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Hydrophilic Copolymers from Multi-Vinyl Monomers via Reversible Addition-Fragmentation Chain Transfer Polymerisation for Hydrogel Applications

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By

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PhD Supervisor: **Dr Hongyun Tai**

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Abstract

The focus of this thesis is to synthesize and develop *in-situ* cross-linkable hydrophilic copolymers using multi-vinyl monomers *via* Reversible Addition-Fragmentation Chain Transfer Polymerisation (RAFT) for hydrogel applications. This thesis comprises six chapters described briefly below:

Chapter 1 provides an introduction to the topics covered in the thesis. General fundamentals of main polymerisation techniques, basic concepts of the polymer chemistry, hyperbranched polymers, hydrogels and their applications are included.

Chapter 2 describes the general experimental procedures and methodology used in this thesis, including the synthesis and characterisation of the precursors of the RAFT agents, final RAFT agents, disulphide diacrylate and the preparation of hydrophilic polymers by conventional and living/controlled radical polymerisation methods. Moreover, methods and analytical techniques used for the characterisation of compounds and polymers are described. The scientific background for interpretation and understanding of the results are also included in this chapter.

Chapter 3 contains two subsections and focuses on the results and discussion on *in-situ* RAFT approach and its applicability in copolymerisation of vinyl monomers.

In section 3.1, an *in-situ* technique of Reversible-Addition Fragment Chain Transfer (*in-situ* RAFT) polymerisation is developed. The kinetic studies on the *in-situ* RAFT polymerisations of methyl methacrylate (MMA) and styrene (St) through a facile one-pot and two-step approach are presented. Where, bis(thiobenzoyl)disulfide and 2,2'-azobis(isobutyronitrile) (AIBN) were used to generate RAFT agent 2-cyanoprop-2-yl dithiobenzoate *in-situ* at 80 ^{o}C, followed by further RAFT polymerisations of MMA or St at 65 ^{o}C. The kinetics of these *in-situ* RAFT polymerisations were studied using Gel Permeation Chromatography (GPC) under different reaction conditions in order to investigate the effects of solvent, temperature, and molar ratio of reactants. The experimental results demonstrated that this *in-situ* approach showed the similar controllability as conventional RAFT polymerisation in terms of the molecular weights and

polydispersity of polymers obtained. The resultant polymers were characterized by proton Nuclear Magnetic Resonance spectroscopy (^{1}H NMR analysis) and GPC, and were successfully used as macro RAFT agents for the preparation of PMMA-b-PSt block copolymers.

In section 3.2, the *in-situ* approach developed in section 3.1 was successfully adopted to copolymerise poly(ethylene glycol) methyl ether methacrylate (PEGMEMA), poly(propylene glycol) methacrylate (PPGMA) and up to 30% of ethylene glycol dimethacrylate (EGDMA) as the branching agent. The characterisation and studies on the properties of prepared responsive copolymers are included. The resultant PEGMEMA-PPGMA-EGDMA copolymers from *in-situ* RAFT were characterised by GPC and ^{1}H NMR analysis. The results confirmed the copolymers with multiple methacrylate groups and hyperbranched structure as well as RAFT functional residues. These water-soluble copolymers with tailored compositions demonstrated tuneable Lower Critical Solution Temperature (LCST) from 22 ^{o}C to 32 ^{o}C. The phase transition temperature can be further altered by post functionalisation through aminolysis of RAFT agent residues in polymer chains.

Chapter 4 describes study on the conventional RAFT copolymerisation of PEGMEMA, PPGMA and bis(2-acryloyl)oxyethyl disulfide (DSDA). A series of polymerisations were carried out to prepare degradable PEGMEMA-PPGMA-DSDA hyperbranched copolymers, using 2-cyanoprop-2-yl dithiobenzoate as the RAFT agent. The molar feed ratios of monomers were varied to adjust polymer properties and manipulate LCST of the final polymers. The copolymers were tailored in order that they could be readily cleavable under mild conditions, physically crosslinked at body temperature and moreover chemically crosslinked with thiol crosslinker (QT) *via* Michael addition reaction. The reactions were monitored by GPC analysis, polymer compositions were calculated from peak integrations according to ^{1}H NMR analysis. In addition, fabrication of hydrogels through Michael addition reaction using PEGMEMA-PPGMA-DSDA copolymers, swelling and degradation studies are also presented.

Chapter 5 focuses on the synthesis of pH responsive dendritic hydrophilic polymers with tailored swelling profile by the use of RAFT polymerisation of 2-hydroxyethyl methacrylate (HEMA) and acrylic acid (AA). The copolymers were synthesised in the presence or absence of EGDMA. 4-Cyano–4-[(dodecylsulfanylthiocarbonyl)sulfanyl]

pentanoic acid was used as the chain transfer agent (CTA), divinyl monomer EGDMA as the branching agent. The hydrogels from the resultant linear and dendritic copolymers demonstrated responsive properties at different pH values and temperatures in swelling studies. The responsive behaviours of these hydrogels have also been compared to the hydrogels prepared directly from crosslinking of AA, HEMA and EDGMA monomers. The resultant copolymers were characterized by GPC and ^{1}H NMR analysis. Moreover, thermal properties of the polymers were evaluated by Thermo-Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). The degrees of swelling of the hydrogels were studied at 20 ^{o}C and 37 ^{o}C in phosphate buffer solution (pH 7.4) and water (pH 4 and pH 7). From these studies, it was found that the hydrogels from copolymers of AA and HEMA demonstrated thermal and pH responsive properties, which were significantly affected by the chemical composition and topological structure of polymer chains.

Chapter 6 summarises the research presented in this thesis and draws the conclusions. Additionally, the vision and possible future work are included.

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For everything, thank you

Anna Tochwin

List of Abbreviations and Acronyms

3D	Three-dimensional
AA	Acrylic acid
ACHN	1,1'-azobis-cyclohexane-carbonitrile
ACBN	1-cyano-1-cyclohexyl dithiobenzoate
AIBN	2,2'-azobis(isobutyronitrile) / Azoisobutyronitrile
AMA	Allyl methacrylate
ATRP	Atom Transfer Radical Polymerisation
bpy	2,2'-bipyridyl
BPO	Benzoyl peroxide
CCTP	Catalytic chain transfer polymerisation
$CDCl_3$	Deuterated chloroform
Conv.	Monomer conversion
CPDB	2-cyanoprop-2-yl dithiobenzoate
Cr	Crude
CRP	Controlled Radical Polymerisation
CSIRO	Commonwealth Scientific and Industrial Research Organization
CTA	Chain Transfer Agent
^oC	Degrees Celsius
DBC	Double bond content
de-ATRP	Deactivation Enhanced Atom Transfer Radical Polymerisation
DLS	Dynamic light scattering
DMF	N,N-dimethylformamide
DMM	Double-monomer methodology
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOB	Degree of branching
DSC	Differential Scanning Calorimetry
DSDA	Bis(2-acryloyl)oxyethyl disulfide (disulfide-based diacrylate)
DSs	Degrees of Swelling
DTBA	Dithiobenzoic acid
DTT	Dithiothreitol
DVB	Divinylbenzene
ECM	Extracellular matrix
EGDMA	Ethylene Glycol Dimethacrylate
ESR	Electron Spin Resonance
FRP	Free Radical Polymerisation
FTIR	Fourier Transform Infra-red
g	Gram
GPC	Gel Permeation Chromatography
GSH	Glutathione
hrs	Hours
HBP	Hyperbranched Polymer
HDDA	1,6-hexanediol diacrylate
HEMA	2-hydroxyethyl methacrylate
HPA	2-hydroxypropyl acrylate
IUPAC	International Union of Pure and Applied Chemistry

KBr	Potassium bromide
LAMs	Less activated monomers
LCST	Lower Critical Solution Temperature
LiBr	Lithium Bromide
LRP	Living Radical Polymerisation
MADIX	Macromolecular Design via Interchange of Xanthates
MALLS	Multiangle Light Scattering detector (mini-Dawn)
MAMs	More activated monomers
MEO_2MA	2-(2-methoxyethoxy)ethyl methacrylate
MFM	Multifunctional monomer
mg	Milligram
min	Minute
MMA	Methyl methacrylate
mL	Millilitre
MVM	Multifunctional vinyl monomer
MWCO	Molecular weight cut off
MWD	Molecular Weight Distribution
M_n	Number-average molecular weight
M_p	Peak molecular weight
M_w	Weight-average molecular weight
NiPAAm	N-isopropyl acrylamide
NMP	Nitroxide-mediated Polymerisation
NMR	Nuclear Magnetic Resonance Spectroscopy
NVP	N-vinylpyrrolidone
OEGMA	Oligo(ethylene glycol) methacrylate copolymers
P	Pure
PAA	Poly(acrylic acid)
PAAm	Polyacrylamide
PBS	Phosphate-buffered saline
PCL	Polycaprolactone
PDI	Polydispersity index = M_w / M_n
PDEAEMA	Poly(2-(diethylamino)ethyl methacrylate)
PDMAEMA	Poly(2-(dimethylamino)ethyl methacrylate)
PE	Polyethylene
PEG	Poly(ethylene) glycol
PEGMEMA	Poly(ethylene glycol) methyl ether methacrylate
PHEMA	Poly(hydroxyethyl methacrylate)
PGA	Poly(glycolic acid)
PLA	Poly(lactic acid)
PLGA	Poly(lactide-co-glycolide)
PMAA	Poly(methacrylic acid)
PMMA	Poly(methyl methacrylate)
PNiPAAm	Poly(N-isopropyl acrylamide)
PP	Polypropylene
PPE	Polyphosphoestres
PPGMA	Poly(propylene glycol) methacrylate
PSt	Polystyrene
PTEGMA	Poly(triethylenglycol monomethacrylate)
PTFE	Polytetrafluoroethylene
PTP	Proton-transfer polymerisation

PVA	Polyvinyl alcohol
PVC	Polyvinylchloride
PVP	Polyvinyl pyrrolidone
QT	Pentaerythritol tetrakis 3-mercaptopropionate
R	Leaving group on RAFT agent
RAFT	Reversible Addition-Fragmentation Chain Transfer
RDRP	Reversible-Deactivation Radical Polymerisation
RI	Refractive Index
RNA	Ribonucleic acid
ROP	Ring Opening Polymerisation
rpm	Rotations per minute
RT	Retention Time
SCROP	Self-condensing ring-opening polymerisation
SCVP	Self-condensing vinyl polymerisation
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
SFRMP	Stable Free Radical Mediated Polymerisation
SMM	Single monomer methodology
St	Styrene
T	Temperature
TE	Tissue engineering
TEMPO	2,2,6,6-tetramethyl-1-piperidynyl-N-oxy
TGA	Thermo-gravimetric Analysis
Tg	Glass transition temperature
THF	Tetrahydrofuran
TLC	Thin layer chromatography
Tm	Melting temperature
TMS	Tetramethylsilane
UCST	Upper Critical Solution Temperature
UV visible	Ultraviolet visible
wt%	Weight %
w/v	weight/volume
Z	Stabilising group on RAFT agent
μm	Micrometer

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Chapter 1 Introduction

In this thesis Multi-Vinyl Monomers (MVMs) are used to prepare *in-situ* cross-linkable, responsive hydrophilic copolymers *via* Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerisation approach for hydrogel applications. In chapter 1 an outline on polymerisations of multi-vinyl monomers and a literature overview covering hyperbranched polymers, hydrogels and their applications as biomedical materials are presented with attention given to preparation methods through controlled/living radical polymerisation techniques.

1.1. Monomers, Polymers, Polymerisations

Intention of this section is to provide an introduction to the topics covered in the thesis. Important aspects on basic concepts of the polymer chemistry, general fundamentals of main polymerisation techniques are included. It is not the intention in this section to provide a detailed descriptions of the topics as this can be found in commonly available literature and relevant textbooks which are covering the detailed knowledge.

1.1.1. Monomers

A polymer is a large molecule made of small repeat units which are covalently bonded to one another. More accurately the repeat unit is called a monomer.[1] The crucial characteristic of a monomer is its poly-functionality i.e. two or more bonding sites with abilities to permit bonding to other monomers to form a polymer chain. We can distinguish between monofunctional, bifunctional, tri- and multifunctional monomers. Mono- and bifunctional monomers can form chain-like, linear polymers, but sub-units of higher functionality can yield hyperbranched or crosslinked network polymeric products. Main monomer categories comprise molecules such as acrylics, alcohols, epoxides and amines.

A number of end-functional polymers and crosslinkers can be used as macro-monomers to create cross-linked or complex polymer architectures. In addition monomers can be classified as "more activated" and "less activated" monomers.[2,3] The "more activated" monomers (MAMs) are those in which double bond is joined to an aromatic ring, a carbonyl group or a nitrile for example styrenes, methacrylates, acrylamides, acrylates (Figure 1-1). The "less activated" monomers (LAMs) are those where double bond is joined to saturated carbon, oxygen, nitrogen lone pair for example vinyl esters, vinyl amides.[4,5]

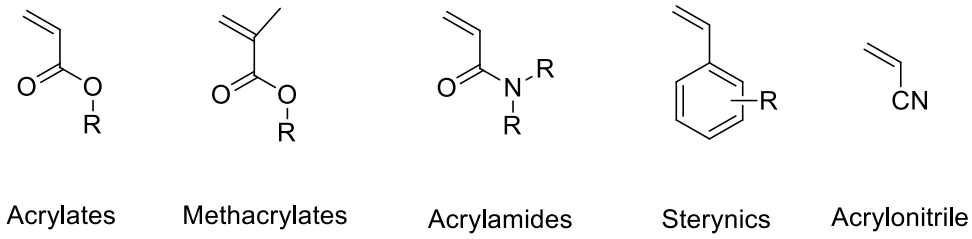


Figure 1-1: Example of activated monomers used in controlled radical polymerisations methods.

These monomers have been successfully polymerised using different polymerisation methods into a wide range of polymers, which can be classified according to their resources and properties, such as natural and synthetic polymers, hydrophilic polymers, responsive polymers, hyperbranched polymers, biodegradable polymers. These polymers will be discussed in the next section.

1.1.2. Polymers

Polymer is large organic molecule formed by combining many smaller molecules in a regular pattern. The type of monomer and type of connection between repeating unit determinates properties of polymers and in consequence their applications. Polymers can be classified according to their source, structure, type of polymerisation, molecular forces, chain growth polymerisation and degradability.[6,1]

1.1.2.1. Natural and Synthetic Polymers

Naturally occurring polymers (biopolymers, Figure 1-2) include carbohydrates (e.g. starch, cellulose, chitin and chitosan), proteins (e.g. gelatin, casein, albumin) and nucleic acids (DNA and RNA).[1] They exist in plants and animals and are degradable, often with poor mechanical strength. By nature these polymers are hydrophilic and to a certain extent crystalline.[7,8]

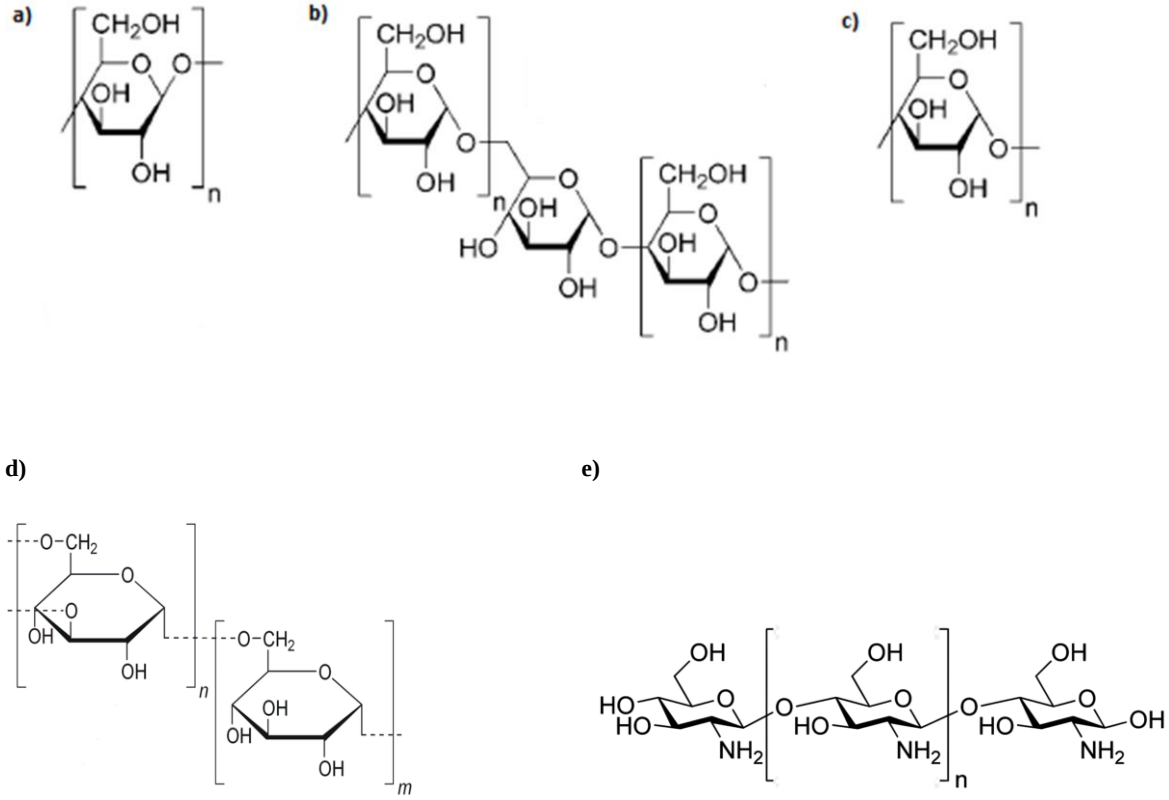


Figure 1-2: Examples of natural polymers a) cellulose, b) amylopectin, c) amylose, d) dextran, e) chitosan.

