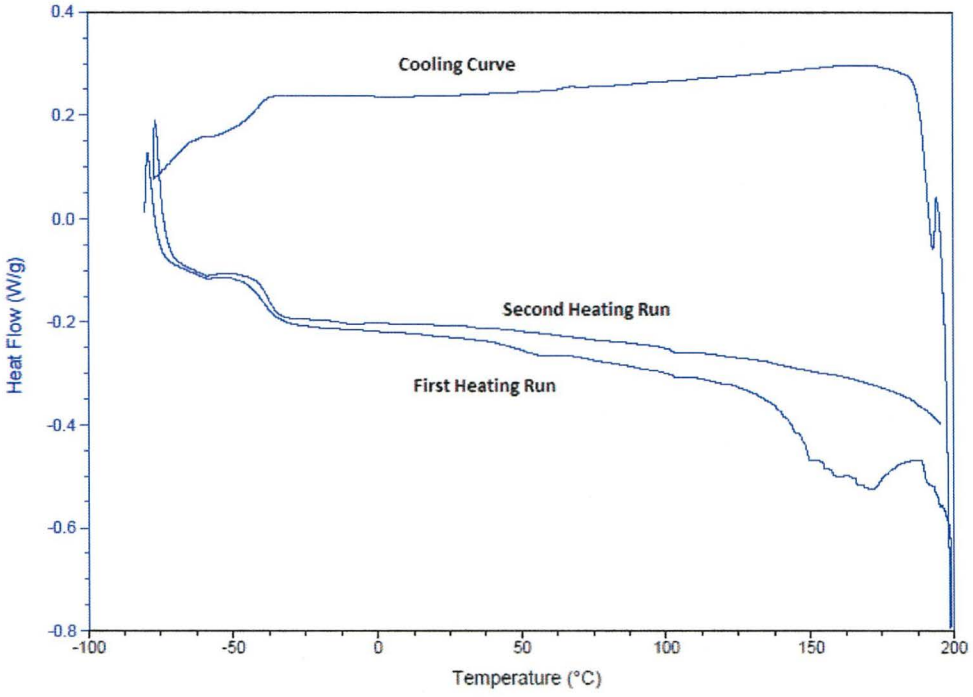


Figure 62: DSC for Macromer 1KL5-1

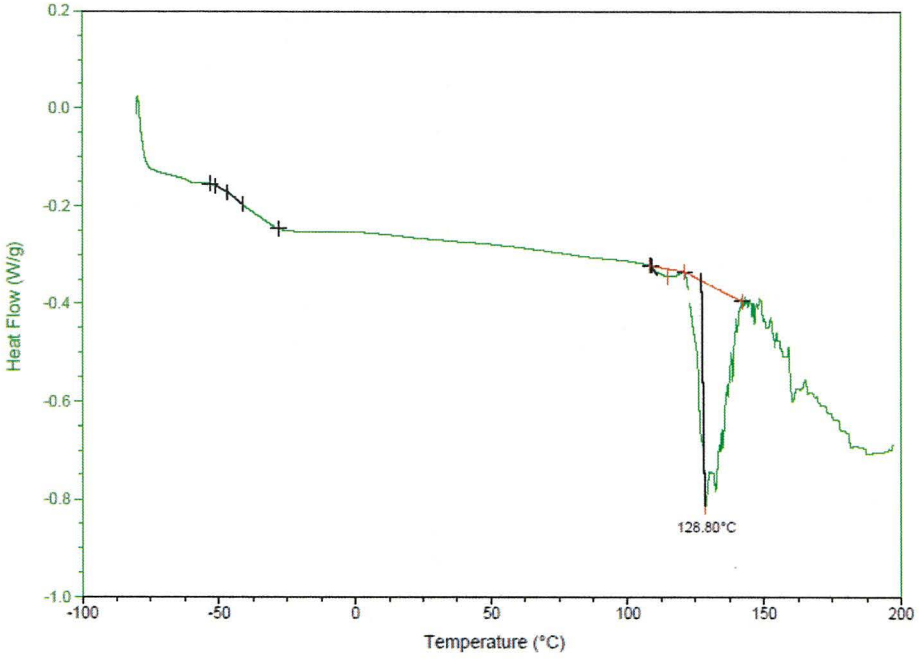


Figure 63: DSC Macromer 1KL5-2 (single heating run)

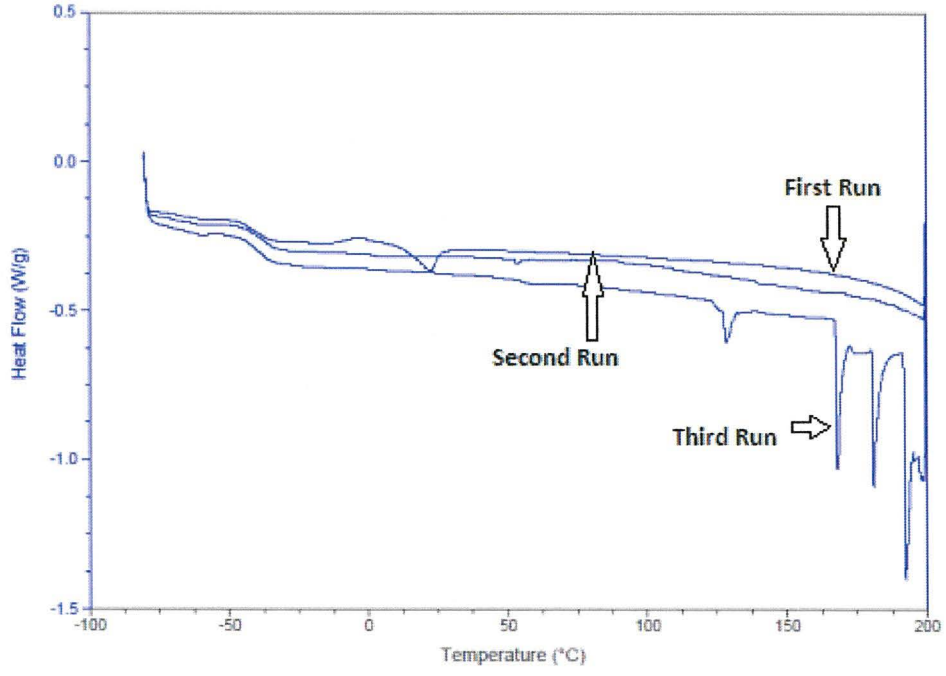


Figure 64: DSC Macromer 1KL5-3 (3 heating runs)

3.2.2 Thermoresponsive Properties-LCST of Co-polymer and Macromer

Varying the ratios of hydrophilic/hydrophobic domains within the co-polymer result in changing the phase transition temperature when in solution in water, the more hydrophilic the copolymer the higher LCST. The LCST for Macromer 1KL10 and co-polymer 1KL10 respectively were determined. The macromer and the copolymer were dissolved in deionized water, and the change in absorbency with increasing temperature was measured at a wavelength of 550 nm. by UV–vis spectrophotometry. The LCST found to be are around 31 °C and 40°C respectively.

These findings also consistent with LCST determinations in which samples of the co-polymer and the Macromer were dissolved in cold de-ionised water which was the heated slowly with stirring. The solutions reversibly became cloudy when the temperature was increased above the LCST for each and the temperature of this transition was ascertained visually. The solutions were transparent below this temperature.The LCST for Macromer 1KL10 and co-polymer 1KL10 by this method were found to be 27°C and 32°C respectively,which is slightly lower than found by spectrophotometry indicating the visual method is a cruder method. Figure 64 shows the temperature scans of the copolymer solutions recorded by the UV spectrophotometer.

The LCSTs of the macromer and the copolymer were measured as about 26 °C and 31°C respectively suggesting they were soluble at temperatures below their LCST, exhibiting a random coil conformation, which undergoes a conformational change, with collapse and aggregation when the temperature is raised above the LCST.

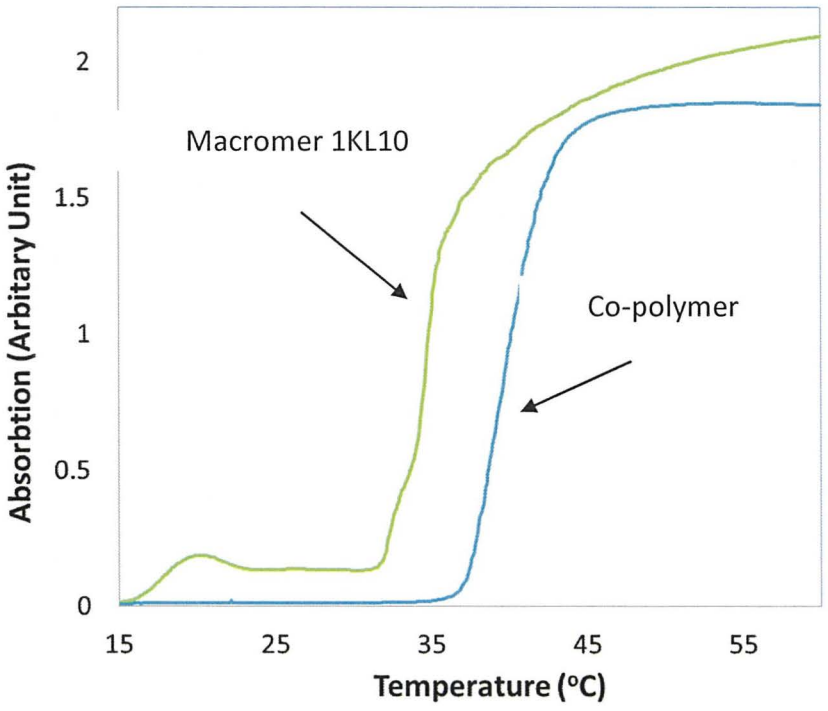


Figure 65: $P_{DL}LA$-Co-PEG-$P_{DL}LA$ 1KL10 co-polymer (blue) and $P_{DL}LA$-Co-PEG-$P_{DL}LA$ co-polymer diacrylate 1KL10 (green).

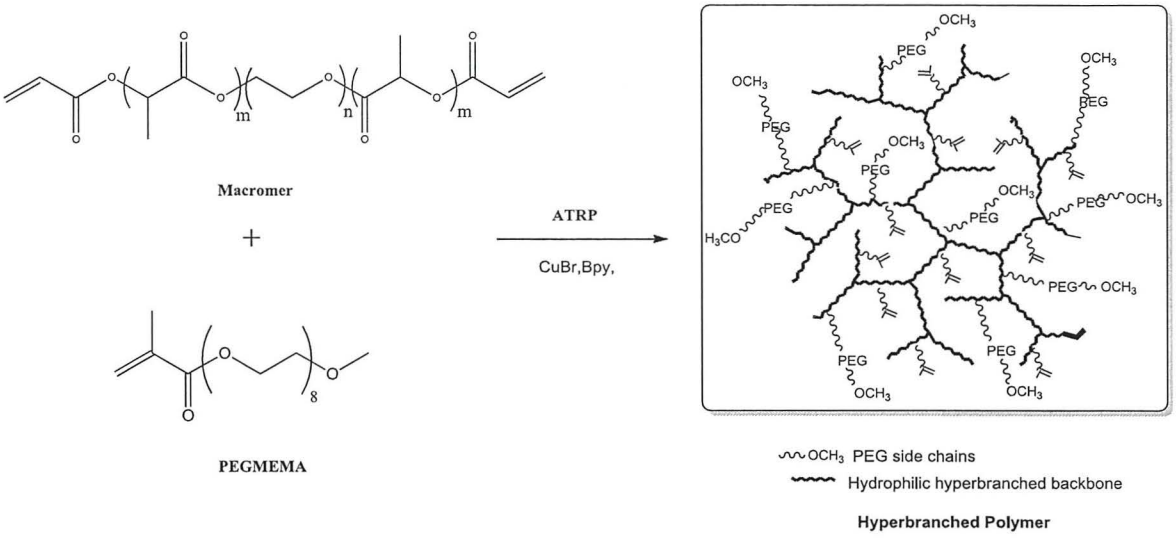
3.3 Synthesis and Characterisation of Hyperbranched Polymers from PEG-co-PLA Diacrylate Macromers

Photocrosslinkable polymers with thermoresponsive properties can be used as injectable scaffolds in tissue engineering. Such stimuli-responsive polymers are required to form stable gels when introduced into defect sites within the tissue and if possible have enhanced mechanical properties[193,194,195,196,197,198] and addition to ease of handling they should retain their shape prior to photo-polymerisation as these are desirable attributes for clinical practice. The Macromer previously synthesized was used as a branching agent when it was co-polymerized with poly(ethylene glycol) methyl ether methylacrylate (PEGMEMA). Free radical polymerisation

(FRP) and atom transfer radical polymerisation (ATRP) were used to achieve a facile one-step polymerisation using PEGMEMA as a monofunctional vinyl monomer and the Macromer as the multifunctional vinyl monomer branching agent. The resultant PEGMEMA-(PEG-lactoyl) hyperbranched copolymer is a highly branched irregular dendritic polymer with a reactive functionality by virtue of surplus vinyl groups and a controlled chain structure. Compared to linear polymers, hyperbranched and dendritic polymers have many advantages, such as low solution and melt viscosity, and high functionality.[198,199,200]

Ideally for clinical practice these co-polymers should be water-soluble co-polymers with a low critical solution temperature (LCST) at *ca.* 37 °C. Such a thermoresponsive polymer which has been widely studied is poly(N-isopropylacrylamide) (PNIPAM) with a rapid coil-to-globule transformation in aqueous solution occurs at around 32 °C.[162]

Thus the targeted hyperbranched copolymers is water soluble by virtue of their hydrophilic PEG domains and cross-linkable by photo-polymerisation due to their multi-vinyl functional groups. Moreover, the cross-linking density of the hyperbranched co-polymers can be finely tuned to tailor their physical properties to yield desirable porous structures and other mechanical properties by adjusting the composition and concentration of the copolymers. The reaction scheme used is as given in Scheme 3 below.



Scheme 3: Preparation of biodegradable hyperbranched polymer.

Variations of the ATRP method were used including Deactivation ATRP in which a halogen-Cu(I)/halogen Cu(II) system is used to deactivate the polymerisation.[117]

A further variation involves the use of a reducing agent such as L-Ascorbic acid in a redox reaction using Cu(II) chloride to generate the Cu(I) ion with the effect of reducing the concentration of the propagating radical (P*). This technique is termed *in situ* de-activation enhanced ATRP (Figure 65).

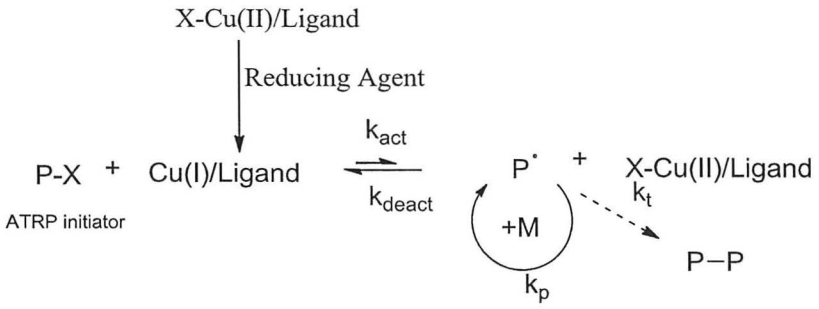


Figure 66: General scheme for *in* situ deactivation enhanced Atom Transfer Free Radical Polymerisation.[115]

Free Radical polymerisation, Atom Transfer Radical Polymerisation (ATRP) (conventional and *in situ* deactivation-enhanced) and RAFT were employed to synthesize the hyperbranched polymers. GPC, FTIR and NMR were used to characterize the products.

The FRP and RAFT initiator used was 1, 1' Azobis(cyanocyclohexane carbonitrile) (Figure 66)

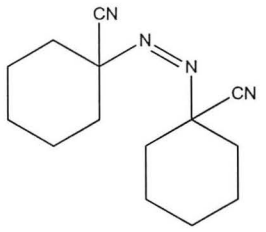


Figure 67: 1, 1' Azobis(cyanocyclohexane carbonitrile)

In contrast the initiator used in ATRP was Ethyl α-bromoisobutyrate (Figure 67).

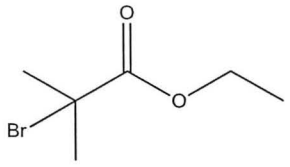


Figure 68: Ethyl α-bromoisobutyrate

3.3.1 Free Radical Polymerisation (FRP) of Macromer and FRP Co-polymerisation of Macromer with PEGMEMA

To demonstrate the capacity of the Macromer to function as a branching agent, a free radical polymerisation was performed with the Macromer alone (homo-polymerisation) and also an FRP was undertaken to co-polymerise the Macromer with another monomer i.e. PEGMEMA, entries 1 and 2 of Table 5 constitute the homo-polymerisations. Polymerisation was found to occur in both solvents DMF and chloroform (entries 1 and 2 in Table 5). However, the conversion was higher in DMF and the PDI is given as 1.03 which indicates a very controlled polymerisation, however, this figure is very suspect and due to incorrect base line consruction. GPC revealed that higher molecular weights were obtained in chloroform but the conversion was 30% lower given that temperature and reaction time were comparable. This difference may reflect the difference in solubility between the two solvents, the chloroform was found to be a good solvent but the DMF yielded a slightly translucent solution because the Macromer 1KL3 was less soluble in DMF.

The co-polymerisations with PEGMEMA are listed in entries 3, 4 and 5 of Table 5. Polymerisation was observed as can be seen from the GPC data, however, the molecular weights obtained were not as high as the homo-polymerisations of the Macromer. If the concentration is reduced, from a monomer to solvent ratio of 1:3 to 1:4, then this caused a lower molecular weight product to be synthesised and this only after doubling the reaction time (see entries 4 and 5 in Table 5). It was also noted that all polymerisations were conducted under heterogeneous conditions.

Table 5: Free Radical Polymerisation (FRP) of Macromer and PEGMEMA

No	Macromer	M/S[(b)]	M/P[(c)]	Solvent	Mw[d] (KDa)	PDI[d]	Conv.(%)[d]	Gel[(e)]	Temp (°C)[(f)]	RT[(g)] (hr)
1	1KL3	1:3	1:0	DMF	224	1.03	29.3	Yes	65	23
2	1KL3	1:3	1:0	Chloroform	551	2.0	19.4	Yes	55	20
3	1KL3	1:3	1:1	Chloroform	269	1.04	13.4	Yes	55	20
4	1KL10	1:1.5	1:9	THF	47	1.49	1.04	Yes	50	21
5	1KL11	1:0.5	1:9	THF	37	1.44	5.00	turbid	50	44

(a) % Acrylation as determined by NMR, (b) Volume Ratio of Total Monomers to solvent, (c) Molar Ratio of Macromer to PEGMEMA, (d) determined by GPC calibrated with PMMA standards at 40°C (e) Gelation observed by inspection (f) temperature in °C. (g) Reaction time in hours.

Figure 68 gives a GPC overlay of the FRP homo-polymerisation of Macromer1KL10 (entry 4) as a comparison with its precursor the co-polymer 1KL10.

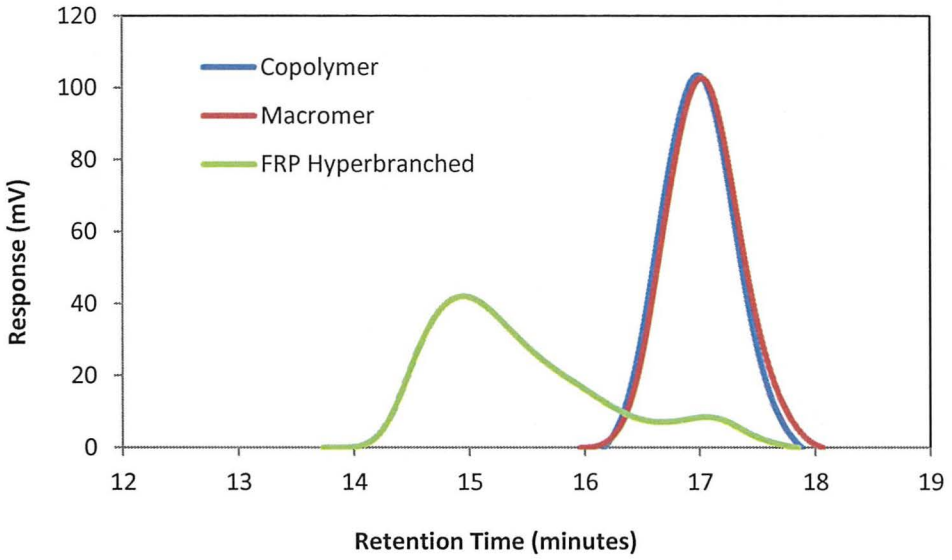


Figure 69: Homo-polymerisation of 1KL10 to produce hyperbranched polymer. GPC overlay with the Macromer only and with the precursor Co-polymer as comparison.

The reactions Table 5 (entries 1-5) were suspension heterogeneous reactions. It is possible that crosslinking of the macromer occurred during storage.

It was found that chloroform as a solvent necessitated its use as the eluent for the GPC column but its refractive index is somewhat closer to the sample under test and may therefore not reveal the full sample signal. The refractive indices (n_D) for the solvents used in this project are: Chloroform-1.446, THF-1.407, DMF-1.431, Acetone-1.359 and Water-1.333.

The project used PEG (M.Wt. 1000) as a component of the co-polymer making up a large fraction of the material. The refractive index (RI) of the PEG is 1.4580 which contrasts with THF of 1.4072, resulting in the greatest RI difference and, therefore, provides greater detection of the PEG based material. Thus, the ideal eluent use would be THF as this gives the greatest contrast in refractive index with the PEG based co-polymers.

Entries 4 and 5 were conducted in THF and the monomer to solvent ratios were generally lower reflecting better solubility. The Macromer was tested in various solvents in the concentration of 0.1grams to 500 μL of solvent and these are tabulated in Table 4.

The PDI for the FRP undertaken were generally fairly narrow and often gave erratic or contradictory figures (see entries 1 and 2). This may be due to the subjective nature of judging the baseline in the chromatograms obtained, but also the GPC samples were obtained upon gelation and, therefore, represent a soluble (selective) extract of the reaction mixture. Consistent figures for PDI were obtained for reactions where THF was used as the solvent, but here the PDI indicates a lack of control over the reaction. The polydispersity (PDI) figures seen reflects a fairly broad population of molecular masses and highlights the uncontrolled nature of FRP. It was therefore decided that a more highly defined polymer was likely if a living polymerisation such as ATRP or RAFT were attempted.

The FTIR spectrum of the PEG (M_W = 1000) copolymer 1KL10 and the co-polymer terminated with acrylate groups (the Macromer)is as shown in Figure 60. The strong absorption at 3510 cm^{-1} in the PEG precursor is due to the terminal hydroxyl group. This signal is reduced in the spectrum of 1KL3 due to acrylation although not eliminated. A strong absorption at 1756 cm^{-1} in the 1KL3 confirms the presence of the ester due to the lactoyl moieties. A signal for the -C=C- in the region 1680-1640 can be seen but is not very strong.

The FTIR of the hyperbranched polymer shows a loss of this signal as the alkene has now cross-linked.

3.3.2 ATRP of Macromers

A Homo-polymerisation of PEGMEMA by Atom Transfer Radical polymerisation (ATRP) was firstly carried out as a comparison to subsequent copolymerisations of PEGMEMA and macromers.

Atom transfer radical polymerisation (ATRP) can be used for the synthesis of a wide range of polymers with increased control over molecular weight and gives a narrow molecular weight distribution (polydispersity, PDI < 1.5). Aqueous ATRP has been found to be generally fast and with products of relatively high polydispersity i.e. poor or no control.[201] The loss of control may be the consequence of several side reactions in aqueous ATRP.[202] Hydrolysis of the ATRP deactivator, X-Cu(II)/Ln, is possibly the main side reaction. This involves an irreversible dissociation of the halide-ligand complex in the higher oxidation state forming a stable inactive species, Cu(II)/Ln. The concentration of the deactivator is decreased thereby reducing the rate of deactivation, resulting in a faster polymerisation and of control. Thus, a sufficient deactivator must be added to the reaction in order to compensate for this. Thus, a modification of the normal ATRP can be made by the addition of a sufficient amount of the halide salts and extra amount of the Cu(II)-halide complex. Also the use of a protic solvent as a co-solvent can lead loss of control over molecular weight distribution.[202] Since the synthesised Macromer is susceptible to acid catalysed hydrolysis the latter should be avoided.

The free-radical polymerisation of poly(ethylene glycol) methyl ether methacrylate (PEGMEMA) has been studied in aqueous media and also using potassium persulfate (KPS) as water soluble initiator.[203] PEGMEMA is of interest as it is biocompatible and thermo-responsible materials.[204]

It was decided, however, to undertake the polymerisation in an organic solvent for two reasons. Firstly, as the reaction is to be a comparison with a similar reaction using the synthesised Macromer this must in a non-aqueous medium as the Macromer is hydrolytically labile. It also appears that no work has been undertaken based on the ATRP technique of PEGMEMA in an aqueous solvent.

Table 6 shows that the ligand used is important as polymerisation occurs when bipyridine (Bpy) is used but PMDTA, when used, must be in a relatively high concentration of at least [I]:[C]:[L]=1:1:4 i.e. when the ligand is at 4 moles for every mole of initiator (see entry 3 in Table 6). Polymerisation occurs in solution but not in bulk (see entries 3 and 4 in Table 6).

Comparing entries 6 and 7, a slightly higher molecular weight was obtained, over a shorter reaction time, for a lower initiator to catalyst/ligand ratio, under the given reaction conditions. However, the difference was not significant.

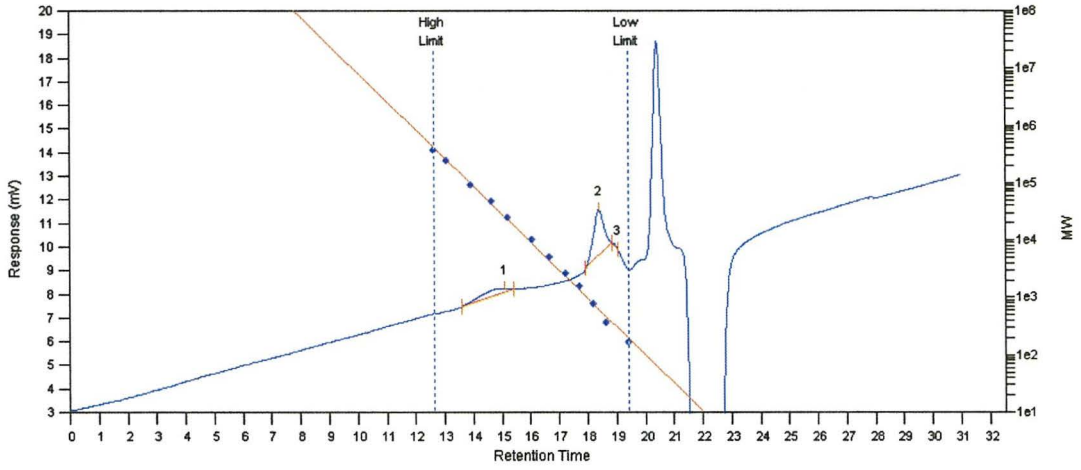
Table 6: Homo-polymerisation of PEGMEMA by conventional ATRP

No	*Solvent volume ratio (1:1)*	*Ligand*	*M_w[a] (daltons)*	*[I]:[C]:[L]*	*Reaction Time (Hr)*
1	Butanone	Bpy	4657	1:2:4	72
2	DMF	PMDTA	No reaction	1:1:2	67
3	DMF	PMDTA	2960	1:1:4	26
4	BULK	PMDTA	No reaction	1:1:2	24
5	DMF	PMDTA	No reaction	1:2:4	117
6	DMF	Bpy	3209	1:2:4	68
7	DMF	Bpy	3594	1:1:2	18

Reaction conditions: 65°C, (a) Molecular weight as determined by GPC, no reaction indicates no polymerisation peak appears in GPC

The deactivation enhanced Atom Transfer Radical Polymerisation (de-ATRP) of Macromer was undertaken. Conventional ATRP uses a halogen/Cu(I)/ligand as the catalyst but in de-ATRP a Cu(I)/halogen -CU(II) mixture is used with the Cu(II) ion deactivating the polymerisation.[116, 145] In *in situ* de-ATRP the Cu(I) is generated within the reaction by the reduction of Cu(II) to the Cu(I) species using a reducing agent such as Ascorbic acid and thus reduces the concentration of the propagating radical (P*).[117] This process is termed AGET –Activator Generated by Electron Transfer ATRP in which a reducing agent is used to generate Cu(I) from Cu(II), and this is found to be a more air tolerant procedure.