Cellulose is the main structural component of plants and contains hundreds or thousands of glucose units.[8] Amylopectin molecule has about 1,000 glucose molecules arranged into branched chains, with a branch at every 24 to 30 glucose units. Complete hydrolysis of amylopectin yields glucose.[7] Amylose is comparable to cellulose, it is a polymer made from glucose monomers, however there is a difference in bonding between the glucose units. The

bonding angles around the oxygen atoms connecting the glucose rings are each 120° in amylose, and 180° in cellulose. Dextran is a complex branched polysaccharide made of many glucose units.[1] The basic chain consists of α-1,6 glycosidic linkages between glucose molecules, while branches begin from α-1,3 linkages. Chitosan is a linear polysaccharide, with available reactive amino and hydroxyl groups.[1,6]

The use of biopolymers has several advantages such as good biocompatibility, biodegradability, in addition there is no cytotoxicity concern and they are generally recognised as a safe.[1] However, limitations such as their poor solubility in solvents and difficulty to process also exist.[7] Natural polymers are very fragile; stability concerns exist due to their poor mechanical properties, and sometimes fast degradation which is not always to advantage when it comes to applications as the control over the rates of degradation is fairly difficult.[9,10]

The synthetic polymeric materials can be made by modification of natural polymers (leather, cellulose derivatives, etc.).[11] However, fully synthetic polymers such as polyethylene (PE), polypropylene (PP), polystyrene (PSt), poly(vinyl chloride) (PVC), polyacrylonitrile (PAN), poly(N-isopropylacrylamide) (PNiPAAm), poly(vinyl acetate) (PVAc) or polytetrafluoroethylene (PTFE) are in common use in daily life and are made by addition polymerisation of petroleum based vinyl monomers.[1,12] Common examples of synthetic polymers are presented in Figure 1-3, p.5.

The addition polymers are produced by the repeated addition of monomer possessing double or triple bonds in their structure.[13] Polymers formed by the polymerisation of a single sub-unit species are known as homopolymers, where the polymers made by addition of two different monomers are known as copolymers. Polymerisation by addition is achieved by adding initiator (with or without catalyst), which provides reactive species (e.g. free radicals). These free radicals are able to attack monomer and form a new free radical which goes on successively adding monomers and therefore chain propagation happens. The final termination of the growing chains leads to a polymer.[13]

The condensation polymers are formed by a repeated condensation reaction between two different bi-functional or tri-functional monomer units.[13] In this type of polymerisation when two or more molecules combine the elimination of a small molecule (by-product) such as water, alcohol, or hydrogen chloride takes place.[13] These condensation polymers

include polyamides, polyesters and polyacetals and unlike addition polymers, they may be biodegradable as acids or enzymes can break the polymer chain into smaller pieces by hydrolysing ester or peptide bonds between monomers.[8]

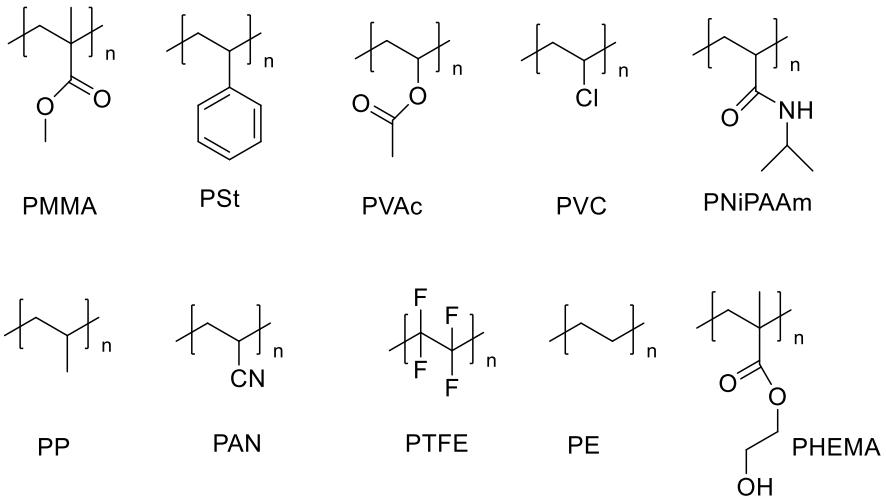


Figure 1-3: Common examples of synthetic polymers.

1.1.2.2. Hydrophilic Polymers

Hydrophilic polymers can dissolve in, or are swollen by, water.[14] They can be natural, semi-synthetic, or synthetic origin. These polymers are categorized by the presence of polar groups (e.g. hydroxyl -OH, carboxyl –COOH, and amino $–NH_2$) attached to polymer backbone. The hydrophilic groups may be non-ionic, cationic, anionic or amphoteric.[6,14] Hydrophilic polymers are often grouped by the chemistry of their structures. The solubility behaviour depends on chain length, amount of inter-molecular crosslinking, and the number and polarity of the side chain substitutes.[15] The crosslinking of hydrophilic polymers/copolymers determinates a predictable expansion ratio. Dehydrated polymers are hard, placing them in water permits hydration of its structure, which depends on the final water uptake capacity of the polymer together with the thickness of the polymer. Hydration takes place until they reach equilibrium. The quantity of the hydrophilic part in copolymers can be easily controlled; therefore the ultimate water content on full hydration may be accurately defined. Examples of hydrophilic polymers include cellulose, proteins, polyamides, polyethylene glycol (PEG), poly(2-hydroxyethyl methacrylate) (PHEMA),

polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), polyacrylic acid (PAA), and polyphosphoesters (PPE). It is known that water sensitivity of hydrophilic polymers will increase when the proportion of polar groups and their polarity increases but water sensitivity of hydrophilic polymers will decrease when chain length and crosslinking increase.

1.1.2.3. Responsive Polymers

Responsive polymers are extensively investigated due to their extraordinary properties. This type of polymer undergoes sharp reversible physical or chemical changes when subjected to modest or small changes of their environment, by chemical and physical stimuli.[16,17,18]

A physical stimulus involves e.g. temperature, mechanical stress or change in electric or magnetic field, where chemical change in the internal or external surroundings includes e.g. pH, ionic strength and chemical agents.[18,19] Modification of the polymer environment can also be facilitated by biochemical means, using stimuli such as antigen, enzyme, ligand and other biochemical agents.[20] Changes in solubility, permeability, phase separation, confirmation, *etc.*, can also occur, and might include not only one response but a combination of several responses simultaneously.[16,21] In responsive polymers/hydrogels the ratio of pH sensitive and/or thermo sensitive polymers must be balanced correctly, to ensure that the polymer can respond in the true physiological settings.[22] Various biomedical applications have been proposed for responsive polymers. As mentioned certain polymers might undergo several responses, which offer the possibility of fabrication of "intelligent" drug/gene delivery systems.[19] In addition, they may be chemically or physically crosslinked and used in fabrication of hydrogels.[22] Research on stimuli-responsive materials is driven by constant need for precisely controlled materials. Though in the beginning, the focus was on polymers having only one responsive moiety, it shifted with time to multiple sensitive functions combined in one polymer.[17]

1.1.2.3.1. Thermo-responsive Polymers

Polymers where a reversible temperature dependent phase transition occurs are known as thermoresponsive polymers (i.e. possesses Lower Critical Solution Temperature (LCST), Upper Critical Solution Temperature (UCST) or a cloud point).[23,24] Thermoresponsive polymers are used extensively in studies focused on hydrogels which are able to respond to temperature in order to be used in tissue engineering and drug delivery.[25] Changes in temperature play an important role, as this stimulus can be easily applied internally and externally. The most popular polymer which fit into this group is Poly(N-isopropyl acrylamide), prepared from monomer NiPAAm. Researchers widely reported the synthesis of PNiPAAm using different polymerisation methods and different reaction conditions.[26,27] It is well known that its LCST in water is in range of 32 ^{o}C.[28] Below this temperature PNiPAAm is swollen, hydrated and hydrophilic, but above this temperature it shrinks, creating collapsed, dehydrated hydrophobic network (Figure 1-4).[29]

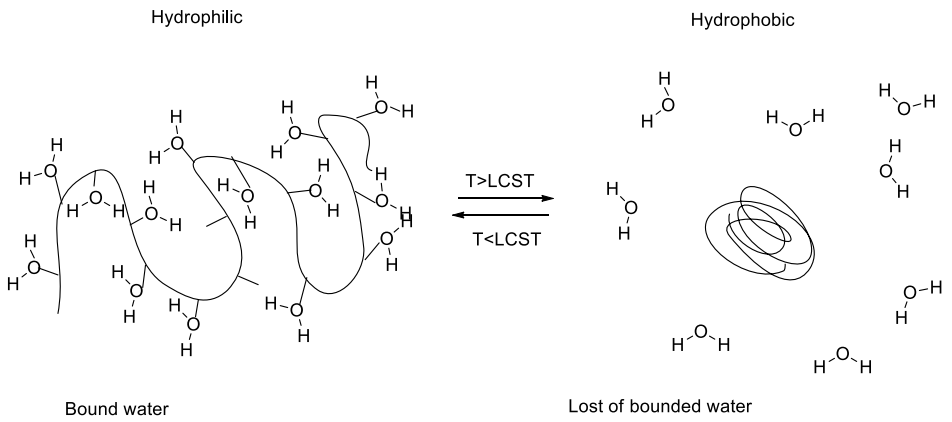


Figure 1-4: LCST response in PNiPAAm upon temperature change (adopted from Ref[29]).

The hydrophilic groups (–CONH–) of PNiPAAm hydrate to form an expanded structure when the temperature is below LCST. However, as the PNiPAAm hydrogel is heated above LCST, the polymer chains collapse suddenly and phase separation occurs.[30] LCST of PNiPAAm can be fine-tuned by modifying the structure, increasing polymer area or introduction of other polymer. Increased numbers of hydrophilic monomers raise the LCST while the incorporation of more hydrophobic units has an opposite effect.[31] Poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) and poly(triethylenglycol monomethacrylate (PTEGMA) are also examples of temperature responsive materials.

Temperature sensitive polymers can also be fabricated by linking hydrophilic and hydrophobic polymeric materials (e.g. poly(ethylene glycol) with (poly[lactic-co-glycolic acid], (PLGA-PEG-PLGA)).[32] The thermal response given by such a tri-block polymer is thermodynamically similar to PNIPAAm. Other examples include 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) and oligo(ethylene glycol) methacrylate copolymers (OEGMA), poly(lactide) (PLA) block copolymers. The LCST of these thermoresponsive polymers strongly depends on the hydrophilic/hydrophobic character of the repeating units. By controlling the hydrophilic/hydrophobic balance of the polymer in copolymerisation process, the LCST temperature range of these copolymers can be tuned.

1.1.2.3.2. pH-responsive Polymers

These type of polymer contain ionisable functional groups and will respond to the changes in the pH of the surrounding environment by donating or accepting protons.[22] This behaviour might affect the dimensions of polymeric materials.

It is known that pH sensitive polymers can be classified as polyacids (with acidic groups e.g. –COOH, $-SO_3H$) and polybases (with basic groups e.g. $–NH_2$). Polyacids will swell in high basic pH, and polybases in low acidic pH. Polyacidic polymers will be unswollen at low pH, since the acidic group will be protonated/unionized. When increasing pH negatively charged polymers will swell. Poly(acrylic acid) and poly(methacrylic acid) (PMAA) are pH responsive polyacids, and the carboxylic group located on side chains accepts protons at low pH, while release them at high pH. Poly(2-(diethylamino)ethyl methacrylate) (PDEAEMA) and poly(vinyl pyridine) are also example of polybases where amine groups gain proton under acidic conditions and release them under basic conditions.[33]

When pH sensitive polymeric chains are forming hydrogels by crosslinking, their behaviour is not only influenced by the nature of ionisable groups, the polymer composition and the hydrophobicity of the backbone, but also by the crosslinking density.[34] The higher the crosslinking density, the lower the permeability, which is especially significant in the case of high molecular weight materials.

Polymers which contain groups cleavable by acids such as anhydrides, acetals or orthoesters are believed to be degradable (pH-labile polymers). They are stable in high pH,

but degrade in low acidic environment. For this instance in acidic conditions acetal groups are hydrolyzed to form aldehyde and hemiacetal with alcohol group, orthoester groups to pentaerythriol and anhydride undergo degradation to acid groups.

Living organisms exhibit different pH environment depending on the organ or system. For example physiological pH is in range of 7.4 – 7.8, but in gastric system pH is around 2 in stomach up to 10 in colon. Unhealthy cells might lower pH in their environment, but even healthy cells express a variety of pH values. Sensitivity of polymers/hydrogels to pH is often a huge advantage in their biological applications; these polymers are found in drug and gene delivery systems, as well as in glucose sensors.[19,35,36]

1.1.2.3.3. Thiol-responsive Polymers

Thiol-responsive polymers have been investigated for use in degradable drug/gene delivery systems. It is known that a reducing agent can cleave disulfide bonds to the corresponding thiols, resulting in the controlled release of drugs/genes. In this process, by exposure to various agents, disulfide bonds are reversibly converted to thiols and undergo disulfide exchange in the presence of other thiols, therefore polymers containing disulfide linkages are thiol (and redox) responsive.[37,38]

There is a wide range of reducing agents in physiological environment (e.g. glutathione, GSH) and in addition there are many synthetic molecules (e.g. dithiothreitol, DTT), with ability to mimic the reducing agents in human body.[39,40,41]

The application of thiol-responsive polymers is based on the varying concentration of reducing agents in the body with regards to disulfide bonds which are common components of many proteins. Often the concentration of these bonds inside cells is significantly higher than outside cells. For example the typical intracellular concentration of GSH is in range of 10 mM, while its concentration in the cellular exterior is 0.002 mM.[42] This noteworthy variation has been used by researchers to design thiol-responsive delivery systems that release drugs upon entry into cells.

1.1.2.3.4. Other Stimuli-responsive Polymers

In addition to thermo-, pH- and thiol-responsive polymers, numerous polymeric/hydrogel materials which are responsive to other stimuli such as field-responsive polymers (i.e. electro, magnetic and photo-responsive materials) are available. Electro responsive polymers are able to offer different swelling, shrinking, bending etc., when exposed to external electric field[43,44] while polymers containing magnetic particles or liquid crystals are able to change shape when exposed to magnetic field.[45]

1.1.2.4. Biodegradable Polymers

Biodegradable polymers can be defined as polymeric systems that are prone to a destructive change in its chemical structure or physical properties under specific conditions.[46] These polymers should be biologically stable and should not generate in their degradation process any substances that are harmful to the environment.[47] There are a wide range of natural, synthetic, and biosynthetic polymers which are bio and environmentally degradable.[48] Such polymers can be produced from natural materials such as polysaccharides, proteins, and polyesters, or they can be generated synthetically through the polymerisation of different natural and synthetic monomers. The degradation behaviour of biodegradable polymers is influenced by many factors which include the chemical composition of the polymer backbone, molecular weight and polydispersity.[49] In addition, environment settings also play an important role.[9] Polymers are degraded in biological systems through enzymatic degradation, hydrolysis and oxidation.[50] It is very complex processes that can occur in a number of ways.[10] An enzymatic degradation applies for naturally occurring polymers, examples of biodegradable natural polymers are shown in Figure 1-2 (p.3). Though for most synthetic polymers, hydrolysis is the most important type of degradation. Certain linkages like ester, amide, urethane, orthoesters, anhydrides etc. are prone to hydrolysis by enzymes and microorganisms. The rate of hydrolysis might be affected by several factors which include the composition of the copolymer and swelling character, water diffusion, the kind of chemical bond, and pH.

Aliphatic poly(esters) such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), polycaprolactone (PCL) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) are few of many examples of degradable synthetic polymers,[8,35] that degrade into naturally

occurring substances.[51] This is due to the presence of highly hydrophilic carbonyl in ester linkage which undergoes hydrolytic and enzymatic chain cleavage to hydroxyacids.[10] Poly(lactide-co-glycolide) (PLGA) is a biodegradable polymer prepared by random copolymerisation of PLA (both L- and D,L-lactide forms) and PGA. Copolymers based on PEG and PLA are known for their biocompatibility and biodegradability, and when functionalized with vinyl groups at the terminal ends have the potential for further polymerisation. The biodegradability of the photocrosslinked hydrogels from linear PEG-PLA diacrylates can be tailored by the composition, thus crosslinking density of the hydrogels.

Synthetic vinyl polymers are generally not prone to hydrolysis and in order to introduce degradation, oxidation process is needed.[7,9] Vinyl polymers which might undergo biodegradation often contain readily oxidizable functional groups. Polyacrylates are also resistant to degradation and biodegradable segments needs to be incorporated into the polymer chains in order to introduce degrability.[8,9]

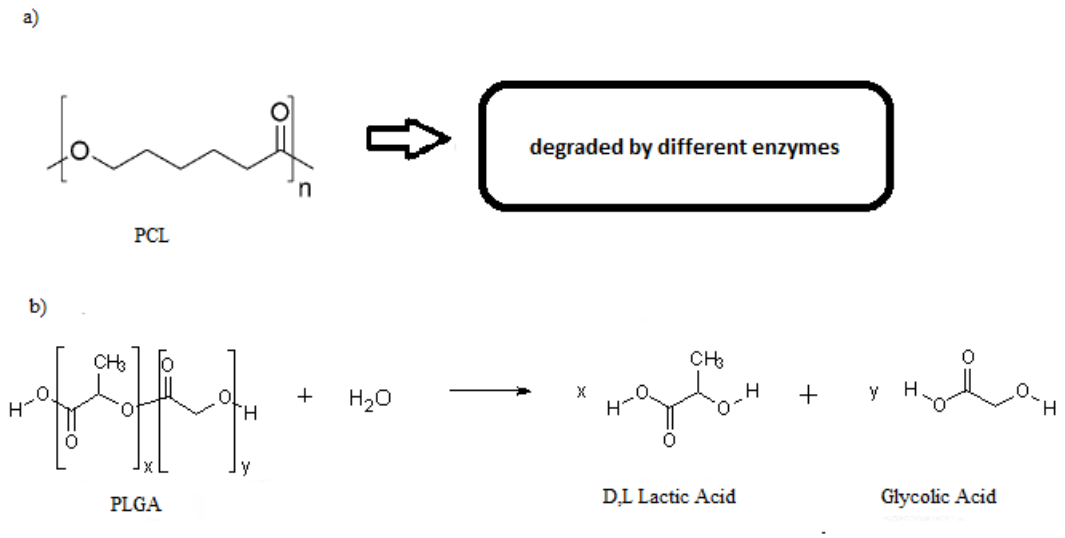


Figure 1-5: Examples of biodegradable synthetic polymers a) Polycaprolactone, b) Poly(lactide-co-glycolide).