A number of reactions undertaken and are listed in Table 7. The reaction was monitored by GPC. Evidence of polymerisation is indicated by GPC (Figure 69). Polymerisation appears to occur giving rise to polymer peak (Peak 1) in the GPC chromatogram. PEGMEMA peak can also be seen (Peaks 2 and 3) at about 500 g/mol. However, the Macromer with molecular weight 2500 was not detected. This indicated that a successful polymerisation occurred. The apparently absent peak for Macromer in GPC curve indicated the Macromer was consumed and should have participated in the copolymerisation. However, homopolymerisation of PEGMEMA is possible. In addition, hydrolysis of Macromer could have occurred and contributed to the peaks 2 and 3 which are low molecular weight macromolecules. The progress of the observed polymerisation is illustrated in Figure 70.



Peak No.	Mp	Mn	Mw	PDI	% Area
1	41691	42398	51064	1.2044	26
2	632	621	643	1.0354	73
3	326	329	330	1.003	<1

Figure 70: GPC of *in-situ* de-ATRP of Macromer 1KL7 and PEGMEMA

Table 7: Reaction conditions for the *in-situ* deactivation enhanced ATRP reactions of Macromers

Entry	Macromer	Mac: PEG	[I]:[M][a]	[I]:[C]:[AA]:[L][b]	[M]:[S][c]	T (°C)
1	1KL5	1:9	1:100	4: 1: 0.2: 1	1:2	50
2	1KL7	1:9	1:100	1: 1: 0.2: 1	1:1	65
3	1KL10	1:9	1:100	4: 1: 0.2: 1	1:2	50
4	1KL10	1:19	1:100	4: 1: 0.2: 1	1:2	50
5	1KL10	1:19	5:100	2: 1: 0.2: 1	1:6	65
6	1KL11	1:19	1:100	2: 1: 0.2: 2	1:1	65
7	1KL10	1:9	1:100	4: 1: 0.2: 1	1:1	55

[a.] The mole ratio of [initiator]:[Monomer]; [b.]The mole ratio of [initiator]:[catalyst]:[ascorbic acid]:[ligand]; [c.]The volume ratio of monomer and solvent

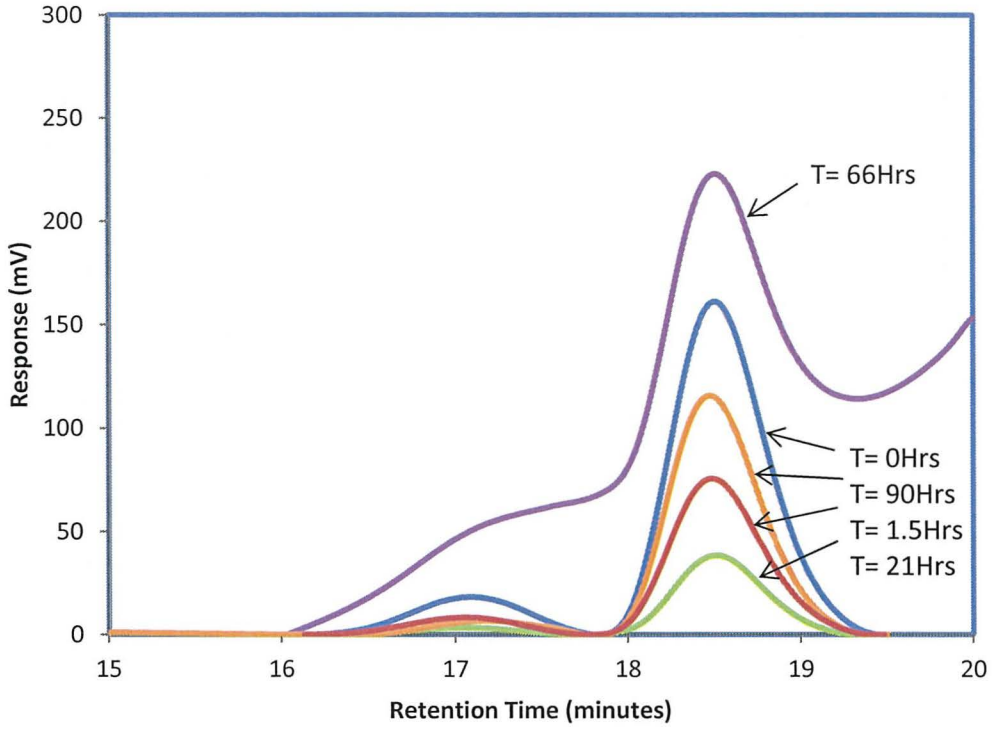


Figure 71: GPC of conventional ATRP of Macromer 1KL10 and PEGMEMA (entry 5 in Table 7)

As can be seen in Figure 70, visual inspection indicates that at T=66 hours polymerisation has occurred as the polymer peak at 17 minutes is relatively high compared to the monomer peak at 18.5 minutes, this is particularly evident when compared to the other curves shown. Normalisation of peaks and samples of the same concentration would permit a quantitative assessment.

ATRP of PEGMEMA with Macromer proved to be a slow reaction with a very low monomer conversion. This could be caused by catalyst poisoning by carboxylic acids which has been documented,[110] because hydrolysis of the macromer can lead to the accumulation of Lactic acid.

Figure 71 demonstrates the comparison of ATRP and FRP from another ATRP experiment to illustrate the more controlled nature of the former. ATRP should give rise to products with a narrower range of molecular weights and the conversion is lower.The FRP (blue curve) gives a very wide range of molecular weights (as shown by the wide range of retention times) as a results of the lack of controllability.

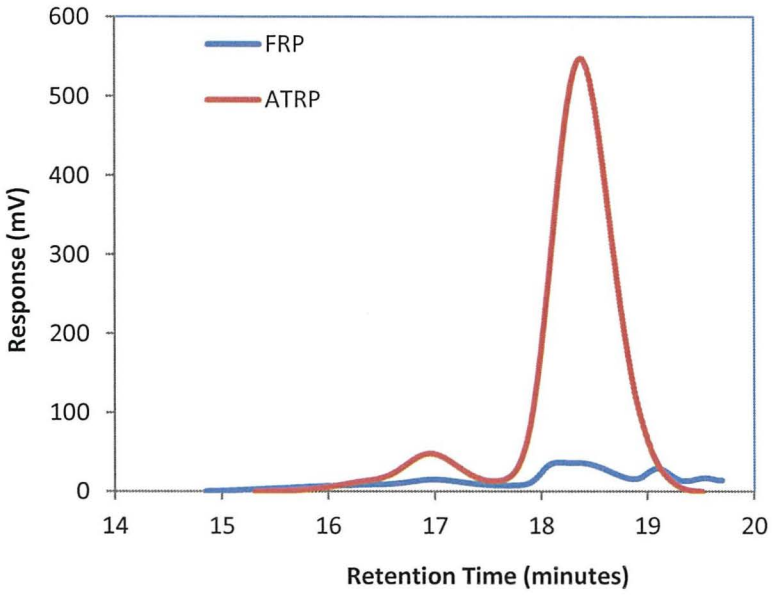


Figure 72: Comparison of ATRP technique with FRP for the Macromer 1KL6 and PEGMEMA

3.3.3 RAFT of Macromers

A further method used in this study was to employ Reversible Addition Fragmentation chain Transfer (RAFT) polymerisation where a chain transfer agent usually a dithio compound is employed. The RAFT agent employed in this project was 2-cyanoprop-2-yl dithiobenzoate (Figure 72) which reversibly traps the majority of the propagating species into the dormant

thiocarbonyl compound. Possible bimolecular termination reactions are thus rendered much less likely.

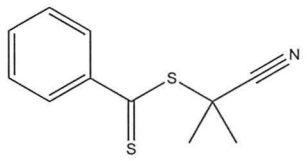


Figure 73: 2-cyanoprop-2-yl dithiobenzoate

The RAFT agent was used in a low concentration of 1% and the aryl and alkyl groups at each end of the molecule are good homolytic leaving groups with respect the propagating polymer chain. The radicals generated must be capable of initiating a polymerisation. Various Macromers were used as branching agents to copolymerise with monomer PEGMEMA. A series of polymerisations were undertaken and are listed in Table 8 below. A polymerisation was observed in entry 1 in which a molecular weight (Mw) of 5374 was attained after 48 hours reaction time. This long reaction time in part reflects the fact that a RAFT polymerisation although a more controlled process with the advantage that the possibility of bi-molecular termination reactions is reduced, is rather slower.

Entry 2 was carried out for a similar Macromer under similar conditions but at a lower temperature with no polymerisation taking place.

Table 8: Reaction conditions of RAFT polymerisations of Macromers with PEGMEMA

Entry No.	Mac.	Mac: PEG ratio	Initiator: Monomers ratio	RAFT/[M][a]	Mon:sol ratio	Reaction time (hrs)	T(°C)	M_w[b] (daltons)
1	1KL10	1:9	1:100	1/100	1:1(THF) heterogeneous	48	65	5374
2	1KL11	1:9	1:100	1/100	1:1(THF) heterogeneous	44	50	None

(a) Ratio of RAFT agent to Monomer (M), (b) Molecular weight (M_w) in daltons, determined by GPC

The GPC trace of Macromer (1KL10) and PEGMEMA is given in Figure 73 below. Macromer 1KL10 and PEGMEMA used as monomers in the molar ratio of 1: 9.The RAFT agent used as a molar percentage of 1% of total monomers. The ratio of monomers to solvent was 1: 1 and solvent used was THF. The initiator 1,1 Azobis (4-cyano valeric acid) was used in the concentration of 1% on a molar basis.

Some evidence of polymerisation was apparent (entry 1 Table 8) and resulted in an increase in molecular weight to M_w 5374 after 48 hours when the reaction was terminated. Peak 3 was the trace for PEGMEMA and peak 2 represents a range of molecular weights that would include the starter Macromer 1KL10 and species resulting from its co-polymerisation with the monomer PEGMEMA. The reaction was not allowed to continue for longer so as to reduce the possibility of hydrolysis.

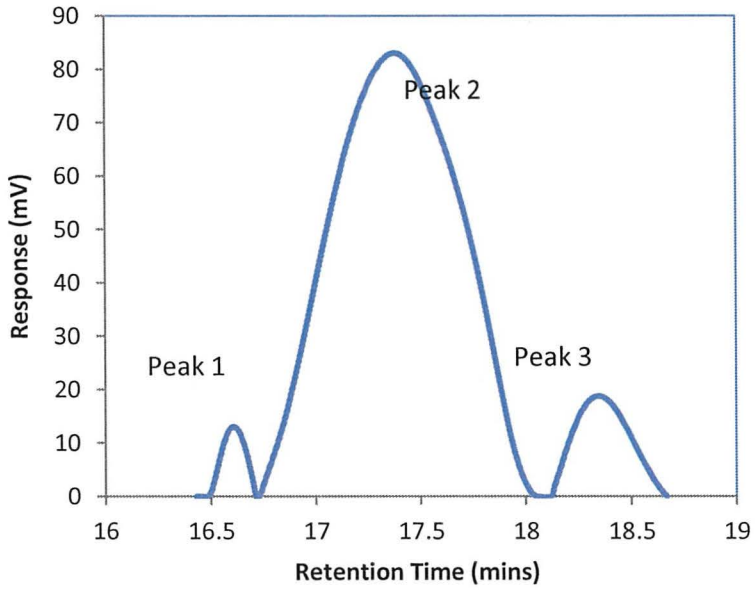


Figure 74: GPC of RAFT of Macromer 1KL10 and PEGMEMA

Table 9: GPC data for Figure 73

Peak No.	Mp	Mn	Mw	PD	% Area
1	5326	5361	5374	1.00	2.57
2	2362	2267	2448	1.08	88.85
3	847	827	838	1.01	8.57

The PEGMEMA peak (peak 3) gives a molecular weight which is has an obviously anomolously high value of Mn = 827 (actual Mn 475). GPC values at this end of the calibration curve are known to be fairly inaccurate. The conversion of monomer to polymer, as indicated by the percentage areas under the peak, are difficult to evaluate as peak 2 contains a population of co-polymers.

The polydispersity index (PDI) of the polymer product in peak 1 is given as unity (1), this is clearly very doubtful in reality. Although RAFT can achieve very narrow PDIs (<1.1)[104] the PDI given is probably an inaccuracy of baseline construction.

The experiment also shows that the reagents used are compatible with the technique which utilises the reversible-fragmentation of a di-thio moiety between active and dormant chains. The technique appears to be tolerant of the acidic conditions pertaining and therefore overcomes the limitation found during the ATRP experiments.

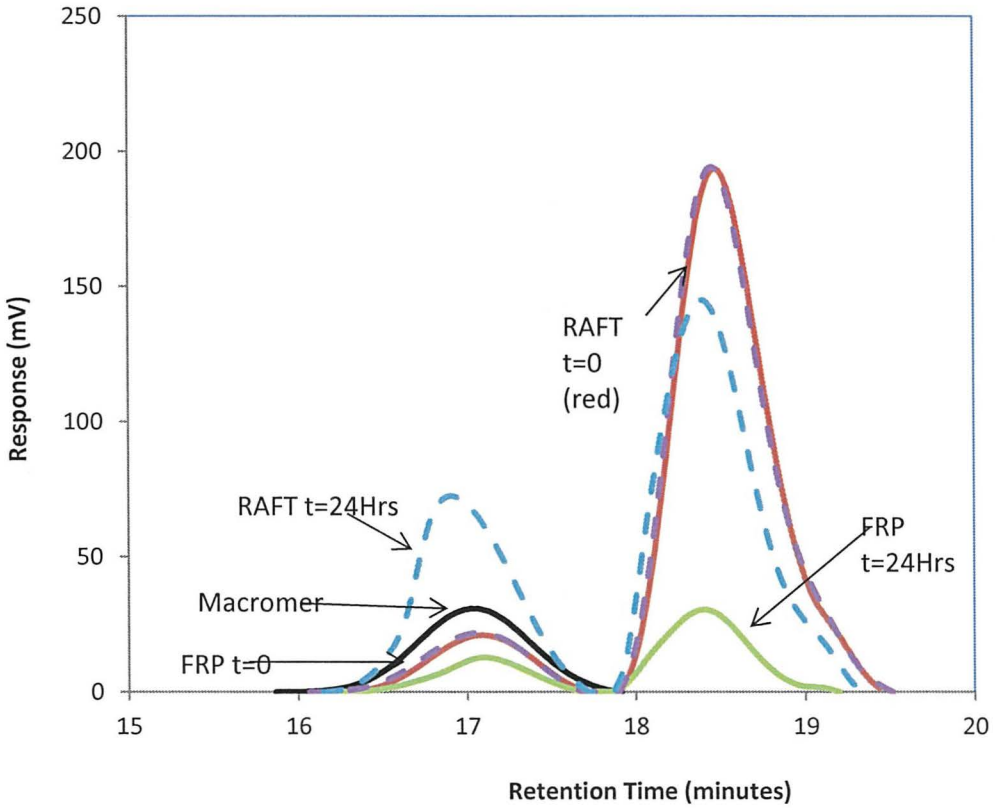


Figure 75: Comparison of GPC traces for FRP and RAFT of Macromer and PEGMEMA

The RAFT technique is designed to produce a living controlled polymerisation to produce narrow poly-dispersities. This is in contrast to Free Radical Polymerisation (FRP) were the poly-dispersity is uncontrolled. To illustrate how both these techniques compare a co-polymerisation of Macromer 1KL10 with PEGMMA was undertaken. An FRP polymerisation and a RAFT polymerisation were performed under the same reaction conditions with the same molar ratio and the results compared.