1.1.3. General fundamentals of main polymerisation techniques

1.1.3.1. Chain-Growth Polymerisation

In chain-growth polymerisation, each polymer chain is initiated by a free radical initiator, a anionic or cationic species, and grows rapidly producing a high molecular weight polymer.[13] Monomer concentration decreases steadily and the growth of a chain takes place by addition of one unit at a time to the active end of polymer chain. Usually it is not possible to have 100% monomer conversion so the mixture of the reaction will contain monomer, high molecular weight polymer and minor quantity of growing polymer chain. Once the propagation within the chain is stopped no further chain growth in polymer occurs. The propagation might be stopped either by chain-transfer or termination step. In this type of polymerisation reactions of initiation, propagation and termination do not have the same mechanisms and rates.

Free radical polymerisation and living/controlled polymerisation are chain-growth polymerisations which will be introduced in more detail in section 1.1.3.3 and section 1.1.3.4.

1.1.3.2. Step-Growth Polymerisation

The polymer molecular weight increases slowly through a single reaction type, and involves mechanism in which bi-functional or multifunctional monomers react (step by step) to form dimers, then trimers, longer oligomers and finally long chain polymers. Monomer disappears in the early stage of the reaction and at least two different monomers need to participate in the reaction. Each phase in step-growth polymerisation involves a reaction between different functional groups, so a dimer is created by the reaction of functional groups of two monomers, and then this dimer can react with monomer and create trimer, in case reaction with another dimer will create tetramer.[13] This process will continue till a high molecular weight polymer is obtained and requires high conversions of monomers. In general, the synthesis of polymers through this method leads to broad molecular weight distribution.

The branching to the polymer synthesized by step-growth polymerisation can be introduced by monomer with functionality of three or more and will eventually form a crosslinked material even at low fractional conversion.

A nonspecific illustration of defined (sections 1.1.3.1 and 1.1.3.2) chain-growth and step-growth polymerisation is presented in the Figure 1-6. To able better visualisation of the processes, single dots represent monomers and dots connected in chains symbolize oligomers and polymers generated during the process.

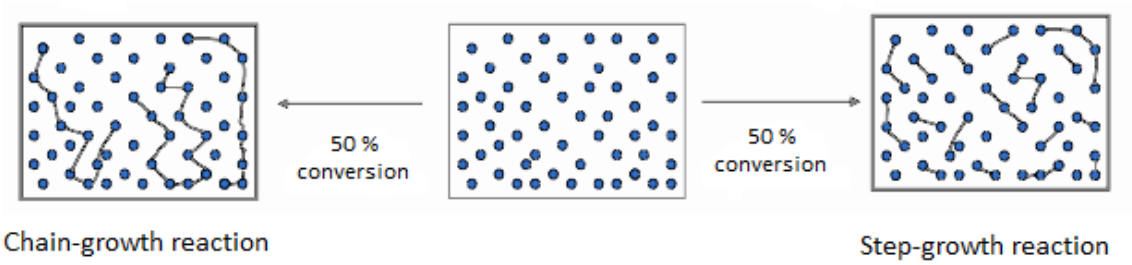


Figure 1-6: Graphical illustration of chain-growth and step-growth polymerisation (Courtesy provided by Dr Becer, University of Warwick).

More specific (representative) examples of chain-growth and step-growth polymerisation are presented in the Figure 1-7.

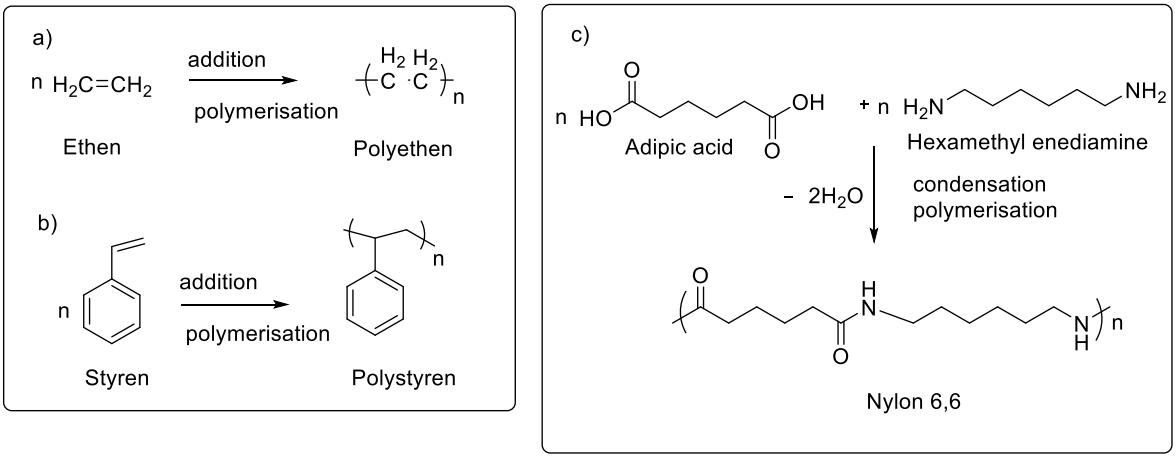


Figure 1-7: General examples of chain (addition) (a, b) and step (condensation) (c) polymerisations.

1.1.3.3. Free Radical Polymerisation

There are many applications in science and industry where strict control over the molecular weight, the molecular weight distribution or the chemical composition of the polymer is not required and these polymers can be easily prepared through free radical polymerisation (FRP). It is one of the easiest and most convenient methods to prepare polymers in a large scale. However, the absence of control over the incorporation of monomer into the polymeric chain structure is the main reason why this method does not apply to well-defined dendritic, branched and hyperbranched structures. Still, the fact is that more than 50% of all plastic materials and more than 70% of vinyl polymers in the modern world are made by FRP.[52,53] This is due to the wide tolerance of the technique towards impurities, water and oxygen. Moreover, the range of monomers which can be used by the use of this method (a vinyl monomers with general structure $CH_2=CR_1R_2$) is greater than those monomers compatible with other techniques. Examples of polymers produced *via* FRP include polystyrene, poly(vinyl acetate), polyethylene, polypropylene, poly(methyl methacrylate). As seen in Figure 1-8, the typical mechanism of FRP is divided into three stages (initiation, propagation, termination) which occurs continuously.[54] The process involves generation of free radicals from an initiator (by thermal decomposition of initiator or by photolysis), which are then added to the monomer. Formation of the radical is in general slower than their addition to monomer and for that reason the first step often determinates the rate of reaction. The propagation step is very fast and addition of the monomer to the growing chain continues. The final step irreversibly terminates the process. As seen on the scheme the termination occurs by several possible ways.

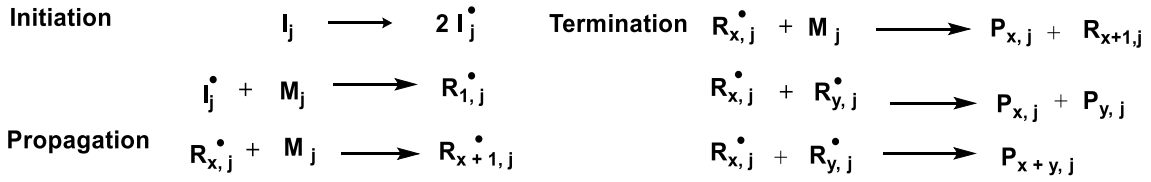


Figure 1-8: Schematic representation of FRP mechanism, including initiation, propagation and terminations steps.

Polymer growing chain termination happens either by combination of two growing chains or disproportionation in which radical transfer from growing chain to another takes place. Combination of an active chain end with an initiator radical will also lead to termination.[55] Propagation rate is higher than initiation rate, formed radicals propagate and terminate in a seconds. Due to continuous initiation and termination, with on-going polymerisation the polymers commonly demonstrate broad mass distribution with polydispersity index (PDI) in range from 3 up to 10.[53]

1.1.3.4. Living/Controlled Radical Polymerisation

In the past ten to fifteen years, there is a debate when it comes to the terms describing radical polymerisations.[56] This dispute is over the terminology and some disagreements started over the use of terms "living" and "controlled" polymerisations.[56] According to the International Union of Pure and Applied Chemistry (IUPAC) recommendations "a living polymerisation is a chain polymerisation in which an irreversible chain transfer and irreversible chain termination (deactivation) are absent". This generally excludes the use of "living" when referring to those processes. Use of "controlled" polymerisation also is controversial. According to IUPAC it is incorrect to use "controlled" when exclusively describing particular form of polymerisation as the word has much broader meaning and usage. Adjectives found in the literature like "controlled living", "controlled/living" and "pseudo-living" are also discouraged. The IUPAC group has recommended the term Controlled Reversible-Deactivation Radical Polymerisation (RDRP), and permitted the use of abbreviated name Controlled Radical Polymerisation (CRP). Living polymerisations are desirable as they do offer control over macro molecular synthesis. The techniques facilitating living polymerisation through reversible deactivation are briefly described in following subsections.

1.1.3.4.1. Nitroxide Mediated Radical Polymerisation

Nitroxide Mediated Radical Polymerisation (NMP) was one of the first Living Radical Polymerisations (LRP) and it is also known as Stable Free Radical Mediated Polymerisation (SFRMP). The technique has its origin in studies of initiation mechanisms as nitroxides were known as radical scavengers. This was due to ability nitroxides to efficiently trap carbon-centred radicals by forming alkoxyamines. In early stage of development, there were a number of studies using nitroxides as a radical trap in polymerisations of monomers with initiator-derived radicals.[57,58] It was observed that under some conditions the trapping of radical by nitroxide was reversible and this was the beginning of further research. Persistent radical effect was described and showed theoretically that NMP could provide narrow polydispersity polymers, and in practice use of TEMPO (stable nitroxy free radical, 2,2,6,6-tetramethyl-1-piperidynyl-N-oxy) as the control agent allowed the polymerisation of PSt with narrow PDI (1.2) at that time.[59,60] This system is fundamentally a radical polymerisation with a thermal initiator (Figure 1-9, mentioned BPO or AIBN in combination with TEMPO). The polymerisation kinetics is determined by the $[\text{nitroxide}]_0/[\text{initiator}]_0$ ratio, as the amount of excess free nitroxide after initiation step shifts the activation–deactivation towards the dormant species, reducing the polymerisation rate.[60]

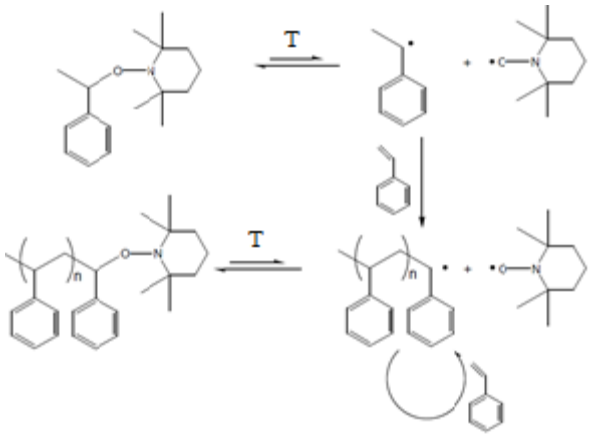


Figure 1-9: Use of TEMPO as the control agent in the polymerisation of styrene.

Thermal initiators are difficult to use as their efficiency to produce primary radicals by thermal decomposition is difficult to assess. The initiating group may cause many of the primary radicals to undergo rearrangement reactions leading to poorly reproducible kinetics in the polymerisation.[60] The nitroxide end-group is retained on the polymer chain after the polymerisation, however it can be removed.

Control in NMP is attained with dynamic equilibration between dormant alkoxyamines and actively propagating radicals. For the controlled polymerisation, equilibrium should be shifted towards dormant species in order to minimalize termination. Since controlled polymerisation of styrene, two strategies were applied to initiate NMP. One of them involves usage of an alkoxyamine as an initiator, and in the other approach alkoxyamine is formed *in-situ* from nitroxide and radicals generated using conventional initiator (Benzoyl peroxide, BPO). The "universal" initiator (which is alkoxyamine) can be successfully employed for a wide numbers of monomers in different conditions. Nitroxide Mediated Radical Polymerisation paved the way to development of ATRP and RAFT.[61]

1.1.3.4.2. Atom Transfer Radical Polymerisation

Atom Transfer Radical Polymerisation (ATRP) is one of the most successful controlled radical polymerisations and it is based on the reversible transfer of an atom X (often halogen) from a dormant initiator to a redox-active transition metal salt (e.g. Cu(I)). This transfer, catalysed by salt, generates an active radical.[62,63] The schematic mechanism is presented in Figure 1-10.

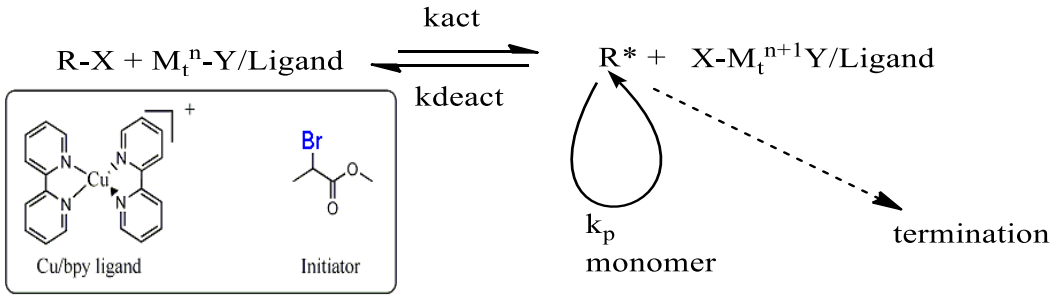


Figure 1-10: Schematic representation of normal ATRP mechanism.

A complex of a copper (I) halide with ligands such as 2,2'-bipyridyl (bpy) is frequently used as metal catalyst and this undergoes a one-electron oxidation by abstraction of the halogen atom from an initiator.[64] In this process an organic radical is generated together with the copper (II) complex. Adjusting the concentrations of transition metal ligand complex allows moving the equilibrium in the direction of deactivation consequently keeping radical concentration low. Initiator generates growing chains so its concentration determinates molecular weight of resultant polymer. Equilibrium of these reactions assures parallel the growing of the chains. Because of the continuous activation-deactivation process, the growth of polymer chain can occur till completing the conversion of the monomer. The advantage is that initiator shows low or no tendency to undergo side reactions. One important but easy rule to follow is that the R- group in the alkyl halide should be similar in structure to that of the monomer. A success of ATRP polymerisation relies on rapid initiation and fast deactivation. This widely used polymerisation method has proven to be a powerful tool in the synthesis of polymers with narrow polydispersities and predictable/controlled molecular weights.[64,65]

Atom Transfer Radical Polymerisation has been successfully performed in bulk, aqueous solution, organic solvents and mini-emulsion by homogenous or heterogeneous reactions.[66] It is essential to remember that the active complexes of the transition metals used are sensitive to solvent effects, which can limit this polymerisation method.[67] Polymerisation in solution is slower when using the same quantities of reactants comparing to polymerisation in bulk. The monomer to be polymerised determines the nature of the other components that can be used in the synthesis. A vast number of vinyl monomers have been successfully polymerised by copper-based ATRP including methacrylates, acrylates and styrenes.

As an improvement of the ATRP method, deactivation-enhanced ATRP (de-ATRP) has been reported and was used for the polymerisation of multifunctional vinyl monomers resulting in water soluble hyperbranched polymers.[23,68,69]

In-situ deactivation enhanced ATRP was further adopted for the preparation of hyperbranched polymers, in which instead of a Cu (I) species, a Cu (II) species were used to form Cu (I) *in-situ* using L-ascorbic acid as a reducing agent.[69] The reduced form of copper was generated in the reaction pot and was able to function as the catalyst in the ATRP process by being oxidised through the transport of halogen radical which was released when a polymer radical was generated.[69]

1.1.3.4.3. Reversible Addition-Fragmentation Chain Transfer Polymerisation

Reversible Addition-Fragmentation Chain Transfer polymerisation has received increasing attention since 1998 and is widely used in the preparation of different polymeric materials.[70] The method was developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO) using sulfur based thiocarbonylthio (S=CS) compounds as chain transfer agents (CTA). These CTAs are providing effective control of radical polymerisation process. Besides, an additional mechanism of RAFT called MADIX (Macromolecular Design by Interchange of Xanthates) was also reported using xanthates RAFT agents.[71] Both, RAFT and MADIX, follow the same mechanism differing only in the polymerisation mediator used.[71] RAFT polymerisations can be used for a large number of monomers in a variety of solvents. It is known that monomers which can undergo FRP can also be polymerised by RAFT.

In principle, the mechanism of the RAFT process involves five stages as presented in Figure 1-11 and includes: initiation, pre-equilibration, re-initiation, chain equilibration and termination.[72] RAFT mediated polymerisation is initiated by the decomposition of an free radical initiator, for example 2,2'-azobis(isobutyronitrile) (AIBN, commonly used RAFT initiator[73]) or 1,1'-azobis(cyclohexanecarbonitrile) (ACHN) to form radicals. These radicals tend to undergo addition to the monomer unit.

The process is based on the simple introduction of small amount of chain transfer agent (CTA) for example dithioesther of standard formula 1 (CTA, Figure 1-11, p.20), into a conventional free radical system (monomer and initiator). The transfer of the CTA between growing radical chains (present at low concentrations), and dormant polymeric chains (present at higher concentrations), will regulate the growth of molecular weight and limit the termination reactions.