The GPC traces are presented as an overlay as given in Figure 75.The reaction proceeded slowly and achieved an increase in molecular weight to Mw 5374 after 72 hours.

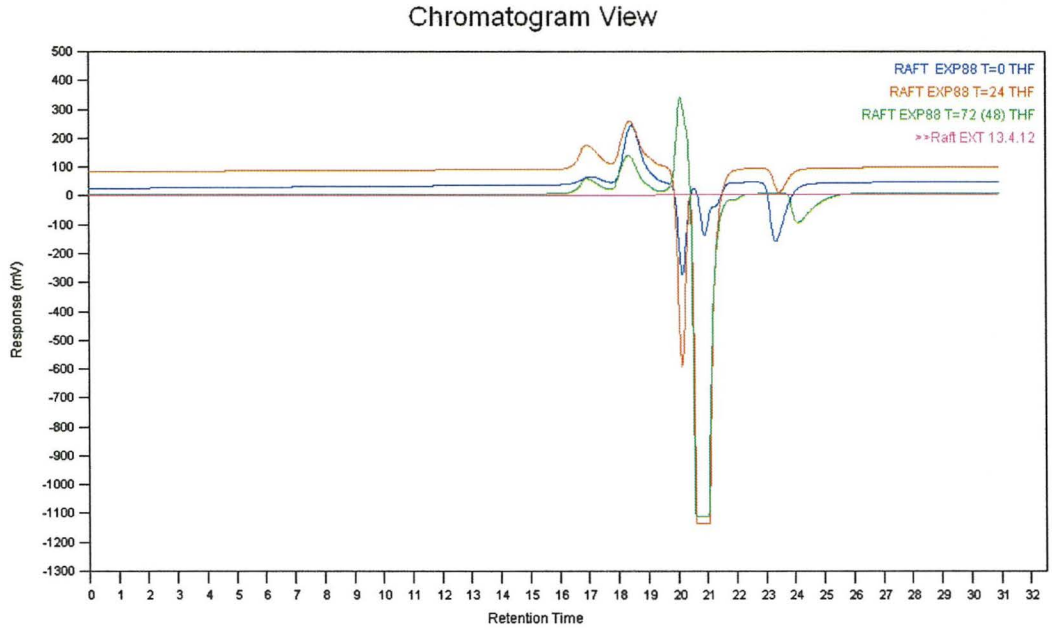


Figure 76: overlay of GPC traces for RAFT method for co-polymerisation of Macromer 1KL10 and PEGMEMA in molar ratio of 1: 9 at 65°C (Initiator and RAFT agent each present in 1% concentration).

The LCST for the Free Radical Polymerisation (FRP) product of co-polymerising Macromer 1KL10 and PEGMEMA was also determined approximately by visual inspection and found to be

11°C but its accurate determination, by thermal analysis profiles, indicate that there were present artefact peaks in the 0-10 ^{0}C region making any establishment of a correlation with temperature difficult. This could be explained by the formation of condensation on the test cuvette in the 0-10 ^{0}C region. This could create light scattering and refraction issues for the samples and with the subsequent evaporation of the condensation at higher temperatures.

3.4 Swelling and Degradation Behavior of Photocrosslinked Hydrogels from Macromers

The PEG-PLA diacrylate Macromer synthesised above was designed for use as a biodegradable branching agent for the preparation of biodegradable hyperbranched polymers. The PEG-PLA diacrylate Macromer was also used as a crosslinking agent to form hydrogels in the presence or absence of another monomer, such as PEGMEMA, which is also water soluble, and to which when co-polymerised with the Macromer has the capacity to form hydrogels when water is added.

With this in mind, a swelling study was undertaken on the hydrogels, derived from the photo-polymerisation of Macromer 1KL3 and on hydrogels derived from the co-polymerisation of Macromer 91 (1KL11) and PEGMEMA. Due to the time constrain of the project, hydrogels have not been prepared using hyperbranched polymers synthesized from PEG-PLA diacrylate Macromer via FRP and CRPs. This will be the following-up further work of this project.

Initially the Macromer was photo-polymerised to make a hydrogel. Table 10 tabulates the Macromer/PEGMEMA ratios used and their feed ratios.

Table 10: Synthesis of hydrogels from photo-polymerisation of Macromers

No	Macromer	Macromer % by Weight	PEGMEMA % by Weight	Concentration[(a)] (%)
1	Macromer 57 (1KL3)	100	0	30
2	Macromer 57 (1KL3)	100	0	54
3	Macromer 91 (1KL11)	100	0	50
4	Macromer 91 (1KL11)	10	90	50
5	Macromer 91 (1KL11)	5	95	50

(a) Concentration of the Macromer/PEGMEMA in water (w/w%)

Photo-polymerisation was conducted at room temperature (25°C) by irradiation with UV light. A 1% aqueous solution was prepared in which the initiator Irgacure 2959 and the Macromer were dissolved. Different concentrations of the Macromer/PEGMMA were subjected to UV exposure. After a given exposure time the resulting solids were gently washed with de-ionised water and dried in a vacuum oven.

Two concentrations were prepared i.e. at 30% and 54% and tested for their swelling characteristics.The dry weight of the material was determined (w_0).

Hydrogels were then made from the co-polymers by the addition of de-ionised water and allowed to soak for a standard time of 24 hours at room temperature (25°C). The excess surface water was gently removed by means of a fine pipette and gently touching the surface with tissue paper ensuring no material is transfered to the tissue. The weight of the swollen gel was taken (w_s).

The swelling ratio was defined as

$$Swelling\ Ratio\ \% = \frac{(w_s - w_0)}{w_0} \times 100 \qquad \text{(Eq.15)}$$

where w_s is the weight of the swollen hydrogel and w_0 is the weight of dried hydrogel.

The swelling ratio was determined. For each time point a plot of the swelling ratio against time was performed (Figure 76).

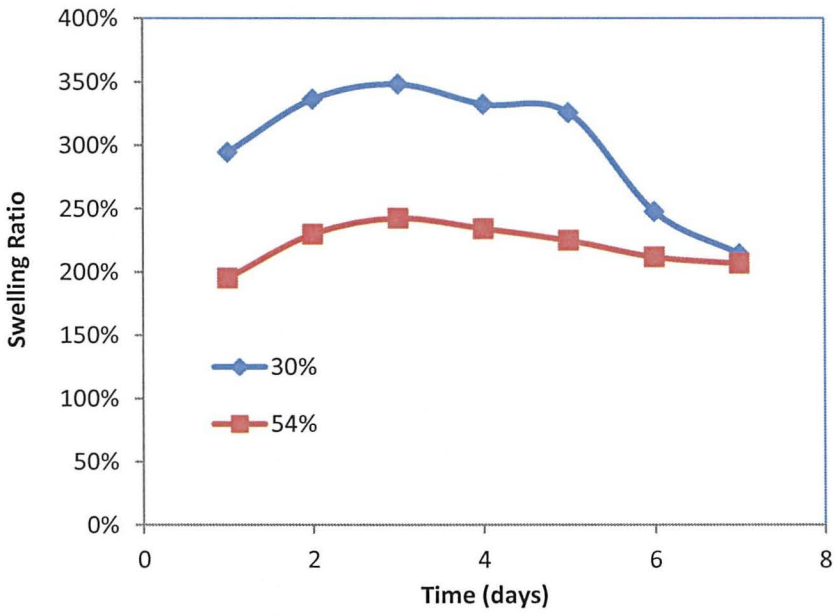


Figure 77: Swelling curves for Macromer 1KL3 in water at room temperture (20 °C) (a) 30% (b) 54%. (The experiments were performed in triplicate and the swelling ratio at each time point was the average of three experimental data)

In Figure 76 a plot is taken of the swelling ratio v Time for Macromer 1KL3 in water, that the 30% hydrogel becomes more swollen than the 54%. This may result from the hydrogel structure being more "open" as a consequence of being a less crosslinked structure formed when less Macromers were used. An open structure with less crosslinking density is likely to be more water absorbant and have a higher swelling ratio.

Both curves have a maximum swelling in 2.5 days approx. when they both start to degrade. This indicates that the 54% hydrogel in not capable of absorbing more water which would be the case if the material were merely slower in uptake due to its density of crosslinking.

It is also observed that, after being fully swollen, degradation occurs but this is more rapid for the 30% hydrogel which is again a consequence of its more open structure. The 30% hydrogel mass with its fewer crosslinking points is likely to disintegrate more rapidly were these links are broken as a result of hydrolysis.

Figure 77(c) shows the swelling curve for a 50% concentration for the Macromer 1KL11 (entry 3 in Table 10) which has a greater lactoyl component than that for Macromer 1KL3 (entry 2). It is observed that entry 3 in Table 10 has higher swelling ratios which could be due to its low crosslinking density compared to entry 2, although the hydrogel from entry 3 has higher lactoyl content which generally confers greater hydrophobicity on the Macromer.

This explanation is given more credence by the fact that 1KL3 (entry 2 in Table 10) could undergo a more rapid degradation than 1KL11 (entry 3) which is due presumably to the greater exposure of its lactoyl domains to water and therefore a greater likelyhood of hydrolytic cleavage.

Hydrogels were then made from co-polymers of Macromer and PEGMEMA (table 10). Referring to Figure 77, we can observe the effect of increasing the PEGMEMA content is to increase the swelling ratio of the material. It is likely that this results from a less compact structure when the Macromer (crosslinking agent) content is reduced. Also the lactoyl content is also reduced thus favouring a greater swelling capacity.

A further set of experiments were performed to determine the swelling/degradation behavior of the co-polymerisation product of Macromer 1KL11 and the monomer PEGMEMA.

The Macromer 1KL11 was used as a crosslinking agent in the synthesis of co-polymers hydrogels in association with the monomer PEGMEMA. The co-polymer hydrogel was synthesised at room temperature (25°C) by photo-polymerisation using UV light, which was used to irradiate a mixture of the Macromer and the PEGMEMA. The monomers were contained in a 1% aqueous solution of the photo-initiator Irgacure 2959. The resulting solids were gently

washed with de-ionised water and dried in a vacuum oven. The dry weight of the material was determined (w_0).

Hydrogels were then soaked in de-ionised water for a standard time of 24 hours at room temperature (25°C). The excess surface water was gently removed by means of a fine pipette and gently touching the surface with tissue paper ensuring no material is transfered to the tissue. The weight of the swollen gel was the taken (w_s).

Various gels were prepared in order to compare the swelling profiles (see Table 11). The swelling ratio was determined for each time point and plotted against time as given in Figure 77.

Table 11: Feed ratios for hydrogels from 1KL 11 used in swelling studies

No.	Mac (g)	PEGMEMA (g)	Weight Ratio	Vol. of water (mL)
1	0.5	Nil	1: 0	1
2	0.05	0.45	1: 9	1
3	0.025	0.475	1:19	1

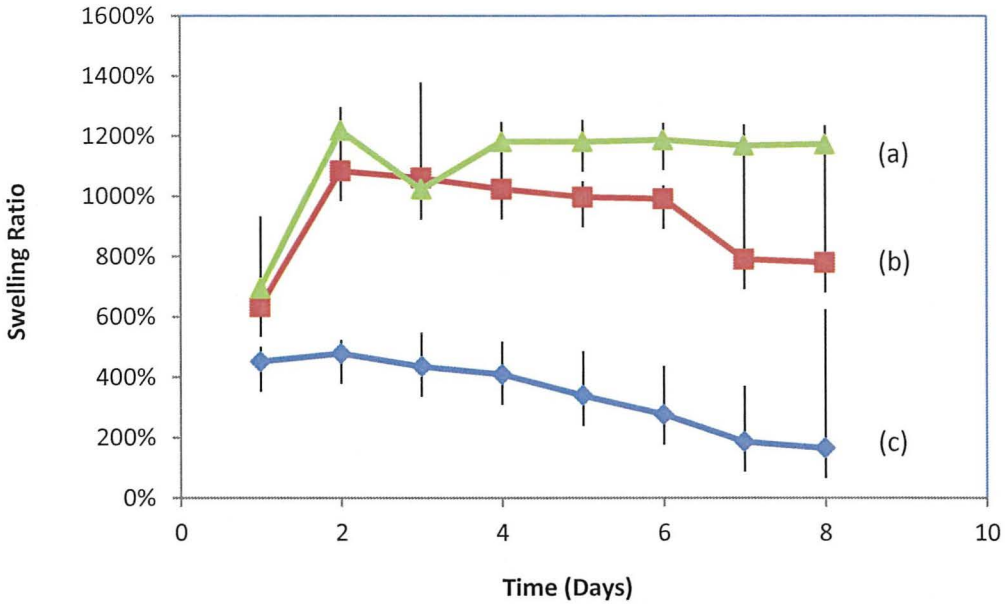


Figure 78: Swelling curves for the three hydrogels used in swelling study i.e. (a) Macromer: PEGMEMA =1 : 19 (w/w), (b) Macromer : PEGMEMA = 1 : 9 (w/w), (c) Macromer only

It may be seen from Figure 77 that both the Macromer and the co-polymers of PEGMEMA absorbed water to achieve swelling. Of the co-polymers, (a) with the higher PEGMEMA content (by molar ratio) had the highest swelling ratio, followed by the other co-polymer and finally the Macromer only exhibited the least swelling (c).

It is thought that the lower concentration of the Macromer crosslinking agent in (a) leads to a more open structure with fewer crosslinking density. This would allow for ready access of water to the hydrophilic regions of the cross linked structure i.e. the PEG domains contained within the Macromer and the PEGMEMA. This would give rise to the highest swelling ratio of the three materials.