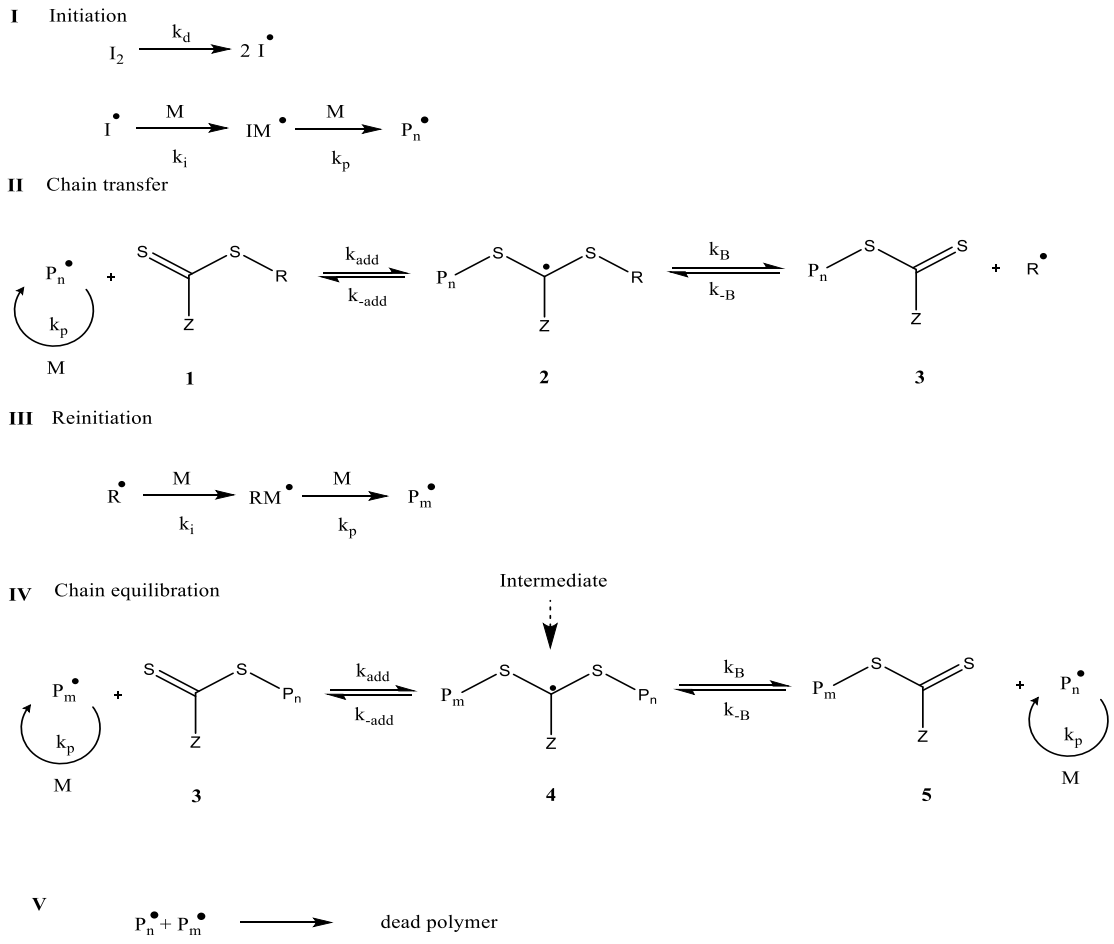


Figure 1-11: General mechanism of the RAFT process (adopted from Ref[71]).

The radical species created from the decomposition of the radical initiator step I (Figure 1-11), reacts with the monomer. Growing polymer chain rapidly adds to reactive bond of the CTA (C=S) to form radical intermediate of generic formula 2 (Figure 1-11). Here, radical initiator might add directly onto CTA, before reacting with any monomer. Step II (Figure 1-11) shows fragmentation of the intermediate occurring reversibly either toward to the initial growing chain or toward to a macro-chain transfer agent of generetic formula 3 (Figure 1-11) and the free re-initiating group (R). The R group can re-initiate the polymerisation by reacting with the monomer and start new polymer chain which will propagate step III or react back on the macro-CTA. When the initial CTA has been consumed the macro-CTA is solely present in the reaction mixture and as seen in step IV (Figure 1-11) rapid balance between active propagating radicals and corresponding dormant species provides equal probability for all chains to grow and allows the preparation of polymers with low PDI values. This is considered as the main equilibrium in RAFT mechanism.

The intermediate radicals presented as a 2 and 4 on Figure 1-11 might get involved in many sides reactions. Step V describes unavoidable termination (either by combination or disproportionation) present in all free radical polymerisation systems. The final product of RAFT process consist mainly polymeric chains showing re-initiating group (R) at one side and thiocarbonyl-thio group at the other side of chains as the side reactions (terminations) are aimed to be kept to minimum. In order for the RAFT process to work efficiently, certain parameters must be above certain limit. For example, the concentration of CTA must be higher than concentration of initiator. This allows maintaining a high concentration of dormant polymer chains over the propagating chains, which reduces the termination reactions and favours constant rate of propagation.

As described it was concluded that RAFT mechanism process differs from ATRP or NMP, as chain growth is based on cooperative chain transfer between polymeric chains instead reversible radical capping.[74] Moreover the majority of the polymeric chains are initiated by the re-initiating CTA group (R group) and terminate by thiocarbonyl-thio group. The source of radicals generates the generative chain transfer which allows chains to grow. In the RAFT process the molar mass is controlled by the stoichiometry of the reaction and it increases in a linear manner with monomer conversion. The rate of polymerisation might be increased by increase in radical concentration, but this will increase the probability of chain termination resulting polymers with higher PDI values. As mentioned, the molecular weight increases linearly with conversion and might be predicted if it is assumed that all CTA reacted and chains initiated by the source of radicals are neglected. Following equation, in an ideal case, allows the theoretical calculation:

$$\boldsymbol{Mn}\ (\mathbf{th}) = \frac{[\boldsymbol{M}]}{[\boldsymbol{CTA}]} * \boldsymbol{Fw}(\boldsymbol{M}) * \boldsymbol{Conv} + \boldsymbol{Fw}(\boldsymbol{CTA}) \qquad \textbf{E.q: 1-1}$$

Where: M – Monomer, CTA – Chain transfer agent; [M] and [CTA] are concentrations of the monomer and CTA; Fw(M) and Fw(CTA) are monomer and CTA formula weights (molecular weights) respectively; Conv is a monomer conversion.

When considering the reaction conditions used for RAFT method, it is worth to keep in mind that three stoichiometric ratios might influence the reaction: [M]:[CTA], [CTA]:[I], [M]:[I].[3] Monomer, CTA and initiator should be soluble in the solvent used. The temperature plays a crucial role in the control of molar mass. Increasing temperature accelerates the rates of fragmentation and polymerisation, in addition the rate of termination reactions. Therefore, at higher temperatures boarder molar mass distributions might be expected. The temperature should be adjusted as a function of the half-life of the initiator used to keep constant radical concentration.

The RAFT is highly tolerant to many functional groups which can be introduced either as the initiator moiety or into the RAFT agent. The obvious is that the methodology has some limitations since the nature of functional groups will affect the stability of dithioesther radical intermediate. Therefore, choice of the CTA is very important in RAFT polymer synthesis.[75] Commonly, dithioesters,[76,77,78] trithiocarbonates,[79,80] dithiocarbamates,[4,81,74] and xanthates[4,81] are used as CTAs (Table 1-1, p.23).

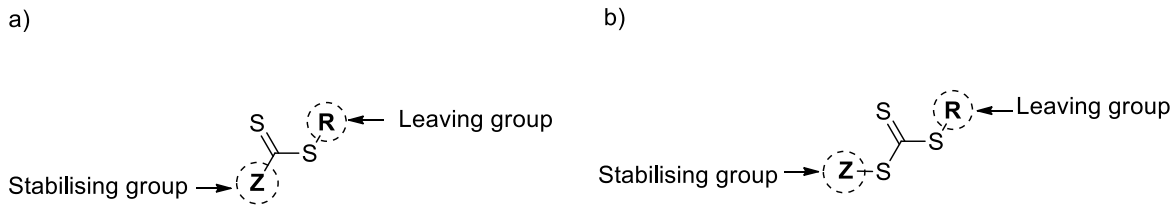
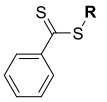
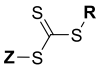


Figure 1-12: The generic structure of dithioester (a) and trithioester (b) used as RAFT agents.

The most reactive are dithioesters and trithiocarbonate with sulphur group next to thiocarbonylothio group. RAFT agents with oxygen or nitrogen next to the thiocarbonyl have considerably lower activity towards radical addition.[4] The general structure of dithioester and trithioester used as RAFT agents are presented in Figure 1-12.

Table 1-1: General types of RAFT agents according to the stabilising group (Z).

RAFT agent type	Z
Dithioester 	**alkyl-, aryl-**
Xanthate 	**alkyl-O-**
Trithiocarbonate 	**alkyl-S-**
Dithiocarbamate 	**Z_1Z_2-N-**

The first compounds used as RAFT agents were dithioesters (e.g. dithiobenzoates, dithioacetates). These compounds have intensive, very offensive smell and pink to dark red colour. Therefore, polymers produced by RAFT process are commonly coloured due to the RAFT agent end group. They exhibit higher activity than trithiocarbonates, dithiocarbamates, and xanthates and their synthesis is not that easy. Xanthates are easier to synthesise and they are colourless and are not that offensive in smell as dithioesters. However they often yield polymers with PDI equal 2 or higher. In the RAFT polymerisation of vinyl monomers, trithiocarbonate – based CTA have been found most effective.[82] Additionally, trithiocarbonate-based RAFT agents are easier in synthesis, easier to handle and show less odour then dithiobenzoates.[83]

Effectiveness of RAFT polymerisation depends on structure of the RAFT agent (which normally comprise of two groups as presented in Figure 1-12). Finest control involves selecting an appropriate RAFT agent for the monomer to be polymerised. The nature of leaving group (R) and stabilising group (Z) is very important.[74,84] Both groups present different properties which contribute to the RAFT living process. It is clear that in majority cases the effect of both groups needs to be considered when designing/choosing most efficient RAFT agent to control the polymerisation of a specific monomer. The (Z) group influences the stability, where the (R) is good free radical leaving group capable of re-

initiating free radical polymerisation successfully. The (R) groups are commonly related to the monomer being used or to the initiators and their inappropriate choice can lead to uncontrolled polymerisation with significant retardation. Strong (Z) groups will increase formation of intermediate and enhance reactivity of S=C bond towards radical addition. However, this needs to be finely tuned in order to favour fragmentation of intermediate, which will free re-initiating group (R). Many versatile RAFT agents are commercially available, and many more have been reported in the literature, also the RAFT agent selection rules have been well described.[4,70,75,5]

In an ideal RAFT polymerisation synthesis, the kinetics of the process is unaffected, tho in typical experimental set up, RAFT process experiences reduction in the polymerisation rate relative to the conventional radical polymerisation. It should be mentioned that in RAFT polymerisation, inhibition and retardation have been observed. Inhibition and retardation vary due to RAFT agent stability. Inhibition describes the situation when chemical reaction does not occur where retardation defines reduction in rate of chemical reaction. Both processes might be credited to either the slow fragmentation of the intermediate radicals in the pre-equilibrium or slow re-initiation of (R) group. There is no need for the radical intermediate to decompose rapidly; slow process can lead to narrower molecular weight distribution.

1.1.3.4.4. Advantages and limitations of controlled radical polymerisations

Controlled radical polymerisations are based on two main principles which include reversible termination and reversible transfer. From the publications in recent years, it is clear that controlled radical polymerisation techniques offer successful synthesis of well–defined polymers with different compositions, topologies, and architectures. Introduction of CRP brought a significant advancement in synthetic polymer chemistry and provided opportunity to control the variety properties of a target material.

Atom Transfer Radical Polymerisation and Nitroxide Mediated Radical Polymerisation are examples of reversible termination, while Reversible Addition-Fragmentation Chain Transfer polymerisation is an example of reversible transfer. To summarise, in reversible termination the polymer chain is end-capped with a moiety that can reversibly undergo

homolytical cleavage; while in processes based on reversible transfer, there is fast exchange of growing radicals by a transfer agent. In ATRP, a halide is the moiety which is reversibly transferred to a transition-metal complex; in NMP, this moiety is a nitroxide. In the RAFT process, dithiocarboxylates are responsible for this exchange, which proceeds by an intermediate radical. The main task of the initiator in CRP is to determinate the number of growing chains and importantly initiation should be fast. In ATRP, alkyl halides of general structure (R-X) are employed as initiators and the rate of initiation is determined by the choice of transition metal catalyst. The transition metal catalyst is the key to determinate the atom transfer equilibrium and dynamics between the dormant and active species. The metal has to have two readily available oxidation states and should have suitable attraction toward a halogen (ligand must complex with metal strongly). The metal catalyst at a lower concentration (Figure 1-10, e.g. Cu (I)) is sensitive to the air and due to lack of the deactivator (e.g. Cu (II)) at the initial stage, polymerisation suffers from bad control. Low poly polydispersity (PDI) should be a characteristic for all CRP methods, however is not always easy to achieve, as this requires the absence of chain transfer and termination. The few of following requirements should be fulfilled in order to obtain polymers with low PDIs.[64,65] The rate of initiation should be competitive with the rate of propagation, which allows the growth of polymer chains. The exchange between species of different reactivity should be faster than propagation which helps the active chain equally react with monomer. The insignificant chain transfer or termination must be present.

In normal ATRP synthesis, a relatively high concentration of metal and ligands must be introduced. Catalyst removal or reduction was, and remains a critical step in the preparation of copolymers. As catalyst removal and recycle process may cause environmental problems this controls economic costs that commercial manufacturers would have to address.

Only RAFT polymerisation allows the control over the polymer synthesis by a simple addition of a single additional compound.[85] Moreover, in RAFT method, the usage of potentially dangerous or toxic metal salts is not essential. Among of mentioned methods only RAFT process tolerates traces of impurities, and is compatible with the broadest range of monomers and reaction conditions.[86] Also an addition of RAFT agent in principle should not have any influence on polymerisation rate and radical concentration.

Theoretically, a cell may be damaged by a polymer, as the impurities from the polymerisation reaction might adversely affect biological system.[85] Catalysts, initiators and

other polymerisation aids may also be sources of toxic impurities. Potential toxicity of polymers prepared by RAFT method is an obvious concern. Possible toxic effects, related to RAFT agent, might depend on the interactions between biological elements, structure of the polymer and RAFT end groups, and the chemical and physical properties of the polymer. However, based on series of studies demonstrating *in vivo* applications of RAFT prepared polymers, it is assumed that apparent toxicity is low and to date no paper noted massively increased toxicity due to presence of RAFT groups.[85] At times, RAFT group removal may be advisable, depending on the RAFT agent employed for *in vitro* and *in vivo* applications.[85]

1.2. Polymerisations of Multi-Vinyl Monomers: Multi-Functional Monomres or Macromers as the crosslinking agents and hyperbranched polymers

Multi-vinyl monomers have been used for long time in the preparation of crosslinked materials. As it was reported, polymerisation of MVMs often lead to insoluble crosslinked networks.[87] Unwanted side reactions often lead to occurrence of gelation. For years, only a low percentage of MVMs were used in copolymerisations. In FRP addition of even small amounts of multi-vinyl monomers would lead to a crosslinked network and conversions of monomer to polymer would be less than 20%.[88,89] For a long time it was regarded as an almost impossible task to control the polymerisation of MVMs.

In order to manage the control over the reactions, CRP methods were adopted in the synthesis of hyperbranched copolymers *via* copolymerisations of multi-vinyl monomers and monovinyl monomers.[90,91] Multi-vinyl monomers used in the syntheses of polymers through CRP methods offered an opportunity to prepare soluble branched polymers with controlled molecular weights, degree of branching, crosslinking density in addition to well-defined 3D macromolecule structures which seemed be impossible to achieve through FRP.[92]

Applying MVMs as branching agents to prepare controlled hyperbranched architectures was reported for a first time by Sherrington et.al..[93,94,95,96] This synthesis was developed as a one-step method and involved the use of chain transfer agent (CTA). It was reported that CTA delayed gelation time and crosslinking as long as molar ratio of CTA and free vinyl groups in MVM is suitable adjusted. The CTA is able to control length of primary polymer

chain to some extent. Organic solvent might also contribute to inhibition of crosslinking. Change of CTA and functionality of multi-functional monomer (MFM) can tailor hyperbranched polymers according to specific requirements. It was reported that in order to achieve soluble HBPs limited molar ratio of MVM to initiator needs to be used. If the ratio exceeds 1, the crosslinking (or even micro gelation) might happen and resultant polymer will not be soluble. So to overcome obvious limitations of the method, further developments were needed. The method was also tested in aqueous conditions. High conversions of methyl methacrylate (MMA) and divinylbenzene (DVB) were carried in emulsion, using potassium persulfate as the conventional free radical initiator.[97] Number of thiols have been investigated in order to inhibit crosslinking and thus favour the formation of branched products. Benzylthiol (BT) has been found to be particularly effective in producing hyperbranched products without crosslinking. Mole ratios of DVB/BT of ≤ 1 ensure that crosslinking is avoided.[97] The radical initiator used to initiate the reaction can also create an issue as some of the polymer chains might bear the radical initiator functionality and some CTA functionality. Soluble, branched poly(methyl methacrylate)s have been also prepared in solution using conventional FRP of MMA in the presence of a branching divinyl comonomer with appropriate levels of dodecanethiol (DDT) chain transfer agent added to inhibit gelation.[98] The branching comonomers employed were ethylene glycol dimethacrylates with varying lengths of PEG chains, DVB, and EGDA.