The homo-polymerised Macromer (c) formed hydrogels with the lowest swelling ratios. This, in line with the above argument, is due to its close linked structure which hinders water access to the interior structure.

Conversely the hydrophobic regions, due to the presence of the lactoyl domains within the Macromer, is found in its highest ratio in (c) and contributes to the lowest degree of swelling.

The lactoyl content is reduced further in (b) and is lowest of all in (a) which has the highest swelling ratio.

The swelling curves also show a reduction in the swelling ratio with time after an optimum swelling point has been reached. The point at which maximal swelling occurs differs with each material and is reached soonest for (c) the Macromer only occurring within 1 day of soaking. For the PEGMEMA co-polymers (a) maximally swells at 6 days and (b) is seen to swell after 4 days. Thus the order correlates inversely to that for the swelling ratio and the hydrophobicity of the material.

After swelling reaches a maximum the hydrogels begin to degrade. The degradation is due to hydrolysis of the lactoyl domains in the material. The macromer with the highest lactoyl content is seen to commence degradation first. The material with the lowest lactoyl content is seen to be the last to commence degradation.

.033.5 Synthesis of Hydrogels from Michael-addition of Macromers.

The diacrylate Macromer with its vinyl functionality was reasoned to have the potential to undergo the Michael addition type reaction. This reaction should be possible when used in a homo-type addition when used on its own with a suitable thio group. The reaction should also be possible when the Macromer is used as a crosslinking agent to copolymerise with the vinyl functional PEGMEMA.

A number of reactions were performed as per Table 12. It can be seen that the homo-polymerisation of Macromer occurred in all the solvents used i.e. water, DMSO and THF (entries 1,2, 3 and 6). However, most of the reactions with PEGMEMA were unsuccessful (entries 4,7 ,8 and 9).

Table 12: Synthesis of hydrogels from Michael-addition of Macromers

No.	Macromer	PEGMEMA	Solvent	Base	QT/M	Gel[a]
1	1KL11	0	water	TEA	1.2:1	Yes
2	1KL11	0	Water	NaOH	1.2:1	yes
3	1KL11	0	DMSO	TEA	1.8:1	Yes
4	1KL11	90%	DMSO	TEA	1.3:1	No
5	1KL11	90%	THF	TEA	1.3:1	Yes
6	1KL11	0	PBS	PBS	1.9:1	Yes
7	1KL11	50	PBS	TEA	1.5:1	No
8	1KL11	50	DMSO	TEA	1.5:1	No
9	0	100%	DMSO	TEA	1.5:1	No

a) gel is defined as a gelation observed by visual inspection and unable to flow when tube inverted.

The Michael addition requires the presence of a base to act as a catalyst.[87] The basic conditions were supplied by the use of PBS buffer (pH 7.4), triethylamine or sodium hydroxide. The reactions with only the Macromer resulted in a white precipitate which was taken to be that the reaction had occurred.

Co-polymerisation with PEGMEMA was also attempted but did not yield any evidence of reaction apart from that undertaken in the solvent THF.

Although the Michael reaction occurred a substantial gel was not obtained. Further studies on gelation conditions are needed.

3.6 Degradation Analysis for Hydrogels from FRP of Macromer 1KL3

During the swelling study, as the hydrogels degraded, samples of the supernatant were taken and an attempt to analyse the breakdown products was made. The supernatant was analysed by HPLC with UV detector at 230nm wavelength. The retention time of known species was also

determined and these are itemised in Table 13. These substances were chosen as they were used in the synthesis and might still be present in the hydrogel. The PEG and the Lactide were also considered as they might be possible breakdown products.

Table 13: Standards used for analysis of products for degradation of hydrogels from FRP of macromer 1KL3

Sample	λ_{max} nm	Retention Time (minutes)
Macromer 1KL3	230	2.614
Lactide	230	2.673
PEG	230	0.783
Acryloyl chloride	230	2.717
Triethylamine	230	0.0508
Irgacure	230	2.680

The supernatant samples were taken from hydrogels prepared by the FRP of a 54% Macromer solution of the Macromer 1KL3 in water using the photo-initiator Irgacure. This hydrogel was the subject of a swelling study as given in 3.4 above. Samples were taken at Day 2,6,7, 9,19 and 21.

The retention times for the sample runs were noted but no correlation could be observed with the above in Table 13 although a PEG signal was found at 230nm. From this limited study we

can conclude that the starter reagents are not present. A minor adjustment to the analysis would have been to include lactic acid as a standard as this is a possible breakdown product .

To proceed with this study it would be necessary to collect the different fractions from the HPLC run and analyse chemically.

Hydrogel degradation studies can also be conducted using SEM to monitor the morphology change and using the balance to monitor the weight loss during degradation.

Chapter 4: Conclusion and Future Work

A series of $P_{DL}LA$-co-PEG-co-$P_{DL}LA$ copolymers are prepared from poly(ethylene glycol) (PEG) (M_w = 1000 g/mol) and poly(D,L)-lactide ($P_{DL}LA$). The various lengths of the PLA oligomers chains are attached to each of the two terminals of a central PEG domain, the flanking chains are of equal size. These linear block copolymers are characterised by NMR, FTIR, Differential Scanning Calorimetry (DSC), Thermogravimetric analysis (TGA) and gel permeation chromatography (GPC) and their basic physical properties ascertained.

The degree of polymerisation of the PLA domains is found to be dependent on the molar feed ratio of reactants i.e. the PEG and the lactide. All the co-polymers synthesised are soluble in water at the low temperature of 4°C. The water solubility of these copolymers also depends on the molar mass. Thus, a high PEG/$P_{DL}LA$ ratio and a low molar mass confers water solubility compared to the same ratio in a high molecular mass compound. The solubility is found to reflect their amphiphilic nature by virtue of possessing both hydophilic and hydrophobic domains. The PLA portion of the molecule being insoluble in water while the PEG domain was water-soluble

confirming the finding that if the ratio of the degree of polymerisation (DP) of the PLA domain to the DP of the PEG domain is less that 0.45 the molecule will be soluble in water.

Other factors being equal, increasing the reaction time gives a higher yield for the the same co-polymer. The yield obtained is, however, very much dependent on the separation procedure. Different co-polymers with their differential solubilities precipitate to slightly different extents.

The co-polymers when characterised using differential Scanning Calorimetry (DSC) shows the presence of short PLA domains in the co-polymer is found to decrease the melting temperature of the PEG domains relative to the pure PEG. This was attributed to the co-polymer being in a highly homogeneous state and can be taken to imply that co-polymerisation has proceeded to completion as any excess material would be expected to be in a localised form and give rise to a T_m.

A series of macromers are prepared from the P_{DL}LA-co-PEG-co-P_{DL}LA co-polymers by functionalising with diacrylate groups. These linear block copolymers are characterised by NMR, FTIR, Differential Scanning Calorimetry (DSC), Thermogravimetric analysis (TGA) and gel permeation chromatography (GPC).

The degree of acrylation increases with increased molar ratios of acryloyl chloride and triethylamine. However, excessive ratios were avoided because of the possibility of causing cleavage of the polymer chain especially as the reaction is exothermic and the need to maintain the reaction at 0°C at least at the early stage. The ice temperature affected the product quality and if not kept low a deep yellow colour results from the exothermic reaction between the acryloyl chloride and the diol groups of the co-polymer.

Hydrophilicity is regarded as important as the Macromer is to be used as either a crosslinking agent or a branching agent for the preparation of hydrogels. Copolymers and Macromers with a low lactoyl content show a greater water solubility. Their solubility in any solvents, including water, appears to be dependent on the relative proportions of the hydrophilic PEG and the hydrophobic lactoyl domains.

With regard to the thermoresponsive properties (LCST) of co-polymer and Macromer, varying the ratios of hydrophilic/hydrophobic domains within the co-polymer results in changing

the phase transition temperature when in solution in water, the more hydrophilic the copolymer the higher LCST. The Lower Critical Solution Temperature (LCST) is determined by both visual and the UV/vis methods for a selected co-polymer and macromer 1KL10. The LCST of co-polymer and Macromer could be further investigated for a wide range of co-polymers.

Diacrylated P_{DL}LA-co-PEG-co-P_{DL}LA copolymers are used as branching agents to synthesize water soluble hyperbranched polymers by copolymerisation with PEG-based monomers for example PEGMEMA via FRP, ATRP and RAFT approaches. The macromers were also used in a homo-polymerisation and hyperbranching obtained by both Free Radical Polymerisation and by the RAFT method. The resulted hyperbranched polymers are designed for use in the preparation of biodegradable hydrogels. Fine tuning of the P_{DL}LA-co-PEG-co-P_{DL}LA macromer constituents and its combination with co-monomers will give the resulting hydrogels a range of hydrophobic and hydrophilic properties and tailored swelling and drug release profiles.

ATRP proved to be unsuccessful in achieving an effective polymerisation. Catalyst poisoning by carboxylic acids is thought to be a contributory factor in the copper based ATRP and these effects have been documented. [110] [109] [105] In ATRP, hydrolysis of the macromer lead to the accumulation of Lactic acid giving rise to acidic conditions. The use of a buffer system should be investigated to see if this can overcome the problem.

References

1. Peppas, N. A.; Hilt, J. Z.; Khademhosseini, A.; Langer, R., Hydrogels in biology and medicine: From molecular principles to bionanotechnology. *Advanced Materials* **2006,** 18, (11), 1345-1360.
2. Thiele, J.; Ma, Y.; Bruekers, S. M. C.; Ma, S.; Huck, W. T. S., 25th Anniversary Articles: Designer Hydrogels for Cell Cultures: A Materials Selectioin Guide. *Advanced Materials* **2014,** 26, 125-148.
3. Censi, R.; Di Martino, P.; Vermonden, T.; Hennink, W. E., Hydrogels for protein delivery in tissue engineering. *J. Control. Release* **2012,** 161, (2), 680-692.
4. Hennink, W. E.; van Nostrum, C. F., Novel crosslinking methods to design hydrogels. *Advanced Drug Delivery Reviews* **2012,** 64, 223-236.
5. Campoccia, D.; Doherty, P.; Radice, M.; Brun, P.; Abatangelo, G.; Williams, D. F., Semisynthetic resorbable materials from hyaluronan esterification. *Biomaterials* **1998,** 19, (23), 2101-2127.
6. Prestwich, G. D.; Marecak, D. M.; Marecek, J. F.; Vercruysse, K. P.; Ziebell, M. R., Controlled chemical modification of hyaluronic acid: synthesis, applications, and biodegradation of hydrazide derivatives. *J. Control. Release* **1998,** 53, (1-3), 93-103.
7. Hoffman, A. S., Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews* **2012,** 64, 18-23.
8. Brondsted, H.; Andersen, C.; Hovgaard, L., Crosslinked dextran - a new capsule material for colon targeting of drugs. *Journal of Controlled Release* **1998,** 53, (1-3), 7-13.
9. Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S., Poly(ethylene glycol) in Drug Delivery: Pros and Cons as Well as Potential Alternatives. *Angewandte Chemie-International Edition* **2010,** 49, (36), 6288-6308.
10. Park, H.; Park, K., Biocompatibility issues of implantable drug delivery systems. *Pharmaceutical Research* **1996,** 13, (12), 1770-1776.
11. Tan, H.; Marra, K. G., Injectable, Biodegradable Hydrogels for Tissue Engineering Applications. *Materials* **2010,** 3, (3), 1746-1767.
12. Kong, H. J.; Wong, E.; Mooney, D. J., Independent control of rigidity and toughness of polymeric hydrogels. *Macromolecules* **2003,** 36, (12), 4582-4588.
13. Lin, C.; Gitsov, I., Preparation and Characterization of Novel Amphiphilic Hydrogels with Covalently Attached Drugs and Fluorescent Markers. *Macromolecules* **2010,** 43, (23), 10017-10030.
14. Censi, R.; Schuurman, W.; Malda, J.; di Dato, G.; Burgisser, P. E.; Dhert, W. J. A.; van Nostrum, C. F.; di Martino, P.; Vermonden, T.; Hennink, W. E., A Printable Photopolymerizable Thermosensitive p(HPMAm-lactate)-PEG Hydrogel for Tissue Engineering. *Advanced Functional Materials* **2011,** 21, (10), 1833-1842.
15. Hunt, N. C.; Grover, L. M., Cell encapsulation using biopolymer gels for regenerative medicine. *Biotechnology Letters* **2010,** 32, (6), 733-742.
16. Mann, C.; Leckband, D., Measuring Traction Forces in Long-Term Cell Cultures. *Cellular and Molecular Bioengineering* **2010,** 3, (1), 40-49.
17. Liu, Y. X.; Chan-Park, M. B., Hydrogel based on interpenetrating polymer networks of dextran and gelatin for vascular tissue engineering. *Biomaterials* **2009,** 30, (2), 196-207.
18. Sawhney, A. S.; Pathak, C. P.; Hubbell, J. A., Bioerodible Hydrogels Based on Photopolymerized Poly(Ethylene Glycol)-Co-Poly(Alpha-Hydroxy Acid) Diacrylate Macromers. *Macromolecules* **1993,** 26, (4), 581-587.
19. Hennink, W. E.; van Nostrum, C. F., Novel crosslinking methods to design hydrogels. *Advanced Drug Delivery Reviews* **2002,** 54, (1), 13-36.