Atom Transfer Radical Polymerisation and Reversible Addition-Fragmentation Chain Transfer Polymerisation methods were introduced in the synthesis of branched structures from multi-vinyl monomers or macromers. With use of one-pot Cu-based ATRP soluble branched structures were prepared from PMMA, also from copolymerisation of EGDMA or disulfide-based dimethacrylate (DSDMA) (branching agents) with hydroxypropyl methacrylate.[39,99] Atom Transfer Radical Polymerisation of monovinyl monomer, and divinyl crosslinker (methyl acrylate and 1,6-hexanediol diacrylate) was also conducted indicating the effect of the dilution on the structure of resultant polymer and number of pendant vinyl group.[100] Atom Transfer Radical Polymerisation was effective in preparing homogeneous polymer networks with a high crosslinking efficiency when copolymerising MMA with EDGMA.[101] Reversible Addition-Fragmentation Chain Transfer polymerisation was successfully conducted to prepare hyperbranched copolymers of MMA and EGDMA.[91] It was the earliest in fact successful application of this method with use of EGDMA as branching agent and near 100% monomer conversion was obtained. The

branched structure of PMMA was well controlled and RAFT functionality was kept in the structure. Furthermore branched acrylic copolymers were prepared from 2-hydroxypropyl acrylate (HPA) with EGDMA and with DSDA.[102] Reversible addition chain transfer polymerisation was also adapted in the synthesis of branched polystyrene with use of asymmetric vinyl monomer.[103] HBPs were also achieved by RAFT from DVB, where the presence of RAFT agents allowed conversions as high as 68% before crosslinking, as an alternative of 15% for conventional FRP.[104] Not only ATRP and RAFT has been studied in the controlled synthesis of MVMs. NMP has also been adopted and using this method HBPs containing up to 12 mol % pendant vinyl groups were prepared.[105] Another successful route to produce vinyl-functionalized HBPs used self-condensing anionic copolymerisation of allyl methacrylate (AMA) and hydroxyethyl methacrylate (HEMA) at room temperature, with high conversion (99.2%), and no gelation.[106]

As seen the controlled radical copolymerisation and homopolymerisation of MVMs enabled the production of a new generation of polymeric materials. It is also clear by now that intramolecular cyclization cannot be ignored. Catalytic chain transfer polymerisation (CCTP) was used to homopolymerise or copolymerises EGDMA to form dendritic/hyperbranched polymers and knot structures. This has been demonstrated as a facile and convenient methodology and could be simply applied as a further chain extension reaction. Soluble dendritic and single cyclized knot polymers were also reported recently and achieved via successful homopolymerisations of MVM by deactivation enhanced atom transfer radical polymerisation (de-ATRP) and RAFT polymerisation.[107,108,109,110,111,112,113,114] High level of vinyl functionality in RAFT was achieved by introducing the large quantity of divinyl monomers and provided those copolymers with advanced capability of *in-situ* gelation through photo-initiated polymerisation or chemical crosslinking.[114] The chain transfer agent considerably delayed the gelation. In those polymers by changing the reaction time and the ratio of the monomer to the chain transfer agent, the control over molecular weight, vinyl content, intermolecular cyclisation and coupling was finely manipulated.[114]

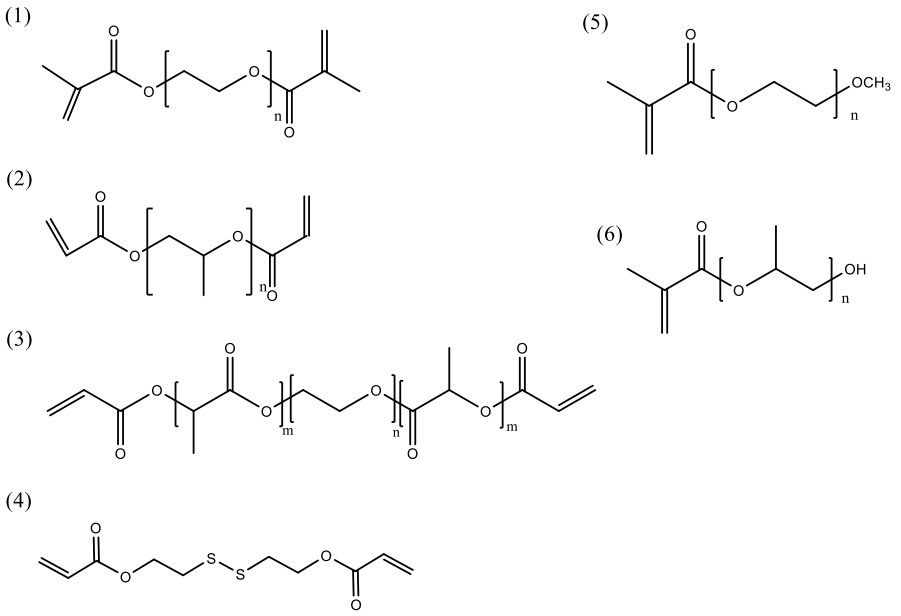


Figure 1-13: Selected examples of multifunctional vinyl monomers (1) PEG, (2) PPG, (3) PLGA, (4) disulphide monomer building block, and monofunctional vinyl monomers (5) PEG, (6) PPG.

To date polymerisation of MVMs (mono and divinyl, examples in Figure 1-13) is still relatively difficult and the vinyl content of free groups in desired structure is limited. Special attention needs to be paid to the synthesis method and the purification steps. Significant competition between intermolecular cyclization and intermolecular branching makes synthesis of hyperbranched polymers with use of MVM very challenging.

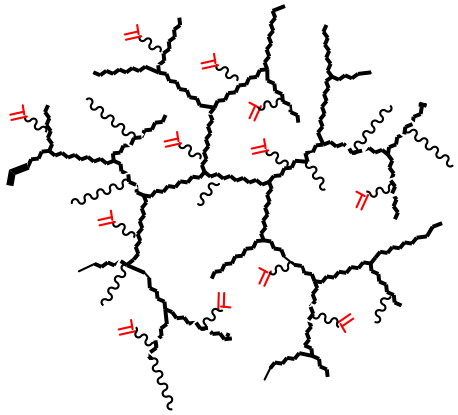


Figure 1-14: General structure of hyperbranched polymer with multi-vinyl functionality.

1.3. Hyperbranched Polymers

Hyperbranched polymers (HBPs) belong to the same group of polymers as dendrimers. They are large and high molecular weight molecules with the ability to be designed for a specific function due to a large number of reactive groups.[115] This group of polymers can provide a range of desirable properties over their linear counterparts. By the use of different chemicals HBP can be tailored in many ways. The present challenge in the synthesis of HBP is the lack of structural control in the polymer product, which significantly limits their potential applications. Modification of functional groups is needed to control their solubility, compatibility, adhesion to various surfaces, self-assembly, chemical recognition.[116] In general, functionalization includes modification of end groups, backbones or hybrid modification. The common end groups at the edge of HBPs contain hydroxyl, carboxyl, amine, thiol, and halide groups.[117] Vinyl-functionalized HBPs (Figure 1-14, p.29) have gained a lot of attention as their pendant vinyl groups can be further modified for a required application. However, the syntheses of these polymers are difficult, because the cross-linking is unavoidable during the polymerisation reaction. Suitable monomers and appropriate polymerisation methods can tailor backbone of the polymers.[118] Typical branched architectures of polymers[115] are illustrated in Figure 1-15.

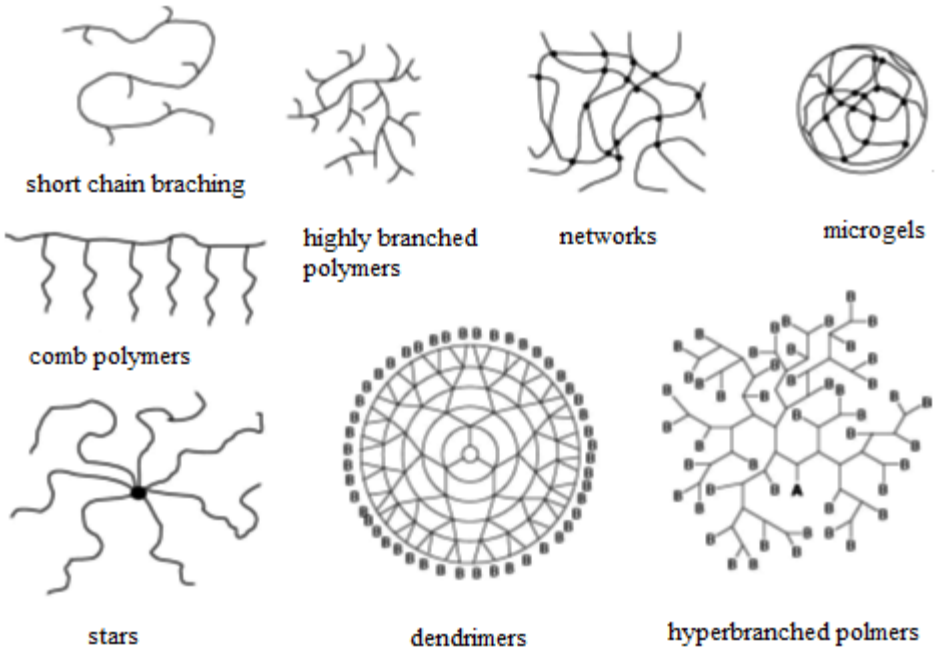


Figure 1-15: Examples of polymer architectures (adopted from Ref[115]).

All hyperbranched polymers (including dendrimers) have tree-like structures. However, hyperbranched polymers possess less regular branching structure than dendrimes but still can retain similar degree of functionality. Hyperbranched polymers have lower viscosity when comparing them to linear polymers with equal molecular weights; also these polymers show broad molar mass distribution as there is almost no control over the size and structure. They often have irregularly branched structure with degree of branching (DOB) lower below 1.0 (typically, 0.4–0.6), high polydispersity of molecular weight (typically, PDI > 3.0), and a high number of functional groups linked.[117] Degree of branching is an important parameter when it comes to HBPs. For an ideal dendrimer DOB is equal 1, and is defined as the ratio of branched terminal and linear units in the polymer.[119] Hyperbranched polymers are often easier and cheaper to prepare (generally in a one-pot reaction) than dendrimes where production requires many stages, drastic purifications between these steps and higher costs.[120,121] For that reason HBPs are convenient for a large scale synthesis in industry.

Applications of hyperbranched polymers can be very wide,[115] and a constant increase in many areas of human life is noticeable, including their usage in hydrogel components for tissue-growth active hydrogels and drug delivery. Within the literature, we can find several good reviews which provide excelent information about the research on structure, proporties, synthesis and applications of hyperbranched polymers. Among of them we have: Fre´chet and Hawker,[122] Hult et al.,[123] Voit,[124] Gao and Yan,[125] Voit,[126] Seiler,[115] England and Rimmer,[127] Fossum[128] and Wang.[117] Time and effort have been put in recent years to develop this area of chemistry. Improvement of facile synthetic techniques that can produce structurally defined hyperbranched polymers in large quantities with low cost is definitely an interesting matter for many researchers.

Synthesis of HBPs can be divided into single monomer methodology (SMM) and double-monomer methodology (DMM).[125] These techniques are based mainly on step growth polymerisation and chain growth polymerisation (see section 1.1.3.1 and 1.1.3.2, p.12-13). The first method of the synthesis is based on polycondensation of AB_x monomers. Where A and B are functionalities that can react with each other but not with themselves. If x = 2 and A reacts only with B, the polymerisation of ABx monomers can results in highly branched polymers. However, according to statistic branching reaches around 50%, so is not as good as in dendrimes where over 90% or even 100% can be achieved.[129] In DMM straight

polymerisation of two types of monomers (A_2 + B_3) generates hyperbranched polymers.[125] Other methods included in SMM are self-condensing vinyl polymerisation (SCVP), self-condensing ring-opening polymerisation (SCROP) and proton-transfer polymerisation (PTP).[125] In SCVP vinyl monomer has second functional group which is able to initiate the polymerisation of other vinyl groups. This method initially used "living cationic" propagation and since have been improved by including "living free radical" and "group transfer".[125] However these methods cannot provide reasonable control of the molecular weight and branched structure for the polymers because of their non-living nature, basics of this method were used by other researchers to investigate copper-mediated living radical polymerisation.[125] In this type of polymerisation the vinyl monomer possesses a second reactive group, e.g. a halogen atom that can be activated and deactivated and, therefore mediate living polymerisation in the presence of a catalyst based on copper (I). In this case propagation may take place at either the double bond or after the initiation of the halogen functionality, resulting in branching points and eventually in hyperbranched polymers.[130] This process requires expensive, tailored vinyl monomers with specific functional groups that require complex syntheses and moreover the polymerisations need to be stopped at low conversions.[125]

Branched copolymers of ethylene glycol dimethacrylate (EGDMA) and methyl methacrylate (MMA) were produced through defining and controlling the number of primary chains according to report, thru ATRP but in this method resultant copolymer had low degree of branching.[98] Another research group adopted a similar procedure using RAFT polymerisation to prepare copolymers with a low degree of branching.[91] Therefore, typical approach in the synthesis of HBPs from MVMs include free radical polymerisation, nitroxide mediated polymerisation, atom transfer radical polymerisation, and reversible addition-fragmentation chain transfer polymerisation. The backgrounds and basics of those methods were explained in previous sections.

Polymers with both thermoresponsive and photo-cross linkable properties have more advantages for scaffolding in tissue engineering and drug deliver than just thermo-responsive or just photo-cross linkable polymers.[131,132] Linear polymers are in common use but mainly due to limited control of polymer modification and non-homogenous crosslinking properties,[133] tree-like macromolecules has gained more interest in research.

1.4. Hydrogels, *In-situ* forming hydrogels

In general hydrogels are 3D networks of crosslinked hydrophilic polymers.[134] They are often used in biomedical applications and they can swell large quantities of water without dissolving the polymer.[135,136] Water swollen polymer matrices in many cases due to their soft and hydrophilic nature are suitable for drug or protein delivery systems likewise cell-entrapping scaffolds in tissue engineering.[137] Due to the fact that cells can respond to the physical properties of the environment choice of the material with corresponding properties is important. There are three classes of macro molecules that can be used to produce hydrogels: synthetic polymers, peptides, polysaccharides and proteins.

Hydrogels can be classified in several ways.[138,139] Due to the origin they can be divided into three groups: natural, synthetic or hybrid.[140,141,142] Most common natural and synthetic monomers used for hydrogel fabrication are listed in Table 1-2 (p.35).

Proteins (e.g., collagen, gelatine and fibrin) and polysaccharides (e.g., alginate chitosan, hyaluronic acid, dextran) are natural polymer-based materials which can be used to create hydrogels. These hydrogels due to excellent biocompatibility have been used in tissue engineering (TE) and drug delivery, however natural derived ECM proteins as a scaffolds carry risk of infections and potential immunogenic reactions, also can demonstrate fairly poor mechanical properties.[142,143,144] Synthetic polymers, such as poly(acrylic acid) (PAA), poly(ethylene) glycol (PEG), poly(vinyl alcohol) (PVA), polyacrylamide (PAAm), and polypeptides[145] are materials used for the synthesis of synthetic hydrogels. PEG and its derivatives are the synthetic hydrogels widely used in medicine and biomedical field. Hybrids are a combination of natural and synthetic polymers. Ability of photo-polymerisation, adjustable mechanical properties, convenient control of scaffolds architecture and chemical compositions[141] gives synthetic hydrogels specific advantages.

Hydrogels due to high water content and their similarity to the native ECM (compositionally and mechanically) are generally highly biocompatible. Due to well-defined structures can be tailored by enzymatic, hydrolytic, or environmental pathways to design their biodegradability and functionality. Due to nature of side groups hydrogels are classified into two groups neutral or ionic,[146] due to preparation method: hydrogels from homo-polymer or co-polymer, due to physical structure of the network as an amorphous, semi-crystalline, hydrogen bonded, super-molecular structures[147,148,134] and hydro-colloidal

aggregates. According to the crosslinking mechanism we have hydrogels: chemically and physically crosslinked.[138] Furthermore, hydrogels can be classified into: *in-situ* forming or preformed hydrogels.[149] As mentioned, hydrogels can be formed by physical or chemical crosslinking. Different interactions methods are presented on Figure 1-16. Interactions such as ionic, van der Walls, hydrophobic and stereocomplexation might cause physical crosslinking, where chemically crosslinked hydrogels will be created by radical chain polymerisation started by redox system or photo initiation. Chemically crosslinked hydrogels are also created by reactions between certain groups including reactions between thiols and acrylates or vinyl sulfones, aldehydes, amines or activated esters. In general physical crosslink is weaker then chemical crosslink in hydrogels, and physically crosslinked materials are more sensitive to changes in environment, which might cause disruption in hydrogel network.

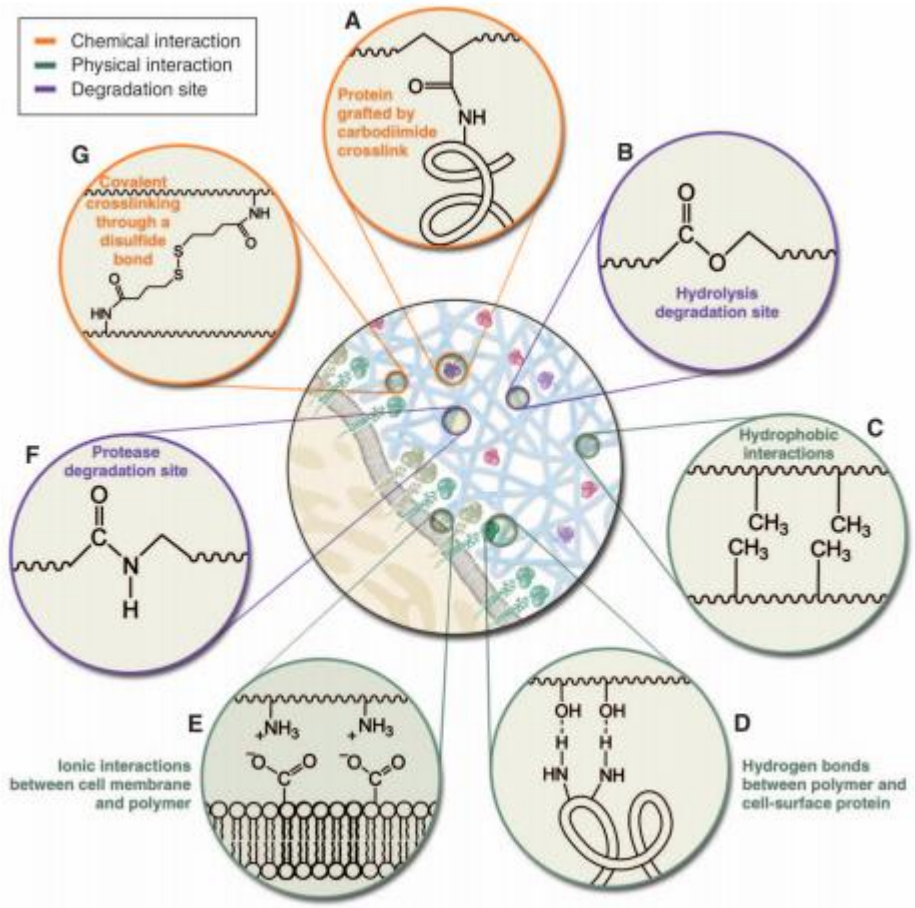
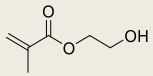
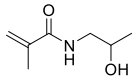
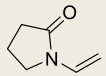
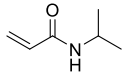
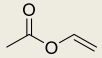
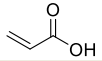
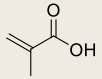
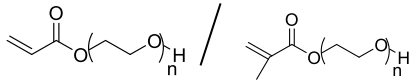
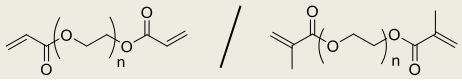
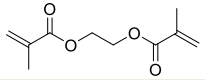
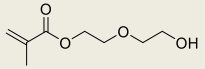
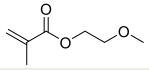
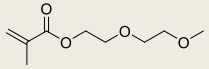
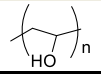


Figure 1-16: A graphical demonstration of a variety of chemical and physical interactions underling hydrogel design (adopted from Ref[142,150]).