20. Herfurth, C.; de Molina, P. M.; Wieland, C.; Rogers, S.; Gradzielski, M.; Laschewsky, A., One-step RAFT synthesis of well-defined amphiphilic star polymers and their self-assembly in aqueous solution. *Polymer Chemistry* **2012,** 3, (6), 1606-1617.
21. Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W., Biodegradable block copolymers as injectable drug-delivery systems. *Nature* **1997,** 388, (6645), 860-862.
22. Jiang, G.; Liu, C.; Liu, X.; Chen, Q.; Zhang, G.; Yang, M.; Liu, F., Network structure and compositional effects on tensile mechanical properties of hydrophobic association hydrogels with high mechanical strength. *Polymer* **2010,** 51, (6), 1507-1515.
23. Gacesa, P., Alginates. *Carbohydrate Polymers* **1988,** 8, (3), 161-182.
24. Goosen, M. F. A.; Oshea, G. M.; Gharapetian, H. M.; Chou, S.; Sun, A. M., Optimization Of Microencapsulation Parameters - Semipermeable Microcapsules As A Bioartificial Pancreas. *Biotechnology and Bioengineering* **1985,** 27, (2), 146-150.
25. Gombotz, W. R.; Wee, S. F., Protein release from alginate matrices. *Advanced Drug Delivery Reviews* **1998,** 31, (3), 267-285.
26. Polk, A.; Amsden, B.; Deyao, K.; Peng, T.; Goosen, M. F. A., Controlled-Release Of Albumin From Chitosan-Alginate Microcapsules. *Journal of Pharmaceutical Sciences* **1994,** 83, (2), 178-185.
27. Liu, L. S.; Liu, S. Q.; Ng, S. Y.; Froix, M.; Ohno, T.; Heller, J., Controlled release of interleukin-2 for tumour immunotherapy using alginate/chitosan porous microspheres. *Journal of Controlled Release* **1997,** 43, (1), 65-74.
28. Lim, F.; Sun, A. M., Microencapsulated Islets As Bioartificial Endocrine Pancreas. *Science* **1980,** 210, (4472), 908-910.
29. Hashidzume, A.; Tomatsu, I.; Harada, A., Interaction of cyclodextrins with side chains of water soluble polymers: A simple model for biological molecular recognition and its utilization for stimuli-responsive systems. *Polymer* **2006,** 47, (17), 6011-6027.
30. Choi, H. S.; Yui, N., Design of rapidly assembling supramolecular systems responsive to synchronized stimuli. *Progress in Polymer Science* **2006,** 31, (2), 121-144.
31. van de Manakker, F.; van der Pot, M.; Vermonden, T.; van Nostrum, C. F.; Hennink, W. E., Self-assembling hydrogels based on beta-cyclodextrin/cholesterol inclusion complexes. *Macromolecules* **2008,** 41, (5), 1766-1773.
32. van de Manakker, F.; Vermonden, T.; el Morabit, N.; van Nostrum, C. F.; Hennink, W. E., Rheological Behavior of Self-Assembling PEG-beta-Cyclodextrin/PEG-Cholesterol Hydrogels. *Langmuir* **2008,** 24, (21), 12559-12567.
33. Hiemstra, C.; Zhong, Z. Y.; Dijkstra, P.; Feijen, J., Stereocomplex mediated gelation of PEG-(PLA)(2) and PEG-(PLA)(8) block copolymers. *Macromolecular Symposia* **2005,** 224, 119-131.
34. Hiemstra, C.; Zhong, Z.; Li, L.; Dijkstra, P. J.; Feijen, J., In-situ formation of biodegradable hydrogels by stereocomplexation of PEG-(PLLA)(8) and PEG-(PDLA)(8) star block copolymers. *Biomacromolecules* **2006,** 7, (10), 2790-2795.
35. Hoare, T. R.; Kohane, D. S., Hydrogels in drug delivery: Progress and challenges. *Polymer* **2008,** 49, (8), 1993-2007.
36. Frisman, I.; Orbach, R.; Seliktar, D.; Bianco-Peled, H., Structural investigation of PEG-fibrinogen conjugates. *Journal of Materials Science-Materials in Medicine* **2010,** 21, (1), 73-80.
37. Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A., Reversible hydrogels from self-assembling artificial proteins. *Science* **1998,** 281, (5375), 389-392.
38. Wang, C.; Stewart, R. J.; Kopecek, J., Hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains. *Nature* **1999,** 397, (6718), 417-420.
39. Tang, A.; Wang, C.; Stewart, R. J.; Kopecek, J., The coiled coils in the design of protein-based constructs: hybrid hydrogels and epitope displays. *Journal of Controlled Release* **2001,** 72, (1-3), 57-70.
40. Forster, S.; Antonietti, M., Amphiphilic block copolymers in structure-controlled nanomaterial hybrids. *Advanced Materials* **1998,** 10, (3), 195-+.

41. Lee, D. S.; Jeong, B.; Bae, Y. H.; Kim, S. W.; Controlled Release, S. O. C., New thermoreversible and biodegradable block copolymer hydrogels. *23rd International Symposium on Controlled Release of Bioactive Materials, 1996 Proceedings* **1996**, 228-229.
42. Jeong, B.; Bae, Y. H.; Kim, S. W., Thermoreversible gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions. *Macromolecules* **1999,** 32, (21), 7064-7069.
43. Jeong, B. M.; Lee, D. S.; Shon, J. I.; Bae, Y. H.; Kim, S. W., Thermoreversible Gelation of Poly(Ethylene Oxide)Biodegradable Polyester Block Copolymers. *Journal of Polymer Science Part a-Polymer Chemistry* **1999,** 37, (6), 751-760.
44. Rashkov, I.; Manolova, N.; Li, S. M.; Espartero, J. L.; Vert, M., Synthesis, characterization, and hydrolytic degradation of PLA/PEO/PLA triblock copolymers with short poly(L-lactic acid) chains. *Macromolecules* **1996,** 29, (1), 50-56.
45. Li, S. M.; Rashkov, I.; Espartero, J. L.; Manolova, N.; Vert, M., Synthesis, characterization, and hydrolytic degradation of PLA/PEO/PLA triblock copolymers with long poly(L-lactic acid) blocks. *Macromolecules* **1996,** 29, (1), 57-62.
46. Hu, D. S. G.; Liu, H. J., Structural-Analysis And Degradation Behavior In Polyethylene-Glycol Poly (L-Lactide) Copolymers. *Journal of Applied Polymer Science* **1994,** 51, (3), 473-482.
47. Kricheldorf, H. R.; Meierhaack, J., Polylactones .22. ABA Triblock Copolymers Of L-Lactide And Poly(Ethylene Glycol). *Makromolekulare Chemie-Macromolecular Chemistry and Physics* **1993,** 194, (2), 715-725.
48. Li, Y. X.; Kissel, T., Synthesis And Properties Of Biodegradable Aba Triblock Copolymers Consisting Of Poly(L-Lactic Acid) Or Poly(L-Lactic-Co-Glycolic Acid) A-Blocks Attached To Central Poly(Oxyethylene) B-Blocks. *Journal of Controlled Release* **1993,** 27, (3), 247-257.
49. Molina, I.; Li, S. M.; Martinez, M. B.; Vert, M., Protein release from physically crosslinked hydrogels of the PLA/PEO/PLA triblock copolymer-type. *Biomaterials* **2001,** 22, (4), 363-369.
50. Huh, K. M.; Bae, Y. H., Synthesis and characterization of poly(ethylene glycol)/poly(L-lactic acid) alternating multiblock copolymers. *Polymer* **1999,** 40, (22), 6147-6155.
51. Bae, Y. H.; Huh, K. M.; Kim, Y.; Park, K. H., Biodegradable amphiphilic multiblock copolymers and their implications for biomedical applications. *Journal of Controlled Release* **2000,** 64, (1-3), 3-13.
52. Eagland, D.; Crowther, N. J.; Butler, C. J., Complexation Between Polyoxyethylene And Polymethacrylic Acid - The Importance Of The Molar-Mass Of Polyoxyethylene. *European Polymer Journal* **1994,** 30, (7), 767-773.
53. Bell, C. L.; Peppas, N. A., Modulation of drug permeation through interpolymer complexed hydrogels for drug delivery applications. *Journal of Controlled Release* **1996,** 39, (2-3), 201-207.
54. Mathur, A. M.; Hammonds, K. F.; Klier, J.; Scranton, A. B., Equilibrium swelling of poly(methacrylic acid-g-ethylene glycol) hydrogels - Effect of swelling medium and synthesis conditions. *Journal of Controlled Release* **1998,** 54, (2), 177-184.
55. Haglund, B. O.; Josi, R.; Himmelstein, K. J., An in situ gelling system for parenteral delivery. *Journal of Controlled Release* **1996,** 41, (3), 229-235.
56. Nagahara, S.; Matsuda, T., Hydrogel formation via hybridization of oligonucleotides derivatized in water-soluble vinyl polymers. *Polymer Gels and Networks* **1996,** 4, (2), 111-127.
57. Wichterle, O.; Lim, D., Hydrophilic Gels for Biological Use. *Nature* **1960,** 185, 2.
58. Langer, R. S.; Peppas, N. A., Present And Future Applications Of Biomaterials In Controlled Drug Delivery Systems. *Biomaterials* **1981,** 2, (4), 201-214.
59. Bettini, R.; Colombo, P.; Peppas, N. A., Solubility Effects On Drug Transport Through Ph-Sensitive, Swelling-Controlled Release Systems - Transport Of Theophylline And Metoclopramide Monohydrochloride. *Journal of Controlled Release* **1995,** 37, (1-2), 105-111.
60. Cicek, H.; Tuncel, A., Immobilization of alpha-chymotrypsin in thermally reversible isopropylacrylamide-hydroxyethylmethacrylate copolymer gel. *Journal of Polymer Science Part a-Polymer Chemistry* **1998,** 36, (4), 543-552.
61. Edman, P.; Ekman, B.; Sjoholm, I., Immobilization Of Proteins In Microspheres Of Biodegradable Polyacryldextran. *Journal of Pharmaceutical Sciences* **1980,** 69, (7), 838-842.

62. Vandijkwolthuis, W. N. E.; Franssen, O.; Talsma, H.; Vansteenbergen, M. J.; Vandenbosch, J. J. K.; Hennink, W. E., Synthesis, Characterization, And Polymerization Of Glycidyl Methacrylate Derivatized Dextran. *Macromolecules* **1995,** 28, (18), 6317-6322.
63. Park, K., Enzyme-Digestible Swelling Hydrogels As Platforms For Long-Term Oral-Drug Delivery - Synthesis And Characterization. *Biomaterials* **1988,** 9, (5), 435-441.
64. Jin, Y.; Yamanaka, J.; Sato, S.; Miyata, I.; Yomota, C.; Yonese, M., Recyclable characteristics of hyaluronate-polyhydroxyethyl acrylate blend hydrogel for controlled releases. *Journal of Controlled Release* **2001,** 73, (2-3), 173-181.
65. Stubbe, B.; Maris, B.; Van den Mooter, G.; De Smedt, S. C.; Demeester, J., The in vitro evaluation of 'azo containing polysaccharide gels' for colon delivery. *Journal of Controlled Release* **2001,** 75, (1-2), 103-114.
66. Ferreira, L.; Vidal, M. M.; Geraldes, C.; Gil, M. H., Preparation and characterisation of gels based on sucrose modified with glycidyl methacrylate. *Carbohydrate Polymers* **2000,** 41, (1), 15-24.
67. Hennink, W. E.; Talsma, H.; Borchert, J. C. H.; DeSmedt, S. C.; Demeester, J., Controlled release of proteins from dextran hydrogels. *J. Control. Release* **1996,** 39, (1), 47-55.
68. vanDijkWolthuis, W. N. E.; Hoogeboom, J. A. M.; vanSteenbergen, M. J.; Tsang, S. K. Y.; Hennink, W. E., Degradation and release behavior of dextran-based hydrogels. *Macromolecules* **1997,** 30, (16), 4639-4645.
69. Peppas, N. A.; Benner, R. E., Proposed Method Of Intracordal Injection And Gelation Of Poly (Vinyl Alcohol) Solution In Vocal Cords - Polymer Considerations. *Biomaterials* **1980,** 1, (3), 158-162.
70. Dai, W. S.; Barbari, T. A., Hydrogel membranes with mesh size asymmetry based on the gradient crosslinking of poly(vinyl alcohol). *Journal of Membrane Science* **1999,** 156, (1), 67-79.
71. Tabata, Y.; Ikada, Y., Synthesis Of Gelatin Microspheres Containing Interferon. *Pharmaceutical Research* **1989,** 6, (5), 422-427.
72. Yamamoto, M.; Tabata, Y.; Hong, L.; Miyamoto, S.; Hashimoto, N.; Ikada, Y., Bone regeneration by transforming growth factor beta 1 released from a biodegradable hydrogel. *Journal of Controlled Release* **2000,** 64, (1-3), 133-142.
73. Jameela, S. R.; Jayakrishnan, A., Glutaraldehyde Cross-Linked Chitosan Microspheres As A Long-Acting Biodegradable Drug-Delivery Vehicle - Studies On The In-Vitro Release Of Mitoxantrone And In-Vivo Degradation Of Microspheres In Rat Muscle. *Biomaterials* **1995,** 16, (10), 769-775.
74. Draye, J. P.; Delaey, B.; Van de Voorde, A.; Van Den Bulcke, A.; Bogdanov, B.; Schacht, E., In vitro release characteristics of bioactive molecules from dextran dialdehyde cross-linked gelatin hydrogel films. *Biomaterials* **1998,** 19, (1-3), 99-107.
75. Bos, G. W.; Jacobs, J. J. L.; Koten, J. W.; Van Tomme, S.; Veldhuis, T.; van Nostrum, C. F.; Den Otter, W.; Hennink, W. E., In situ crosslinked biodegradable hydrogels loaded with IL-2 are effective tools for local IL-2 therapy. *European Journal of Pharmaceutical Sciences* **2004,** 21, (4), 561-567.
76. Brondsted, H.; Hovgaard, L.; Simonsen, L., Dextran Hydrogels For Colon-Specific Drug-Delivery .4. Comparative Release Study Of Hydrocortisone And Prednisolone Sodium-Phosphate. *Stp Pharma Sciences* **1995,** 5, (1), 65-69.
77. Gehrke, S. H.; Uhden, L. H.; McBride, J. F., Enhanced loading and activity retention of bioactive proteins in hydrogel delivery systems. *Journal of Controlled Release* **1998,** 55, (1), 21-33.
78. Kamath, K. R.; Park, K., Biodegradable Hydrogels In Drug-Delivery. *Advanced Drug Delivery Reviews* **1993,** 11, (1-2), 59-84.
79. Elbert, D. L.; Pratt, A. B.; Lutolf, M. P.; Halstenberg, S.; Hubbell, J. A., Protein delivery from materials formed by self-selective conjugate addition reactions. *J. Control. Release* **2001,** 76, (1-2), 11-25.
80. Kuijpers, A. J.; van Wachem, P. B.; van Luyn, M. J. A.; Engbers, G. H. M.; Krijgsveld, J.; Zaat, S. A. J.; Dankert, J.; Feijen, J., In vivo and in vitro release of lysozyme from cross-linked gelatin hydrogels: a model system for the delivery of antibacterial proteins from prosthetic heart valves. *Journal of Controlled Release* **2000,** 67, (2-3), 323-336.
81. Eiselt, P.; Lee, K. Y.; Mooney, D. J., Rigidity of two-component hydrogels prepared from alginate and poly(ethylene glycol)-diamines. *Macromolecules* **1999,** 32, (17), 5561-5566.