Table 1-2: Synthetic and natural monomers/polymers used for hydrogel construction.[134, 142,143,146,151]

Synthetic monomer/polymer	Structure	Natural polymer	Structure
Hydroxyethyl methacrylate (HEMA)		Chitosan	example structures of natural polymers are given in section 1.1.2.1
N-(2-hydroxypropyl) methacrylate (HPMA)		Alginate	
N-vinyl-2-pyrrolidone (NVP)		Fibrin	
N-isopropyl acrylamide (NIPAAm)		Collagen	
Vinyl acetate (VAc)		Gelatin	
Acrylic acid (AA)		Hyaluronic acid	
Methacrylic acid (MAA)		Dextran	
Polyethylene glycol acrylate/methacrylate (PEGA/PEGMA)			
Polyethylene glycol diacrylate/dimethacrylate (PEGDA/PEGDMA)			
Ethylene glycol dimethacrylate (EGDMA)			
Hydroxyethoxyethyl methacrylate (HEEMA)			
Methoxyethyl methacrylate (MEMA)			
Methoxyethoxyethyl methacrylate (MDEEMA)			
Poly(vinyl alcohol) (PVA)			

Hydrogels made from natural polymers as mentioned, have in-built biocompatibility but hydrogels made from synthetic materials allow better control over the structure of the design artificial tissue. In order to obtain good balance in hydrogels used for biomedical applications, both natural and synthetic polymers have been combined into hybrids.[152]

***In-situ* forming hydrogels.** These hydrogels form in the body after injection of the precursors (Figure 1-17), in contrast to preformed hydrogels that have to be implanted by surgery. To find suitable materials that can solidify *in-situ*, preferably by self-assembly of the building blocks, and have desired biological and mechanical properties is challenging.

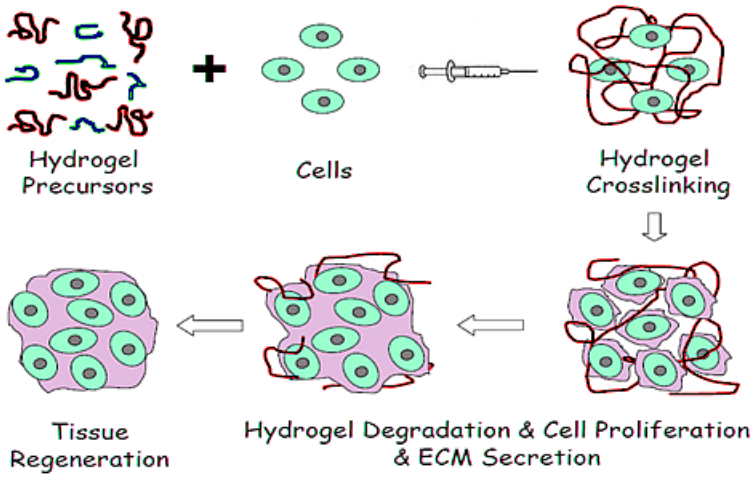


Figure 1-17: Schematic illustration of *in-situ* forming hydrogel (adopted from Ref[149]).

The gel precursors are injectable, non-toxic fluids introduced to body prior to gelation. Gelation should not cause any toxicity or significant temperature rise; this ensures a good fit and contact with surrounding tissue as scaffolds.[153] Hydrogel precursors can be introduced directly into tissue with irregular shapes and sizes.[151] A number of *in-situ* forming hydrogels have been reported in literature.[141,154,155,156,157,158,159] These hydrogels may be separated into two groups: physical and chemical gels in regards to their gelation mechanism.[160,161,159] In many of *in-situ* forming hydrogels, the formation of the gel does not occur for several minutes after injection, which can create side effect as the hydrogel may flow out of the defective area and cause pain to human or animal.[162,156] The hydrated structure of the *in-situ* forming hydrogel is similar to the native tissue. That is why

continuous work and improvement of gelation time of different hydrogel systems is in heart of many scientists.

Physically crosslinked hydrogels have been commonly prepared *in-situ* by self-assembly of thermosensitive amphiphilic block copolymers, however chemically crosslinked hydrogels may also be formed in this way.[163] In physical crosslinking no reactive groups, (photo) initiators or crosslinking agents are required. So the requirements to create physical crosslinking are generally mild. It is possible to reverse many physical interactions, so created gels are weak, and by changing external environment (e.g. temperature, pH) it is possible to raise disruption of the hydrogel. Hydrogels made by physical crosslinking are mechanically unstable to be used for scaffolds or drug delivery. In contrast, better performance can be presented by chemical crosslinking gelation of macromonomers, as their structures are controllable by designing module units resulting with gel possessing preferred mechanical properties. In concerns to biological components of hydrogels, chemical crosslinking can be sometimes harsh procedure.[164] Stimuli responsive polymers that, as it was mentioned earlier, change in respond to external stimuli are easy to apply in hydrogel fabrication and its development, they can also form self-assembly hydrogels.[164,24,165] Smart polymers have been engineered with idea to meet requirements in developing materials that support the attachment and proliferation of cells *in-vitro* and *in-situ*.

Synthetic hydrogels have advantages over natural hydrogels and a large number of commonly used hydrogels are based on linear structures such as poly(ethylene glycol) (PEG), poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and poly(lactide-co-glycolide) (PLGA). Poly(ethylene glycol) is non-toxic, hydrophilic, highly adaptable compound, easily soluble in water and other solvents. Poly(ethylene glycol) based hydrogels are biocompatible, non-immunogenic, resistant to protein absorption and easy to modify,[146,145] therefore they are very attractive scaffolds to provide 3D networks. Poly(ethylene glycol) can have linear or brunched structure. Both temperature-responsive and cross-linked polymers have been fabricated from PEG. To form relatively stable gels from either PEG or its derivatives, various methods of gelation can be used (e.g. physical, ionic or covalent). Chemically crosslinked hydrogels are formed by covalent bonds and there is a reaction between functional groups. Hydrogels based on PEG can be made by crosslinking methods including free radical polimerysation of PEG acrylates, Michael type addition or click

chemistry. Among of different methods photopolymerisation is commonly used. This method uses visible light (or UV-irradiation) and can convert a liquid monomer or macromer to a hydrogel by free radical polymerisation in a fast, controllable manner under physiological conditions. PEGylation is a technique common known and constantly being developed. It involves attachment of PEG polymer chains to molecules and macrostructures, such as a drug or therapeutic protein. The covalent attachment of PEG can "mask" the agent from the host's immune system. PEGylation can also provide water solubility to hydrophobic drugs and proteins.

Hydrophilic monomers and polymers provide diverse advantages in both fabrication and application of hydrogel systems. For this reason photo-cross-linkable precursors like PEG or poly(lactic acid) hydrogels (based on linear multi-vinyl macromers), branched and star polymers, dendrimers with acrylate end groups have the potential to be used for in vitro and in vivo applications.[166] Introduction of degradable linkers into the covalent-crosslinking allows fabrication of well-defined networks with tailorable properties.

1.5. Applications of hydrogels

Tissue engineering (TE), also known as regenerative medicine is one of the important line of biomedical research. It is evident that this research area has many obstacles due to the complexity and many unknowns in living systems. However, challenges, applications and wide area of this research field make it more interesting for scientists around the world. Regenerative medicine has expanded over the years and to date no tissue or organ structure has been excluded from active research. Scientists have managed to restore the function of several tissues such as blood vessels, nerve, liver, skin, cartilage and bone. However, over last few decades, only a few products have entered clinical trials, including bladder, skin and cartilage for repair of joint defects.[167,168,169,12]

The basic idea behind TE is to regenerate natural tissues from living cells to replace defective or lost tissues and organs. This can be done either by growing autologous cells *in vitro* guided by a scaffold, or by implanting an acellular scaffold *in vivo* and allowing the cells to repair the tissue guided by the scaffold.[170] Direct injection of cell suspensions without biomaterial source has been used in some cases,[171,172] but in these cases attempts to control the localization of transplanted cells were problematic. Many disciplines go hand in

hand for effective tissue engineering because work in this area combines the principles of materials science, engineering, chemistry, physics and life science.[173] The aim is to fabricate biological substitutes in material similar to ECM that can maintain, restore and replace damaged or dysfunctional organ in the human body.[174] TE avoids the risks of immunological responses such as rejections, as well as infection, by using autologous cells which re-creating the situation in which the donor and recipient are the same person.[167]

The field of TE is still developing. Novel scaffold structures and also reproducible fabrication techniques have become of primary importance.[175] At a time when cellular therapies are becoming very popular, TE approaches steer towards the ability to combine cells with scaffolds material.[176]

Drug delivery is a major market and hydrogel based devices are already available. It aims to deliver the right drug to the right place at the right concentration for a specific period of time. Protein, peptide, DNA based drugs can be delivered thru hydrogel carriers. Hydrogels used in drug delivery generally are fabricated outside of the body, filled with drugs and then as a complex (hydrogel – drug) introduced to the body. In order to deliver drugs in this way "an opening" must be created, which could potentially cause discomfort and with the risk of infection to the patient, for that reason researchers are trying to develop injectable materials. An understanding of cell biology and understanding of delivery mechanisms point towards the need for very specific targeting. Drugs need to reach the problematic area in a specific manner and quantity. A number of synthetic hydrogels (including PHEMA, PMA, PEG and PVA) have been investigated for use in drug delivery.[21] The use of hydrogel allows not only drug delivery but also controlled release.

Importance of hydrogels in drug delivery and TE applications is such as to have led to the development of many novel and promising preparation strategies.[137] In addition, the application of hyperbranched polymers and hydrogels in biological systems has experienced rapid growth.[177,178,179]

Obviously the choice of a suitable material in above applications is important. The material must be biocompatible and preferably biodegradable to avoid the risk of complications that may be associated with the long-term presence of a foreign material in the body. Polymers/Hydrogels should also include good cell specific adhesion, high porosity and without immunogenic reactions. Therefore, it is desirable that prospective polymeric

material possess cell specific adhesion and enzyme sensitive degradation, to promote cellular functions.[169] Biomaterials can naturally possess cell-adhesion-promoting factors or this elements can also be incorporated into the synthetic biomaterial.[180,181] Many hydrogels are non-adhesive to cells meaning that surface modification techniques are needed. To produce a cell responsive matrix biomolecules can be attached to hydrogels. Cells can recognize physical properties and chemical structure of hydrogel scaffolds and regulate their behaviour accordingly. Cell adhesion is the first event which can be observed in the cellular response. Incorporation of adhesive unit such as an adhesion peptide can produce desirable biological interactions.[182] The peptide length, the sequence of amino acids together with peptide immobilization method on to scaffolds are the key aspects in tissue regeneration process and can be well controlled by chemical methods.[183] The most commonly used cell adhesion peptide for cell-adhesive modification is the RGD sequence and its modifications. This peptide sequence is most effective and most often incorporated to non-adhesive synthetic polymers to stimulate cell adhesion.

1.6. Conclusions

Hydrophilic hyperbranched responsive copolymers with controlled chain structure and high density of end functional vinyl groups could open up new potentials in the design of new materials for hydrogel applications. This type of new (branched) materials could be used in TE, drug delivery, wound hilling and other biomedical applications.

Living control polymerisation techniques enabled synthesis of polymers with high number of free vinyl end groups, which can be considered in medical applications. This methods offer control over the structure and molecular weights of the polymers which was impossible in synthesis of non-living nature. However, polymerisation of MVMs (mono and divinyl) is still relatively difficult and the vinyl content of free groups in desired structure is limited.

In recent years RAFT process has become very effective tool in polymer synthesis in comparison to other controlled radical polymerisation methods such as mentioned earlier e.g. ATRP and de-ATRP. The possibility of its usage with wide range of monomers, ability to synthesise structures with targeted molecular weights and narrow PDIs and synthesis in relatively mild conditions provides a huge advantage. The method offers the possibility to

use readily available and inexpensive MVMs in synthesis of responsive, hydrophilic, hyperbranched polymers with tailored degree of branching, fairly high number of free vinyl end groups and further possibility of post modifications.

1.7. Aims and Objectives of This Thesis

The overall aim of this project is to develop and synthesise of hydrophilic polymers using multi-vinyl monomers *via* the RAFT polymerisation approach for hydrogel and other biomedical applications. The work presented in this thesis focuses on four specific objectives:

→ Firstly, on the development of an *in-situ* RAFT approach by modification of conventional RAFT polymerisation with an assessment of applicability of *in-situ* RAFT.

→ Secondly, on the use of *in-situ* RAFT approach in the synthesis of new PEG based hyperbranched polymers with both: thermal responsive and photocrosslinking properties.

→ Thirdly, on advancing the polymer/hydrogel system prepared *via* RAFT polymerisation through introducing biodegradability by incorporating disulfide based multi-vinyl monomer as the branching agent.

→ Fourthly, on synthesis of pH responsive dendritic hydrophilic polymers *via* RAFT polymerisation where HEMA and AA copolymers were synthesized in the presence or absence of EGDMA, with tailored swelling profiles.

Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerisation has proved to be very applicable to the majority of monomers subjected to radical polymerisation.[70] The main objective of this project is to investigate preparation of hydrophilic hyperbranched/dendritic copolymers from commercially available MVMs for hydrogel applications. Advantages of the RAFT over other polymerisation methods were listed

earlier in the introduction; however the application of RAFT technique is more complicated when it comes to MVMs.

As previously reported by Tai et.al.,[23] successful copolymerisation of PEGMEMA, PPGMA and EGDMA was achieved by the use of a one-step de-ATRP approach.
This thesis develops (as discussed in chapter 3, section 3.1 and 3.2) an *in-situ* RAFT approach to synthesise novel hyperbranched PEGMEMA-PPGMA-EGDMA copolymers, and we propose a pathway which can be an alternative to the conventional RAFT synthesis. The developments of an *in-situ* RAFT approach by modification of conventional RAFT might simplify and ease the process. The purpose of this study was to increase the understanding of the disulphide based RAFT mechanism and to connect *in-situ* synthesis of RAFT agent with the polymerisation process. The goal was to be able to predict the optimal conditions for well controlled proposed *in-situ* polymerisation approach. Furthermore, to introduce biodegradability, novel degradable and thermal responsive PEGMEMA-PPGMA-DSDA hyperbranched copolymers are synthesized (chapter 4) *via* RAFT method. In addition, RAFT polymerisations have been used (chapter 5) to obtain dendritic copolymers containing AA, HEMA and EGDMA, as to our knowledge this type of copolymers prepared in this fashion has not been reported. Our approach, involved preparation of linear and dendritic copolymers, study of the kinetics to determine the appropriate experimental parameters for the synthesis of reported copolymers and report on thermal properties, and the degrees of swelling (DSs).

Presented in this thesis polymers were synthesised by the use of *in-situ* and conventional RAFT polymerisation and bear RAFT end groups giving polymer structures which have great potential to be further modified with additional functionalities (e.g. cell adhesion peptides, proteins) in order to be utilized as drug delivery systems, cell carriers, and scaffolds for tissue engineering applications.

Chapter 2 Experimental Procedures and Methodology

2.1. Introduction

This chapter describes the general experimental procedures and methodology used in this thesis, including the synthesis and characterisation of the precursors of the RAFT agents, final RAFT agents, disulphide diacrylate and the preparation of hydrophilic polymers by conventional and living/controlled radical polymerisation methods. A facile one-pot and two-step polymerisation approach is described in addition to standard procedures of polymerisation and hydrogel fabrication. Moreover, methods and analytical techniques used for the characterisation of compounds and polymers are described. The scientific background for interpretation and understanding of the results are also included in this chapter.

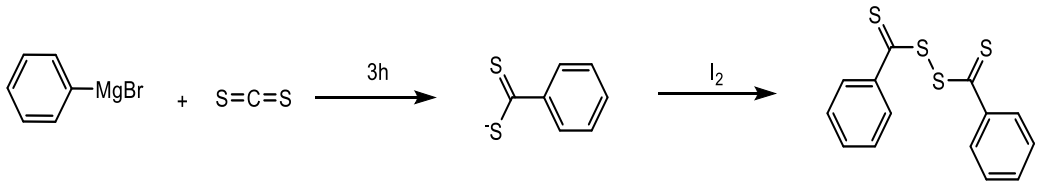
2.2. Materials and chemicals

Commercially available reagents and solvents were used as received unless stated. The monomers employed in this work include methyl methacrylate (MMA, 99%, ≤30 ppm MEHQ as inhibitor, Aldrich), styrene (St, <15 ppm 4-tert-butylcatachol as stabilizer, ≥99% Aldrich), acrylic acid (AA, Aldrich), the 2-hydroxyethyl methacrylate (HEMA, Aldrich), polyethylene glycol methacrylate (PEGMA, M_n = 526, Aldrich), poly (ethylene glycol) methyl ether methacrylate (PEGMEMA, M_n = 475, Aldrich) and poly (propylene glycol) methacrylate (PPGMA, M_n = 375, Aldrich). Divinyl monomer ethylene glycol dimethacrylate (EGDMA, Aldrich) was used (as received) as the branching agent. 1,1'-azobis(cyclohexanecarbonitrile) (ACHN, analytical grade, Aldrich), 4,4` azobis(4-cyanovaleric acid) (Aldrich), and azobisisobutyronitrile (AIBN, analytical grade, Aldrich) were used as the initiators after being purified by re-crystallisation from methanol. Bis(thiobenzoyl) disulfide, 2-cyanoprop-2-yl dithiobenzoate, 1-cyano-1-cyclohexyl

dithiobenzoate, bis(2-acryloyl)oxyethyl disulfide, bis(dodecylsulfanylthiocarbonyl) disulphide, 4-cyano–4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid were synthesized and purified according to published methods[184,185,186,187] and characterised by ^{1}H and ^{13}C NMR. The experimental procedures are described in this chapter (section 2.3.1 – 2.3.6). The other chemicals used in this work include bis-2 hydroxyethyl disulphide (Sigma – Aldrich), acryloyl chloride (Sigma – Aldrich), triethylamine (Fisher Scientific), sodium hydrogen carbonate (Fisher Scientific), sodium chloride (Fisher Scientific), anhydrous tetrahydrofuran (Sigma – Aldrich), dichloromethane (Sigma – Aldrich), cysteamine hydrochloride (Sigma – Aldrich), sodium thiosulfate (anhydrous, Fisher Scientific), sodium sulfate (Fisher Scientific), 1N iodine aqueous solution (Aldrich), pentaerythritol tetrakis (QT) (3-mercaptopropionate) (Sigma – Aldrich), methylene chloride (Fisher Scientific), phenylmagnesium bromide (Aldrich), carbon disulphide (Aldrich), ethyl acetate (Fisher Scientific), ethyl ether (Fisher Scientific), hexane (Aldrich), petroleum spirits 40-60 ^{o}C, tetrahydrofuran (THF, Aldrich), n-butanone (Aldrich), deuterated chloroform ($CDCl_3$, 99,8%, FluoroChem), dimethyl sulfoxide-d6 (DMSO-d6, Aldrich), phosphate buffer powder (pH = 7.44, Sigma - Aldrich), solid iodine (Sigma - Aldrich), sodium hydride (60% in oil) (Aldrich), n-dodecylthiol (Aldrich), N, N-dimethylformamide (HPLC grade, Fisher Scientific), 2-hydroxy-4'-(2-hydoxy-ethoxy)-2-methyl-propriophenone (Irgacure 2959, Aldrich), and dry IR-grade potassium bromide (KBr). Silica gel (60A, FluoroChem) was used for flash chromatography purification.

2.3. Synthesis and polymerisation procedures

2.3.1. Synthesis of bis(thiobenzoyl) disulphide

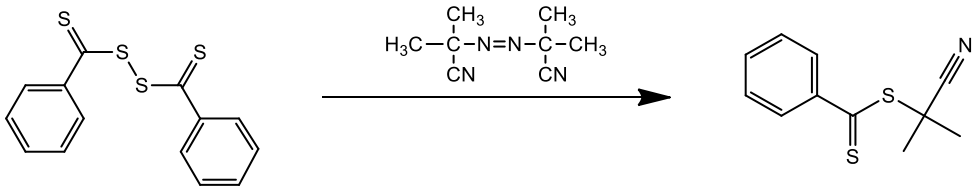


Scheme 2-1: Synthesis of bis(thiobenzoyl) disulfide.[184]

The compound was prepared by placing 1M solution of phenylmagnesium bromide (100 mL, 1M in THF) in a two-necked round-bottom flask equipped with condenser, magnetic stirrer and nitrogen atmosphere. Flask was cooled in ice bath and carbon disulfide (8.36 mL, 139 mmol) was added drop wise over 20 min. The solution was stirred for 1h in 0 ^{o}C and for another 2.5 hrs in room temperature. The solvent was then removed under vacuum and the resulting deep red viscous liquid was dissolved in a diluted K_2CO_3 solution (8 g in 200 mL), filtered and washed with ethyl ether (2 x 100 mL). Aqueous phase was collected and poured in a round-bottom flask equipped with magnetic stirrer. Aqueous solution of iodine 1.0 N (90 mL, 100 mmol) was then added dropwise over 30 min. During the addition, the solution started to change colour from dark red/purple to pink as the disulfide precipitated. After elimination of excess I_2 with crystals of $Na_2S_2O_3$, the mixture was extracted with methylene chloride, dried over sodium sulfate, evaporated and dried in vacuum oven. A pink/light red powder was obtained (Scheme 2-1, yield 70.7%) and characterised by ^{1}H NMR. The crude product was used for the subsequent reaction without further purification.

^{1}H NMR (400 MHz, $CDCl_3$), δ(PPM): 7.48 (m, *m*-ArH, 4H); 7.64 (m, *p*-ArH, 2H); 8.11 (m, *o*-ArH, 4H).

2.3.2. Synthesis of 2-cyanoprop-2-yl dithiobenzoate

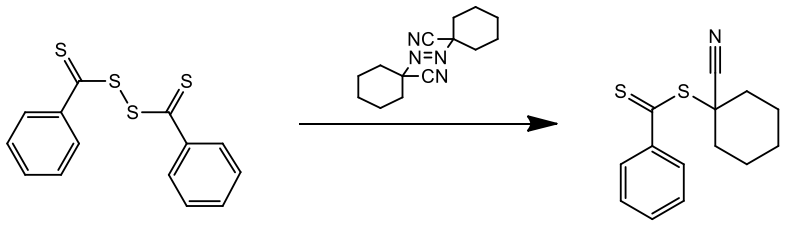


Scheme 2-2: Synthesis of 2-cyanoprop-2-yl dithiobenzoate.[184,186]

The solution of bis(thiobenzoyl) disulfide (858 mg, 2.8 mmol, synthesized according to protocol in section 2.3.1) and the azo compound (AIBN) (532 mg, 3.24 mmol) in ethyl acetate (100 mL) was degassed in a 250 mL round-bottom flask equipped with condenser and magnetic stirring and then refluxed in nitrogen atmosphere for 20 hrs. Then the solvent was removed and the product purified by flash chromatography, eluent: hexane/DCM 3:2. A red-purple oily liquid (Scheme 2-2, yield 68%) was obtained. The product was characterised by ^{1}H NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ(PPM): 1.97 (s, 6H, C*H3*), 7.41 (m, 2H, Ar*H*), 7.58 (m, 1H, Ar*H*), 7.94 (m, 2H, Ar*H*).

2.3.3. Synthesis of 1-cyano-1-cyclohexyl dithiobenzoate



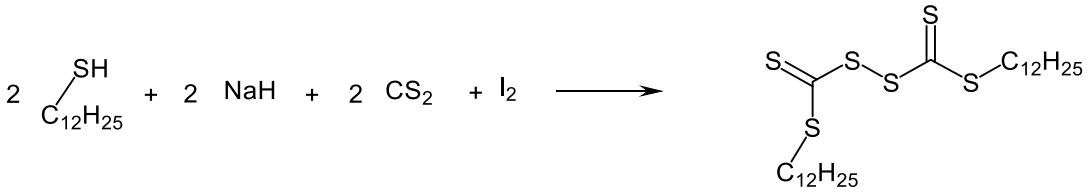
Scheme 2-3: Synthesis of 1-cyano-1-cyclohexyl dithiobenzoate.[184]

A solution of bis(thiobenzoyl) disulfide (815 mg, 2.6 mmol, synthesized according to protocol in section 2.3.1) and 1,1'-azobis(cyclohexanecarbonitrile) (ACHN, 140 mg, 5.48 mmol) in ethyl acetate (10 mL) was prepared. The mixture was degassed and refluxed in a round bottom flask equipped with condenser, magnetic stirring and inner atmosphere. Reaction was monitored by TLC, run for 52 hours. Then solvent was removed under

reduced pressure and the product purified on a silica-gel column using a mixture of ethyl acetate/petroleum spirits 40-60 ^{o}C (1:9), a purple oil (Scheme 2-3, yield 50.8%) was obtained. The product, 1-cyano-1-cyclohexyl dithiobenzoate (ACBN), was characterised by ^{1}H NMR.

^{1}H NMR (400 MHz, $CDCl_3$), 7.48 (m, *m*-ArH, 4H); 7.64 (m, *p*-ArH, 2H); 8.11 (m, *o*-ArH, 4H).

2.3.4. Synthesis of bis(dodecylsulfanylthiocarbonyl) disulphide



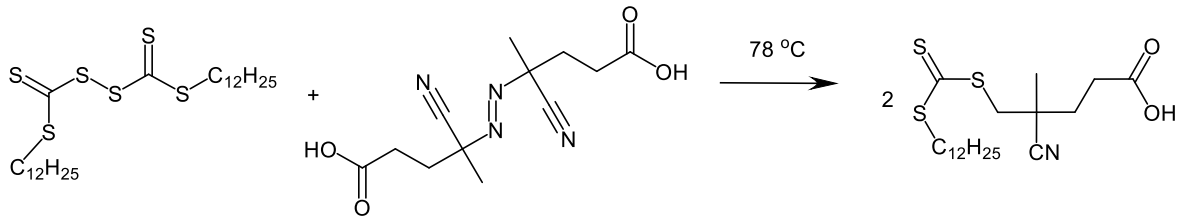
Scheme 2-4: Synthesis of bis-(dodecylsulfanylthiocarbonyl) disulfide.[82,188]

The compound was prepared by placing suspension of sodium hydride (60% in oil) (12.59 g, 0.525 mol) in diethyl ether (600 mL) in round-bottom flask, then adding n-dodecylthiol (61.60 g, 0.304 mol, 72.9 mL) over 25 min constantly stirring at a temperature between 5 and 10 ^{o}C. A vigorous evolution of hydrogen was observed and the greyish sodium hydride was transformed to dense white slurry of sodium thiododecylate. In the next step the reaction mixture was cooled to 0 ^{o}C and carbon disulfide (24.00 g, 0.316 mol, 19 mL) was added slowly. A thick yellow precipitate of sodium S-dodecyl trithiocarbonate was obtained. Product was collected by filtration, dried in vacuum oven (room temperature) and the crude product was used for the subsequent reaction without further purification.

A suspension of sodium S-dodecyl trithiocarbonate (81.36 g, 0.273 mol) in diethyl ether (100 mL) was treated by addition of solid iodine (34.41 g, 0.137 mol). The reaction mixture was then stirred at room temperature for 1 h when the white sodium iodide which settled was removed by filtration. The yellow–brown filtrate was washed with an aqueous solution of sodium thiosulfate to remove excess of iodine and then dried over sodium sulphate and filtered. The solvent was evaporated to provide a solid of bis-(dodecylsulfanylthiocarbonyl) disulphide (Mp. 33-35 ^{o}C, 62.60 g). The product was characterised by ^{1}H NMR.

^{1}H NMR ($CDCl_3$): δ 0.90 (t, 6H); 1.34 (br s, 36H); 1.70 (m, 4H); 3.35 (t, 4H).

2.3.5. Synthesis of 4-cyano-4-(dodecylsulfanylthiocarbonyl) sulfanyl pentanoic acid

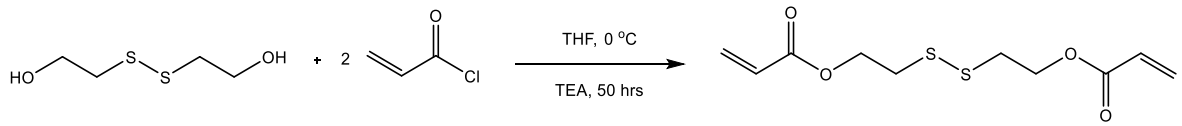


Scheme 2-5: Synthesis of 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid.[82,188]

4,4'-azobis(4-cyanopentanoic acid) (15.20 g, 0.054 mol), bis-(dodecylsulfanylthiocarbonyl) disulfide (20.00 g, 0.036 mol, prepared according to protocol in section 2.3.4) were mixed, ethyl acetate (300 mL) was added and solution was heated at reflux for 22 hrs. After removal of the volatiles under the reduced pressure, the crude product of 4-cyano-4-(dodecylsulfanylthiocarbonyl) sulfanyl pentanoic acid was washed with water (5 x 100 mL). End product was afforded as a pale yellow solid by recrystallization from heptane and characterised by ^{1}H NMR, (Mp. 58-59 ^{o}C, 14.40 g, 87% yield).

^{1}H NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3); 1.30 (br s, 18H); 1.71 (m, 2H); 1.92 (s, 3H, CH_3); 2.40-2.80 (m, 4H, CH_2CH_2); 3.36 (t, 2H, CH_2S).

2.3.6. Synthesis of bis(2-acryloyl)oxyethyl disulfide - disulphide diacrylate



Scheme 2-6: Synthesis of disulphide diacrylate.[185]

The chemicals used for this synthesis were provided with the highest purity available and were used as received.

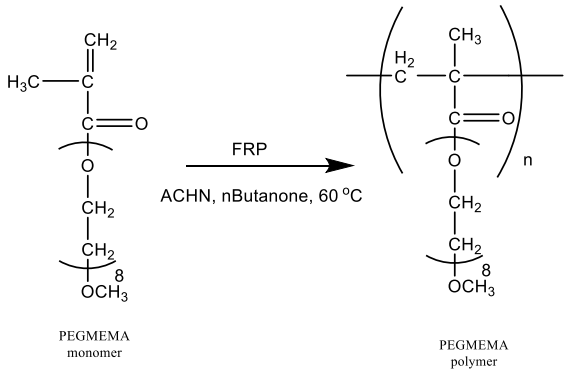
Bis-2 hydroxyethyl disulphide (7.70 g, 0.05 mol) and triethylamine (40.5 g, 0.4 mol) were weighted into a round-bottom flask, equipped with a magnetic stirrer bar. Anhydrous THF (150 mL) was added into flask, immersed in an ice bath and purged with nitrogen for 20 minutes, at 0 °C. As a next step acryloyl chloride (16.3 mL, 0.2 mol) was added drop wise to the reaction mixture, and the heterogeneous solution was left to stir for 50 hours, and then filtered to remove the triethylamine hydrochloride byproduct. The solvent was removed by rotary evaporation, and the crude product (brownish viscous oil) was dissolved in chloroform. Purification required washing organic phase with deionised water (3 x 300 mL), sodium hydrogen carbonate (3 x 300 mL), brine (3 x 300 mL). In order to dry, organic phase was subsequently stirred with anhydrous magnesium sulphate for 28 hrs. The crude product (9.82 g, yield = 75%) was filtered to remove the magnesium sulphate, and was purified on column using a silica gel as the stationary phase and dichloromethane as the eluent. The final disulfide diacrylate (DSDA) product was obtained (after removing the volatiles) as yellowish oil (4.80 g, yield 37%) and stored in the freezer, under nitrogen, in absence of light prior to use. The purified DSDA was characterised by ^{1}H NMR.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.44 (d, 1H); 6.13 (dd, 1H); 5.86 (d, 1H); 4.43 (t, 2H); 2.98 (t, 2H).

2.3.7. Typical procedures for Free Radical and Reversible Addition-Fragmentation Chain Transfer Polymerisations

All polymerisations presented in this thesis were carried out in a round bottom flask either in bulk /or solution. The Schlenk technique allowed inert atmosphere of nitrogen or argon. The RAFT polymerisation experiments were typically stopped at the desired time point or at high conversions, i.e. when the reaction mixture became too viscous to be withdrawn from the flask through a sample syringe needle. In all cases the polymerisations were terminated by rapid cooling to 20 ^{o}C and exposing to air. The purification procedures involved the selective dissolution/precipitation method for polymer mixture separation and dialysis in some cases. The syntheses of polymers involved monitoring the polymerisations using Gel Permeation Chromatography/Size Exclusion Chromatography (GPC)/(SEC) to obtain monomer conversions, molecular weights and polydispersity indexes of analysed samples. The results of this experiments and their discussion are included in chapter 3 of this thesis.

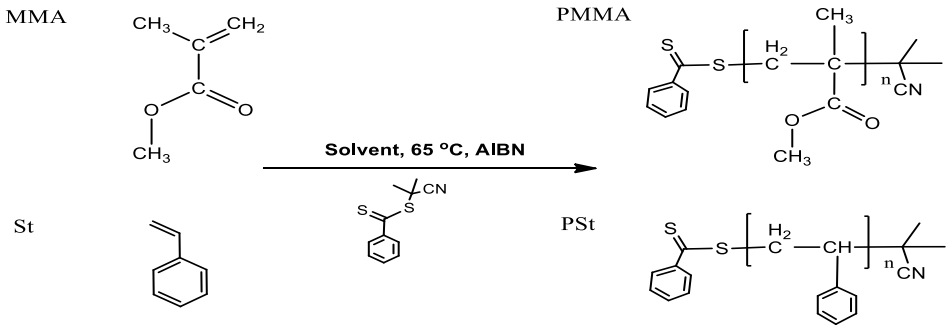
2.3.7.1. Free Radical Polymerisation of Poly(ethylene glycol) methyl ether methacrylate, Methyl methacrylate and Styrene



Scheme 2-7: FRP of PEGMEMA using ACHN as the initiator.