82. Valdesaguilera, O.; Pathak, C. P.; Shi, J.; Watson, D.; Neckers, D. C., Photopolymerization Studies Using Visible-Light Photoinitiators. *Macromolecules* **1992,** 25, (2), 541-547.
83. Elisseeff, J.; Anseth, K.; Sims, D.; McIntosh, W.; Randolph, M.; Langer, R., Transdermal photopolymerization for minimally invasive implantation. *Proceedings of the National Academy of Sciences of the United States of America* **1999,** 96, (6), 3104-3107.
84. Lu, S. X.; Anseth, K. S., Photopolymerization of multilaminated poly(HEMA) hydrogels for controlled release. *J. Control. Release* **1999,** 57, (3), 291-300.
85. Ward, J. H.; Peppas, N. A., Preparation of controlled release systems by free-radical UV polymerizations in the presence of a drug. *Journal of Controlled Release* **2001,** 71, (2), 183-192.
86. Bryant, S. J.; Nuttelman, C. R.; Anseth, K. S., Cytocompatibility of UV and visible light photoinitiating systems on cultured NIH/3T3 fibroblasts in vitro. *Journal of Biomaterials Science-Polymer Edition* **2000,** 11, (5), 439-457.
87. Mather, B. D.; Viswanathan, K.; Miller, K. M.; Long, T. E., Michael addition reactions in macromolecular design for emerging technologies. *Prog. Polym. Sci.* **2006,** 31, (5), 487-531.
88. Vermonden, T.; Censi, R.; Hennink, W. E., Hydrogels for Protein Delivery. *Chemical Reviews* **2012,** 112, (5).
89. Cheng, V.; Lee, B. H.; Pauken, C.; Vernon, B. L., Poly(N-isopropylacrylamide-co-Poly(ethylene glycol))-acrylate simultaneously physically and chemically gelling polymer systems. *Journal of Applied Polymer Science* **2007,** 106, (2), 1201-1207.
90. Vernon, B.; Tirelli, N.; Bachi, T.; Haldimann, D.; Hubbell, J. A., Water-borne, in situ crosslinked biomaterials from phase-segregated precursors. *Journal of Biomedical Materials Research Part A* **2003,** 64A, (3), 447-456.
91. Dong, Y.; Hassan, W.; Zheng, Y.; Saeed, A. O.; Cao, H.; Tai, H.; Pandit, A.; Wang, W., Thermoresponsive hyperbranched copolymer with multi acrylate functionality for in situ cross-linkable hyaluronic acid composite semi-IPN hydrogel. *Journal of Materials Science-Materials in Medicine* **2012,** 23, (1), 25-35.
92. Malkoch, M.; Vestberg, R.; Gupta, N.; Mespouille, L.; Dubois, P.; Mason, A. F.; Hedrick, J. L.; Liao, Q.; Frank, C. W.; Kingsbury, K.; Hawker, C. J., Synthesis of well-defined hydrogel networks using Click chemistry. *Chemical Communications* **2006,** (26), 2774-2776.
93. Clark, M.; Kiser, P., In situ crosslinked hydrogels formed using Cu(I)-free Huisgen cycloaddition reaction. *Polymer International* **2009,** 58, (10), 1190-1195.
94. J.M.G., C.; Valeria, A., *Polymers: Chemistry and Physics of Modern Materials*. 2008.
95. Cowie, J. M. G., *Polymers: Chemistry and Physics of Modern Materials*. 2nd ed.; Chapman and Hall: New York, 1991.
96. Hageman, H. J., Photoinitiators For Free-Radical Polymerization. *Progress in Organic Coatings* **1985,** 13, (2), 123-150.
97. Stevens, M. P., *Polymer Chemistry : An introduction*. 3rd ed.; Oxford University Press: New York, 1999; p xix, 551 p.
98. Greszta, D.; Mardare, D.; Matyjaszewski, K., LIVING RADICAL POLYMERIZATION .1. POSSIBILITIES AND LIMITATIONS. *Macromolecules* **1994,** 27, (3), 638-644.
99. Patten, T. E.; Matyjaszewski, K., Atom transfer radical polymerization and the synthesis of polymeric materials. *Advanced Materials* **1998,** 10, (12), 901-+.
100. Siegwart, D. J.; Oh, J. K.; Matyjaszewski, K., ATRP in the design of functional materials for biomedical applications. *Progress in Polymer Science* **2012,** 37, (1), 18-37.
101. Kwak, Y.; Nicolay, R.; Matyjaszewski, K., Synergistic Interaction Between ATRP and RAFT: Taking the Best of Each World. *Australian Journal of Chemistry* **2009,** 62, (11), 1384-1401.
102. Braunecker, W. A.; Matyjaszewski, K., Controlled/living radical polymerization: Features, developments, and perspectives. *Progress in Polymer Science* **2007,** 32, (1), 93-146.
103. Matyjaszewski, K.; Xia, J. H., Atom transfer radical polymerization. *Chemical Reviews* **2001,** 101, (9), 2921-2990.

104. Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H., Living free-radical polymerization by reversible addition-fragmentation chain transfer: The RAFT process. *Macromolecules* **1998,** 31, (16), 5559-5562.
105. Nicolas, J.; Guillaneuf, Y.; Lefay, C.; Bertin, D.; Gigmes, D.; Charleux, B., Nitroxide-mediated polymerization. *Progress in Polymer Science* **2013,** 38, (1), 63-235.
106. Ahmed, M.; Narain, R., Progress of RAFT based polymers in gene delivery. *Progress in Polymer Science* **2013,** 38, (5), 767-790.
107. Matyjaszewski, K., Atom Transfer Radical Polymerization: From Mechanisms to Applications. *Israel Journal of Chemistry* **2012,** 52, (3-4), 206-220.
108. Li, Y.; Wu, Y.-F.; Yuan, L.; Liu, S.-Q., Application of Atom Transfer Radical Polymerization in Biosensing. *Chinese Journal of Analytical Chemistry* **2012,** 40, (12), 1797-1802.
109. Webster, O. W., Living Polymerization Methods. *Science* **1991,** 251, (4996), 887-893.
110. Matyjaszewski, K.; Patten, T. E.; Xia, J. H., Controlled/"living" radical polymerization. Kinetics of the homogeneous atom transfer radical polymerization of styrene. *J. Am. Chem. Soc.* **1997,** 119, (4), 674-680.
111. Curran, D. P., The Design and Application of Free-Radical Chain Reactions in Organic-Synthesis .2. *Synthesis-Stuttgart* **1988,** (7), 489-513.
112. Tang, W.; Kwak, Y.; Braunecker, W.; Tsarevsky, N. V.; Coote, M. L.; Matyjaszewski, K., Understanding atom transfer radical polymerization: Effect of ligand and initiator structures on the equilibrium constants. *J. Am. Chem. Soc.* **2008,** 130, (32), 10702-10713.
113. Matyjaszewski, K.; Davis, K.; Patten, T. E.; Wei, M. L., Observation and analysis of a slow termination process in the atom transfer radical polymerization of styrene. *Tetrahedron* **1997,** 53, (45), 15321-15329.
114. Patten, T. E.; Xia, J. H.; Abernathy, T.; Matyjaszewski, K., Polymers with very low polydispersities from atom transfer radical polymerization. *Science* **1996,** 272, (5263), 866-868.
115. Wang, W.; Zheng, Y.; Roberts, E.; Duxbury, C. J.; Ding, L.; Irvine, D. J.; Howdle, S. M., Controlling chain growth: A new strategy to hyperbranched materials. *Macromolecules* **2007,** 40, (20), 7184-7194.
116. Tai, H. Y.; Wang, W. X.; Vermonden, T.; Heath, F.; Hennink, W. E.; Alexander, C.; Shakesheff, K. M.; Howdle, S. M., Thermoresponsive and Photocrosslinkable PEGMEMA-PPGMA-EGDMA Copolymers from a One-Step ATRP Synthesis. *Biomacromolecules* **2009,** 10, (4), 822-828.
117. Dong, Y.; Gunning, P.; Cao, H.; Mathew, A.; Newland, B.; Saeed, A. O.; Magnusson, J. P.; Alexander, C.; Tai, H.; Pandit, A.; Wang, W., Dual stimuli responsive PEG based hyperbranched polymers. *Polymer Chemistry* **2010,** 1, (6), 827-830.
118. Rizzardo, E.; Chiefari, J.; Chong, B. Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Thang, S. H., Tailored polymers by free radical processes. *Macromolecular Symposia* **1999,** 143, 291-307.
119. Chong, Y. K.; Le, T. P. T.; Moad, G.; Rizzardo, E.; Thang, S. H., A more versatile route to block copolymers and other polymers of complex architecture by living radical polymerization: The RAFT process. *Macromolecules* **1999,** 32, (6), 2071-2074.
120. Moad, G.; Rizzardo, E.; Thang, S. H., Living radical polymerization by the RAFT process. *Australian Journal of Chemistry* **2005,** 58, (6), 379-410.
121. McCormack, C. L.; Lowe, A. B., Aqueous RAFT polymerization: Recent developments in synthesis of functional water-soluble (Co)polymers with controlled structures. *Accounts of Chemical Research* **2004,** 37, (5), 312-325.
122. Fischer, H., The persistent radical effect: A principle for selective radical reactions and living radical polymerizations. *Chemical Reviews* **2001,** 101, (12), 3581-3610.
123. Hawker, C. J., MOLECULAR-WEIGHT CONTROL BY A LIVING FREE-RADICAL POLYMERIZATION PROCESS. *Journal of the American Chemical Society* **1994,** 116, (24), 11185-11186.

124. Nuyken, O.; Pask, S. D., Ring-Opening Polymerization-An Introductory Review. *Polymers* **2013,** 5, (2), 361-403.
125. Gombotz, W. R.; Guanghui, W.; Hoffman, A. S., Immobilization Of Poly(Ethylene Oxide) On Poly(Ethylene-Terephthalate) Using A Plasma Polymerization Process. *Journal of Applied Polymer Science* **1989,** 37, (1), 91-107.
126. Fuertges, F.; Abuchowski, A., The Clinical Efficacy Of Poly(Ethylene Glycol)-Modified Proteins. *Journal of Controlled Release* **1990,** 11, (1-3), 139-148.
127. Salinas, C. N.; Anseth, K. S., The enhancement of chondrogenic differentiation of human mesenchymal stem cells by enzymatically regulated RGD functionalities. *Biomaterials* **2008,** 29, (15), 2370-2377.
128. Yamaoka, T.; Tabata, Y.; Ikada, Y., Distribution And Tissue Uptake Of Poly(Ethylene Glycol) With Different Molecular-Weights After Intravenous Administration To Mice. *Journal of Pharmaceutical Sciences* **1994,** 83, (4), 601-606.
129. Hubbell, J. A.; Massia, S. P.; Desai, N. P.; Drumheller, P. D., Endothelial Cell-Selective Materials for Tissue Engineering in the Vascular Graft Via a New Receptor. *Bio-Technology* **1991,** 9, (6), 568-572.
130. Asadi, H.; Rostamizadeh, K.; Salari, D.; Hamidi, M., Preparation and characterization of tri-block poly(lactide)-poly(ethylene glycol)-poly(lactide) nanogels for controlled release of naltrexone. *International Journal of Pharmaceutics* **2011,** 416, (1), 356-364.
131. Ruan, G.; Feng, S. S., Preparation and characterization of poly(lactic acid)-poly(ethylene glycol)-poly(lactic acid) (PLA-PEG-PLA) microspheres for controlled release of paclitaxel. *Biomaterials* **2003,** 24, (27), 5037-5044.
132. Langer, R., New Methods Of Drug Delivery. *Science* **1990,** 249, (4976), 1527-1533.
133. Tamada, J.; Langer, R., The Development Of Polyanhydrides For Drug Delivery Applications. *Journal of Biomaterials Science-Polymer Edition* **1992,** 3, (4), 315-353.
134. Cruise, G. M.; Scharp, D. S.; Hubbell, J. A., Characterization of permeability and network structure of interfacially photopolymerized poly(ethylene glycol) diacrylate hydrogels. *Biomaterials* **1998,** 19, (14), 1287-1294.
135. West, J. L.; Hubbell, J. A., Polymeric biomaterials with degradation sites for proteases involved in cell migration. *Macromolecules* **1999,** 32, (1), 241-244.
136. Mann, B. K.; Gobin, A. S.; Tsai, A. T.; Schmedlen, R. H.; West, J. L., Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: synthetic ECM analogs for tissue engineering. *Biomaterials* **2001,** 22, (22), 3045-3051.
137. Bryant, S. J.; Anseth, K. S., The effects of scaffold thickness on tissue engineered cartilage in photocrosslinked poly(ethylene oxide) hydrogels. *Biomaterials* **2001,** 22, (6), 619-626.
138. Metters, A. T.; Anseth, K. S.; Bowman, C. N., Fundamental studies of a novel, biodegradable PEG-b-PLA hydrogel. *Polymer* **2000,** 41, (11), 3993-4004.
139. Tai, H. Y.; Zheng, Y.; Wang, W. X., Hyperbranched Copolymers Synthesized by Cocondensation and Radical Copolymerization. In *Hyperbranched Polymers: Synthesis, Properties, and Applications*, Deyue Yan, C. G., Holger Frey, Ed. John Wiley & Sons, Inc: Hoboken, NJ, USA, 2011; pp 203-226.
140. Gao, C.; Yan, D., Hyperbranched polymers: from synthesis to applications. *Prog. Polym. Sci.* **2004,** 29, (3), 183-275.
141. Jikei, M.; Kakimoto, M., Hyperbranched polymers: a promising new class of materials. *Prog. Polym. Sci.* **2001,** 26, (8), 1233-1285.
142. Yates, C. R.; Hayes, W., Synthesis and applications of hyperbranched polymers. *Eur. Polym. J.* **2004,** 40, (7), 1257-1281.
143. Costello, P. A.; Martin, I. K.; Slark, A. T.; Sherrington, D. C.; Titterton, A., Branched methacrylate copolymers from multifunctional monomers: chemical composition and physical architecture distributions. *Polymer* **2002,** 43, (2), 245-254.
144. Guan, Z., Control of polymer topology through transition-metal catalysis: Synthesis of hyperbranched polymers by cobalt-mediated free radical polymerization. *J. Am. Chem. Soc.* **2002,** 124, (20), 5616-5617.

145. Tai, H. Y.; Howard, D.; Takae, S.; Wang, W. X.; Vermonden, T.; Hennink, W. E.; Stayton, P. S.; Hoffman, A. S.; Endruweit, A.; Alexander, C.; Howdle, S. M.; Shakesheff, K. M., Photo-Cross-Linked Hydrogels from Thermoresponsive PEGMEMA-PPGMA-EGDMA Copolymers Containing Multiple Methacrylate Groups: Mechanical Property, Swelling, Protein Release, and Cytotoxicity. *Biomacromolecules* **2009,** 10, (10), 2895-2903.
146. Tai, H.; Tochwin, A.; Wang, W., Thermoresponsive hyperbranched polymers via In Situ RAFT copolymerization of peg-based monomethacrylate and dimethacrylate monomers. *Journal of Polymer Science Part a-Polymer Chemistry* **2013,** 51, (17), 3751-3761.
147. Dong, Y.; Saeed, A. O.; Hassan, W.; Keigher, C.; Zheng, Y.; Tai, H.; Pandit, A.; Wang, W., "One-step" Preparation of Thiol-Ene Clickable PEG-Based Thermoresponsive Hyperbranched Copolymer for In Situ Crosslinking Hybrid Hydrogel. *Macromolecular Rapid Communications* **2012,** 33, (2), 120-126.
148. Zalipsky, S., Chemistry Of Polyethylene-Glycol Conjugates With Biologically-Active Molecules. *Advanced Drug Delivery Reviews* **1995,** 16, (2-3).
149. Greenwald, R. B.; Choe, Y. H.; McGuire, J.; Conover, C. D., Effective drug delivery by PEGylated drug conjugates. *Advanced Drug Delivery Reviews* **2003,** 55, (2).
150. Nair, L. S.; Laurencin, C. T., Biodegradable polymers as biomaterials. *Progress in Polymer Science* **2007,** 32, (8-9), 762-798.
151. Williams, D. F., On the mechanisms of biocompatibility. *Biomaterials* **2008,** 29, (20), 2941-2953.
152. Lenz, R. W., Biodegradable Polymers. *Advances in Polymer Science* **1993,** 107, 1-40.
153. Tomihata, K.; Burczak, K.; Shiraki, K.; Ikada, Y., Cross-Linking And Biodegradation Of Native And Denatured Collagen. *Abstracts of Papers of the American Chemical Society* **1992,** 204, 326-POLY.
154. Li, S. M.; Vert, M., Crystalline Oligomeric Stereocomplex As An Intermediate Compound In Racemic Poly(Dl-Lactic Acid) Degradation. *Polymer International* **1994,** 33, (1), 37-41.
155. Gopferich, A.; Langer, R., The Influence Of Microstructure And Monomer Properties On The Erosion Mechanism Of A Class Of Polyanhydrides. *Journal of Polymer Science Part a-Polymer Chemistry* **1993,** 31, (10), 2445-2458.
156. Laitinen, O.; Tormala, P.; Taurio, R.; Skutnabb, K.; Saarelainen, K.; Iivonen, T.; Vainionpaa, S., Mechanical-Properties Of Biodegradable Ligament Augmentation Device Of Poly(L-Lactide) Invitro And Invivo. *Biomaterials* **1992,** 13, (14), 1012-1016.
157. Sawhney, A. S.; Hubbell, J. A., Rapidly Degraded Terpolymers Of Dl-Lactide, Glycolide, And Epsilon-Caprolactone With Increased Hydrophilicity By Copolymerization With Polyethers. *Journal of Biomedical Materials Research* **1990,** 24, (10), 1397-1411.
158. Domb, A. J.; Gallardo, C. F.; Langer, R., Poly(Anhydrides) .3. Poly(Anhydrides) Based On Aliphatic Aromatic Diacids. *Macromolecules* **1989,** 22, (8), 3200-3204.
159. Domb, A. J.; Mathiowitz, E.; Ron, E.; Giannos, S.; Langer, R., Polyanhydrides .4. Unsaturated And Cross-Linked Polyanhydrides. *Journal of Polymer Science Part a-Polymer Chemistry* **1991,** 29, (4), 571-579.
160. Hern, D. L.; Hubbell, J. A., Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. *Journal of Biomedical Materials Research* **1998,** 39, (2), 266-276.
161. Hoffman, A. S., Stimuli-responsive polymers: Biomedical applications and challenges for clinical translation. *Advanced Drug Delivery Reviews* **2013,** 65, (1), 10-16.
162. Schild, H. G., Poly (N-Isopropylacrylamide) - Experiment, Theory And Application. *Progress in Polymer Science* **1992,** 17, (2), 163-249.
163. Yoshida, R.; Uchida, K.; Kaneko, Y.; Sakai, K.; Kikuchi, A.; Sakurai, Y.; Okano, T., Comb-Type Grafted Hydrogels With Rapid De-Swelling Response To Temperature-Changes. *Nature* **1995,** 374, (6519), 240-242.
164. Bulmus, V.; Ding, Z. L.; Long, C. J.; Stayton, P. S.; Hoffman, A. S., Site-specific polymer-streptavidin bioconjugate for pH-controlled binding and triggered release of biotin. *Bioconjugate Chemistry* **2000,** 11, (1), 78-83.

165. Lee, D. S.; Shim, M. S.; Kim, S. W.; Lee, H.; Park, I.; Chang, T. Y., Novel thermoreversible gelation of biodegradable PLGA-block-PEO-block-PLGA triblock copolymers in aqueous solution. *Macromolecular Rapid Communications* **2001,** 22, (8), 587-592.
166. Aamer, K. A.; Sardinha, H.; Bhatia, S. R.; Tew, G. N., Rheological studies of PLLA-PEO-PLLA triblock copolymer hydrogels. *Biomaterials* **2004,** 25, (6), 1087-1093.
167. Bae, S. J.; Suh, J. M.; Sohn, Y. S.; Bae, Y. H.; Kim, S. W.; Jeong, B., Thermogelling poly(caprolactone-b-ethylene glycol-b-caprolactone) aqueous solutions. *Macromolecules* **2005,** 38, (12), 5260-5265.
168. Smith, G. D.; Bedrov, D., Roles of enthalpy, entropy, and hydrogen bonding in the lower critical solution temperature behavior of poly(ethylene oxide)/water solutions. *Journal of Physical Chemistry B* **2003,** 107, (14), 3095-3097.
169. Rice, C. V., Phase-transition thermodynamics of N-isopropylacrylamide hydrogels. *Biomacromolecules* **2006,** 7, (10), 2923-2925.
170. Zhang, X. C.; Wyss, U. P.; Pichora, D.; Goosen, M. F. A., An Investigation Of The Synthesis And Thermal-Stability Of Poly(Dl-Lactide). *Polymer Bulletin* **1992,** 27, (6), 623-629.
171. Andini, S.; Ferrara, L.; Maglio, G.; Palumbo, R., Synthesis Of Block Polyesteramides Containing Biodegradable Poly(L,L-Lactide) Segments. *Makromolekulare Chemie-Rapid Communications* **1988,** 9, (3), 119-124.
172. Dai, X.; Chen, X.; Yang, L.; Foster, S.; Coury, A. J.; Jozefiak, T. H., Free radical polymerization of poly(ethylene glycol) diacrylate macromers: Impact of macromer hydrophobicity and initiator chemistry on polymerization efficiency. *Acta Biomaterialia* **2011,** 7, (5), 1965-1972.
173. Benaglia, M.; Rizzardo, E.; Alberti, A.; Guerra, M., Searching for more effective agents and conditions for the RAFT polymerization of MMA: Influence of dithioester substituents, solvent, and temperature. *Macromolecules* **2005,** 38, (8), 3129-3140.
174. Stevens, M. P., *Polymer Chemistry: An Introduction*. 3rd. ed.; Oxford University Press: New York: 1999.
175. Tai, H. Y. Free radical heterogeneous polymerisation of vinylidene fluoride in supercritial carbon dioxide. the University of Nottingham, 2005.
176. Munk, P.; Aminaabhavi, T. M., *Introduction to Macromolecular Science*. 2nd ed.; Wiley: New York, 2002.
177. A, S. D.; J, L. J., *Principles of Instrumental Analysis*. Fourth Edition ed.; Saunders College Publishing: 1971.
178. Chu, B.; Xu, R. L.; Wang, Z. L.; Zuo, J., Critical Phenomena And Polymer Coil-To-Globule Transition. *Journal of Applied Crystallography* **1988,** 21, 707-714.
179. Park, I. H.; Wang, Q. W.; Chu, B., Transition Of Linear Polymer Dimensions From Theta To Collapsed Regime .1. Polystyrene Cyclohexane System. *Macromolecules* **1987,** 20, (8), 1965-1975.
180. Du, Y. J.; Lemstra, P. J.; Nijenhuis, A. J.; Vanaert, H. A. M.; Bastiaansen, C., ABA Type Copolymers Of Lactide With Poly(Ethylene Glycol) - Kinetic, Mechanistic, And Model Studies. *Macromolecules* **1995,** 28, (7), 2124-2132.
181. Yu, Y.; Storti, G.; Morbidelli, M., Ring-Opening Polymerization of L,L-Lactide: Kinetic and Modeling Study. *Macromolecules* **2009,** 42, (21), 8187-8197.
182. Kricheldorf, H. R., Syntheses and application of polylactides. *Chemosphere* **2001,** 43, (1), 49-54.
183. Kowalski, A.; Duda, A.; Penczek, S., Kinetics and mechanism of cyclic esters polymerization initiated with tin(II) octoate. 3. Polymerization of L,L-dilactide. *Macromolecules* **2000,** 33, (20), 7359-7370.
184. Kowalski, A.; Libiszowski, J.; Biela, T.; Cypryk, M.; Duda, A.; Penczek, S., Kinetics and mechanism of cyclic esters polymerization initiated with tin(II) octoate. Polymerization of epsilon-caprolactone and L,L-lactide co-initiated with primary amines. *Macromolecules* **2005,** 38, (20), 8170-8176.

185. Zhu, K. J.; Lin, X. Z.; Yang, S. L., Preparation, Characterization, And Properties Of Polylactide (Pla) Poly(Ethylene Glycol) (Peg) Copolymers - A Potential-Drug Carrier. *Journal of Applied Polymer Science* **1990,** 39, (1), 1-9.
186. Deng, X. M.; Xiong, C. D.; Cheng, L. M.; Xu, R. P., Synthesis And Characterization Of Block Copolymers From D,L-Lactide And Poly(Ethylene Glycol) With Stannous Chloride. *Journal of Polymer Science Part C-Polymer Letters* **1990,** 28, (13), 411-416.
187. Tanzi, M. C.; Verderio, P.; Lampugnani, M. G.; Resnati, M.; Dejana, E.; Sturani, E., Cytotoxicity Of Some Catalysts Commonly Used In The Synthesis Of Copolymers For Biomedical Use. *Journal of Materials Science-Materials in Medicine* **1994,** 5, (6-7).
188. Dust, J. M.; Fang, Z. H.; Harris, J. M., Proton Nmr Characterization Of Poly(Ethylene Glycols) And Derivatives. *Macromolecules* **1990,** 23, (16), 3742-3746.
189. Hiltunen, K.; Harkonen, M.; Seppala, J. V.; Vaananen, T., Synthesis and characterization of lactic acid based telechelic prepolymers. *Macromolecules* **1996,** 29, (27), 8677-8682.
190. Han, D. K.; Hubbell, J. A., Synthesis of polymer network scaffolds from L-lactide and poly(ethylene glycol) and their interaction with cells. *Macromolecules* **1997,** 30, (20), 6077-6083.
191. Du, Y. J.; Nijenhuis, A. J.; Bastiaansen, C.; Lemstra, P. J., New Approaches To Ring-Opening Polymerization-Evidences Of Step Transfer Polymerization. *Journal of Macromolecular Science-Pure and Applied Chemistry* **1995,** A32.
192. Zhang, Y. L.; Won, C. Y.; Chu, C. C., Synthesis and characterization of biodegradable network hydrogels having both hydrophobic and hydrophilic components with controlled swelling behavior. *Journal of Polymer Science Part a-Polymer Chemistry* **1999,** 37, (24), 4554-4569.
193. Freiberg, S.; Zhu, X., Polymer microspheres for controlled drug release. *International Journal of Pharmaceutics* **2004,** 282, (1-2), 1-18.
194. Hou, Q. P.; De Bank, P. A.; Shakesheff, K. M., Injectable scaffolds for tissue regeneration. *Journal of Materials Chemistry* **2004,** 14, (13), 1915-1923.
195. Liu, R.; Fraylich, M.; Saunders, B. R., Thermoresponsive copolymers: from fundamental studies to applications. *Colloid and Polymer Science* **2009,** 287, (6).
196. Kharlampieva, E.; Kozlovskaya, V.; Tyutina, J.; Sukhishvili, S. A., Hydrogen-bonded multilayers of thermoresponsive polymers. *Macromolecules* **2005,** 38, (25).
197. Kavanagh, C. A.; Rochev, Y. A.; Gallagher, W. A.; Dawson, K. A.; Keenan, A. K., Local drug delivery in restenosis injury: thermoresponsive co-polymers as potential drug delivery systems. *Pharmacology & Therapeutics* **2004,** 102, (1).
198. Hruby, M.; Kucka, J.; Mackova, H.; Lebeda, O.; Ulbrich, K., Thermoresponsive polymers - From laboratory curiosity to advanced materials for medicinal applications. *Chemicke Listy* **2008,** 102, (1).
199. Voit, B., New developments in hyperbranched polymers. *Journal of Polymer Science Part a-Polymer Chemistry* **2000,** 38, (14).
200. Seiler, M., Hyperbranched polymers: Phase behavior and new applications in the field of chemical engineering. *Fluid Phase Equilibria* **2006,** 241, (1-2).
201. Perrier, S.; Haddleton, D. M., Effect of water on copper mediated living radical polymerization. *Macromolecular Symposia* **2002,** 182, 261-272.
202. Tsarevsky, N. V.; Pintauer, T.; Matyjaszewski, K., Deactivation efficiency and degree of control over polymerization in ATRP in protic solvents. *Macromolecules* **2004,** 37, (26), 9768-9778.
203. Najafi, V.; Ziaee, F.; Kabiri, K.; Mehr, M. J. Z.; Abdollahi, H.; Nezhad, P. M.; Jalilian, S. M.; Nouri, A., Aqueous free-radical polymerization of PEGMEMA macromer: kinetic studies via an on-line H-1 NMR technique. *Iranian Polymer Journal* **2012,** 21, (10), 683-688.
204. Lutz, J.-F., Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials. *Journal of Polymer Science Part a-Polymer Chemistry* **2008,** 46, (11), 3459-3470.