Free radical polymerisation was used to conduct homopolymerisations of poly(ethylene glycol) methyl ether methacrylate (PEGMEMA), methyl methacrylate (MMA) and styrene (St). Herein, a typical procedure for FRP polymerisation is described, an example of FRP of PEGMEMA using ACHN as the initiator is presented in scheme 2-7. Monomer e.g. PEGMEMA (10 g, 5.2 mmol) and initiator e.g. ACHN (51.31 mg, 0.21 mmol) were mixed in n-butanone (10 mL) in a round bottom flask. The mixture was degassed by purging solution with nitrogen/ or argon to remove any dissolved oxygen which inhibits the reaction. In order to start polymerisation the flask was immersed in an oil bath at 60 ^{o}C, stirred (400 rpm). Aliquots (0.5 mL) were taken out throughout the reaction time, filtered through a PVDF filter (pore size 0.2 μm) to remove any insoluble species and taken for GPC analysis to monitor the polymerisation progress. The polymerisations were terminated; resulted polymers were purified by dissolving the products in n-butanone/ethyl acetate and precipitating the polymers into large excess of hexane (3x), and then dried in a vacuum oven at 20 ^{o}C.

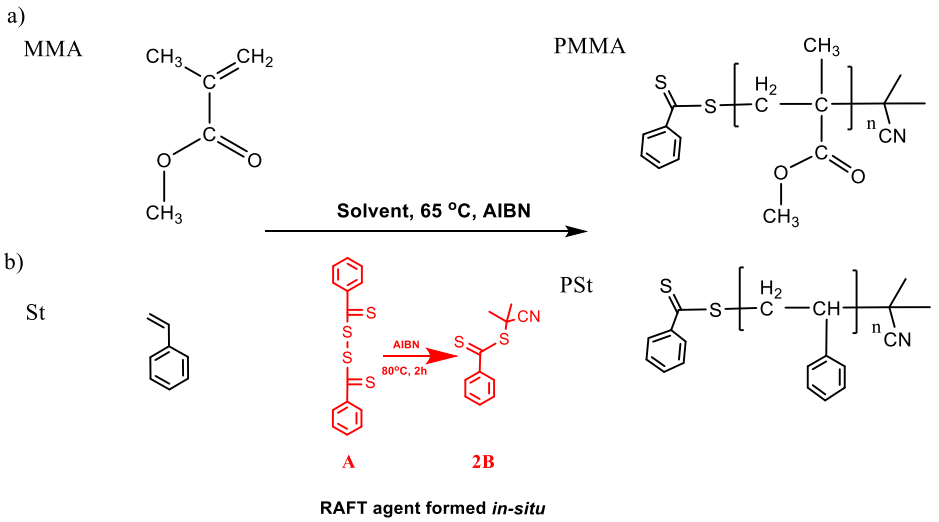
2.3.7.2. Conventional Reversible Addition-Fragmentation Chain Transfer homopolymerisation of Methyl methacrylate and Styrene



Scheme 2-8: Synthesis of PMMA and PSt through conventional RAFT polymerisation. 2-cyanoprop-2-yl dithiobenzoate prepared prior to polymerisation.

The conventional RAFT homopolymerisation of methyl methacrylate and styrene were conducted using 2-cyanoprop-2-yl dithiobenzoate and 1-cyano-1-cyclohexyl dithiobenzoate (prepared according to procedures described in section 2.3.2 and section 2.3.3, p.46) as the RAFT agents. A typical procedure for conventional RAFT polymerisation is described as following: Calculated amount of monomer (MMA or St) was dissolved in solvent (n-butanone or ethyl acetate) under stirring at room temperature. The RAFT agent 2-cyanoprop-2-yl dithiobenzoate (CTA) and the initiator azobisisobutyronitrile were introduced at a relevant molar ratio (e.g. 1:0.2). Solutions were bubbled with nitrogen using Schlenk line to deoxygenate the reaction mixture. After degassing, flasks were immersed in an oil bath (at 65 ^{o}C) and stirred at 400 rpm. Aliquots (0.5 mL) were withdrawn at different time intervals, filtered through a PVDF filter (pore size 0.2 μm) to remove any insoluble species and then given for GPC analysis to monitor polymerisation progress.

2.3.7.3. *In-situ* Reversible-Addition Fragmentation Chain Transfer homopolymerisation of Methyl methacrylate and Styrene



Scheme 2-9: Synthesis of PMMA (a) and PSt (b) *via in-situ* RAFT polymerisation. Bis(thiobenzoyl)disulfide (A) was used to form RAFT agent 2-cyanoprop-2-yl dithiobenzoate (B) *in-situ*.

The reactions followed the similar procedures as for conventional RAFT described in section 2.3.7.2, with difference that RAFT agent (B in Scheme 2-9) is created *in-situ* in the reaction mixture, and then involved in polymerisation process as the chain transfer agent (CTA). A typical procedure for *in-situ* RAFT reaction is described as following: Calculated amount of monomer was dissolved in solvent (n-butanone or ethyl acetate) under stirring at room temperature. Bis(thiobenzoyl)disulfide (a precursor of CTA) and azobisisobutyronitrile were introduced at a different molar ratio of (e.g. 5:7). Solution was bubbled with nitrogen using Schlenk line, to deoxygenate the reaction mixture. After degassing, flasks were immersed in an oil bath at 80 ^{o}C for required time to form the 2-cyanoprop-2-yl dithiobenzoate and then immersed in an oil bath at 65 ^{o}C, and stirred at 400 rpm. Aliquots (0.5 mL) were withdrawn at different time intervals, filtered through a PVDF filter (pore size 0.2 µm) to remove any insoluble species and then subjected to GPC analysis to monitor the polymerisation progress. The polymerisation was terminated; homopolymers produced were purified by dissolving the products in n-butanone/ethyl acetate and precipitating the polymers into large excess of hexane (3x), and then dried in a vacuum oven at 20 ^{o}C.

2.3.7.4. Chain extension using Poly(methyl methacrylate) and Polystyrene as macro Reversible-Addition Fragment Chain Transfer agents

For chain extension polymerisations the same procedures as conventional RAFT polymerisation of MMA and Styrene were adopted. The difference is that the 2-cyanoprop-2-yl dithiobenzoate RAFT agent was replaced by PMMA or PSt macro RAFT agents which were obtained from previous RAFT homopolymerisation of MMA and Styrene. The polymerisations were terminated; the block copolymers produced in the synthesis were purified by dissolving the products in n-butanone/ethyl acetate and precipitating the polymers into large excess of hexane (3x), and then dried in a vacuum oven at 20 ^{o}C.

2.3.8. Preparation of thermoresponsive hyperbranched copolymers *via* conventional Reversible-Addition Fragmentation Chain Transfer polymerisation using 2-cyanoprop-2-yl dithiobenzoate

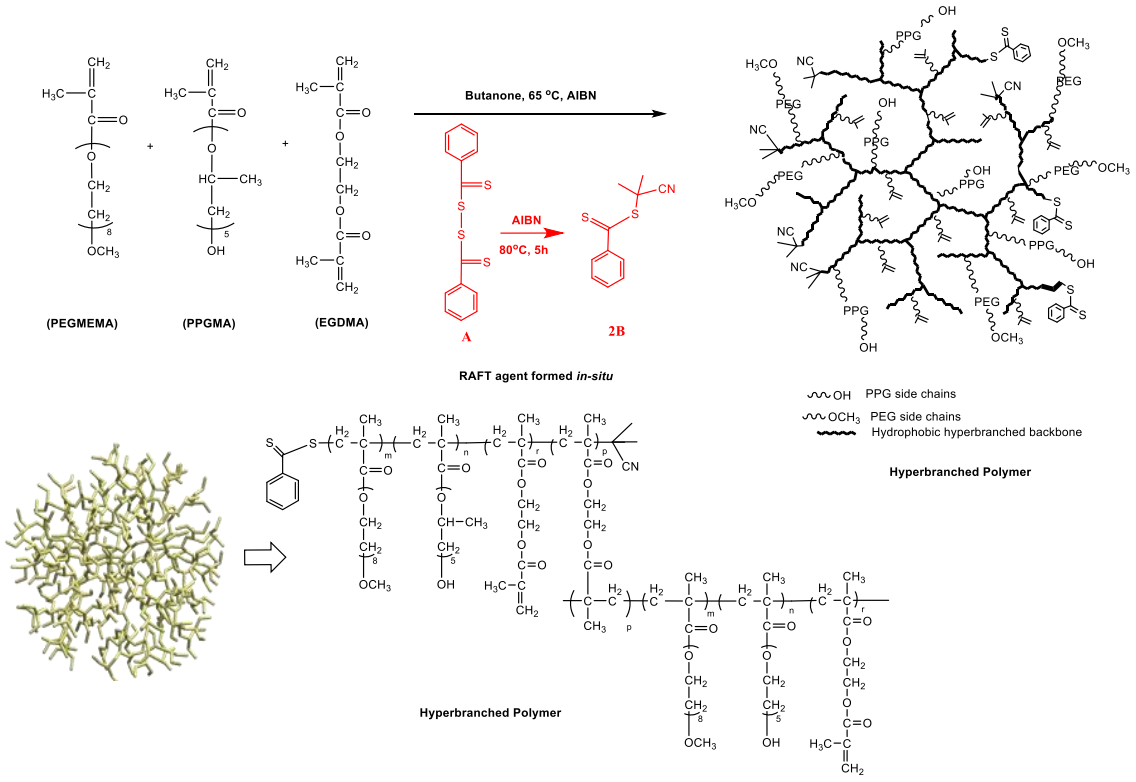
The conventional RAFT copolymerisation of PEGMEMA, PPGMA and EGDMA was conducted using 2-cyanoprop-2-yl dithiobenzoate prepared previously (section 2.3.2, p.46) as the RAFT agent. A typical reaction procedure is described as following: monomers PEGMEMA, PPGMA and EGDMA were prepared at the desired molar feed ratio where [total monomer]/RAFT agent /[AIBN] is equal to 25/1/0.2, i.e. PEGMEMA (4.16 g, 8.75 mmol), PPGMA (3.28 g, 8.75 mmol), EGDMA (1.49 g, 7.5 mmol), AIBN (0.0328 g, 0.2 mmol), RAFT agent 2-cyanoprop-2-yl dithiobenzoate (1 mmol), and n-butanone (10 mL). The reaction flask was degassed for 20 minutes with argon; the solution was stirred at 500 rpm and then immersed into oil bath at 65 ^{o}C and further stirred at 500 rpm. During the reaction, samples were withdrawn for GPC analysis (taken at required time points, filtered through a PVDF filter 0.2 μm pore size and then analysed). At required time, polymerisations were terminated. To remove unreacted PPGMA and EGDMA the final polymer solutions were precipitated into a large excess of hexane. After this step, unreacted PEGMEMA was still present in the precipitated mixture of the polymer; in order to remove it, the sample was purified by dialysis (MWCO 3500) against fresh deionised water, which was changed regularly. The pure polymer samples were obtained after freeze drying as pink tacky solids and then taken for further characterisations and property evaluations.

2.3.9. Preparation of thermoresponsive hyperbranched copolymers *via* Reversible-Addition Fragmentation Chain Transfer polymerisation using bis(thiobenzoyl) disulfide to form 2-cyanoprop-2-yl dithiobenzoate *in-situ*

Bis(thiobenzoyl) disulfide (a precursor of RAFT agent, A in Scheme 2-10, p.55) was used to form 2-cyanoprop-2-yl dithiobenzoate (RAFT agent, B in Scheme 2-10) *in-situ* through a one-pot and two-stage approach.

The reactions followed the similar procedures as for conventional method described in section 2.3.8, p.53. The difference is that RAFT agent (B in Scheme 2-10) is created *in-situ* in reaction mixture, and then involved in polymerisation process as the chain transfer agent.

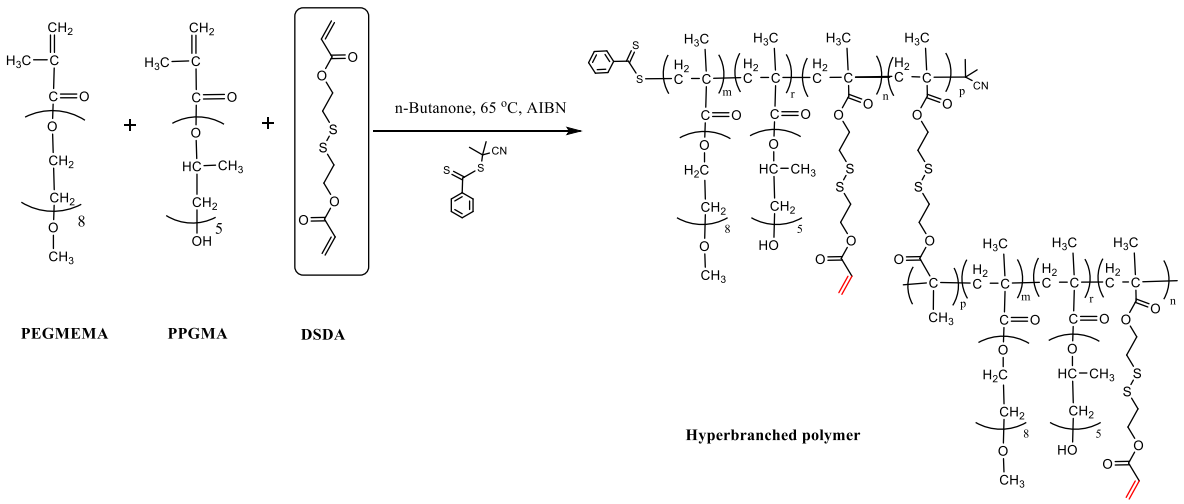
A typical reaction procedure is described as following: monomers PEGMEMA (5.94 g, 12.5 mmol), PPGMA (8.44 g, 22.5 mmol) and EGDMA (2.97 g, 15 mmol) were prepared at the desired molar feed ratio of i.e. 25:45:30. Bis(thiobenzoyl) disulfide (A) (1 mmol) and azobisisobutyronitrile (AIBN) (1.4 mmol) were introduced at a molar ratio of 5:7. Substances were dissolved in 20 mL n-butanone and set up on a Schlenk line. Argon was bubbled through the system; the solution was stirred at 500 rpm. The reaction was run for 5 h at 80 °C to form the RAFT agent 2-cyanoprop-2-yl dithiobenzoate (B) *in-situ* and then left for a further 30 h at 65 °C to allow RAFT polymerisation. The polymerisations were monitored at required time points using GPC. The resulting polymers were purified and dried according to the aforementioned procedures (section 2.3.8). The pure polymer samples were taken for further characterisations and property evaluations.



Scheme 2-10: Synthesis of thermoresponsive hyperbranched polymers with multivinyl functionality *via in-situ* RAFT polymerisation. The RAFT agent 2-cyanoprop-2-yl dithiobenzoate (B) was formed *in-situ* from bis(thiobenzoyl) disulfide (A).[111]

2.3.10. Introducing biodegradability - copolymerisation of Poly(ethylene glycol) methyl ether methacrylate, Poly(propylene glycol) methacrylate and bis(2-acryloyl)oxyethyl disulfide

The conventional RAFT copolymerisation of PEGMEMA, PPGMA and DSDA was conducted using 2-cyanoprop-2-yl dithiobenzoate prepared previously (section 2.3.2, p.46) as the RAFT agent. The series of polymerisations were run according to Scheme 2-11 (p.56) and the molar feed ratios of monomers were varied to adjust polymer properties and manipulate LCST of the final polymers.



Scheme 2-11: Synthesis of biodegradable thermoresponsive hyperbranched polymers with multivinyl functionality *via* conventional RAFT copolymerisation of PEGMEMA, PPGMA and DSDA.

A typical reaction procedure is described as following:

PEGMEMA, PPGMA and DSDA were prepared in 100 mL flask i.e. [PEGMEMA]/[PPGMA]/[DSDA]/RAFTagent/[AIBN] is equal to 20/70/10/1/0.2, mol ratio i.e. PEGMEMA (1.80 g, 3.80 mmol), PPGMA (4.99 g, 13.30 mmol), DSDA (0.5 g, 1.90 mmol), RAFT agent 2-cyanoprop-2-yl dithiobenzoate (0.04 g, 0.19 mmol), and n-butanone (10 mL). The reaction flask was degassed for 10 minutes with argon, than AIBN (0.0328 g, 0.2 mmol) was added. The mixture was bubbled further for the following 5 minutes. The solution was stirred at 500 rpm and then immersed into oil bath at 65 ^{o}C and further stirred at 500 rpm. During the reaction, samples were withdrawn for GPC analysis (taken at required time points, filtered through a PVDF filter 0.2 μm pore size and then analysed). At required time, polymerisations were terminated. Samples of the resultant polymers were purified by selective dissolution and participation. Prepared polymers were lyophilised and then taken for further characterisations and property evaluations. The purified polymers were characterised by GPC and ^{1}H NMR.

2.3.11. Preparation of pH responsive copolymers of acrylic acid and 2-hydroxyethyl methacrylate in the presence or absence of branching agent *via* Reversible Addition-Fragmentation Chain Transfer and Free Radical Polymerisations

A series of RAFT polymerisation reactions (Scheme 2-12, p.58) have been conducted either in bulk or in organic solvents, varying their conditions to analyse the influence of the solvent, initiator, RAFT agent (4-cyano–4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid) and incorporation of branching agent ethylene glycol dimethacrylate on the synthesis of pH responsive copolymers. Moreover, the conventional FRP polymerisations were also conducted for the comparison. The kinetic of the polymerisations were studied at different reaction conditions using GPC in order to obtain the copolymers with tailored composition, average molecular weight and molecular distribution. The majority of copolymers produced while working on the polymerisations of acrylic acid and 2-hydroxyethyl methacrylate were purified by dissolving the products in methanol and precipitating the polymers into diethyl ether. In cases where polymers were not soluble in methanol, they were washed and extracted with water, methanol and ethanol respectively to remove unreacted monomers, and then dried in a vacuum oven at 20 ^{o}C.

2.3.11.1. Reversible-Addition Fragmentation Chain Transfer polymerisation in bulk

In RAFT bulk polymerisations, calculated volume ratios of monomers, CTA and initiator (according to Table 5-1, Table 5-2, p.143) were placed in flasks, mixed at room temperature, stirred and degassed with nitrogen. After degassing, flasks were immersed in an oil bath at 65 ^{o}C, stirred (500 rpm). Aliquots (0.5 mL) were taken out for GPC analysis throughout the reaction.

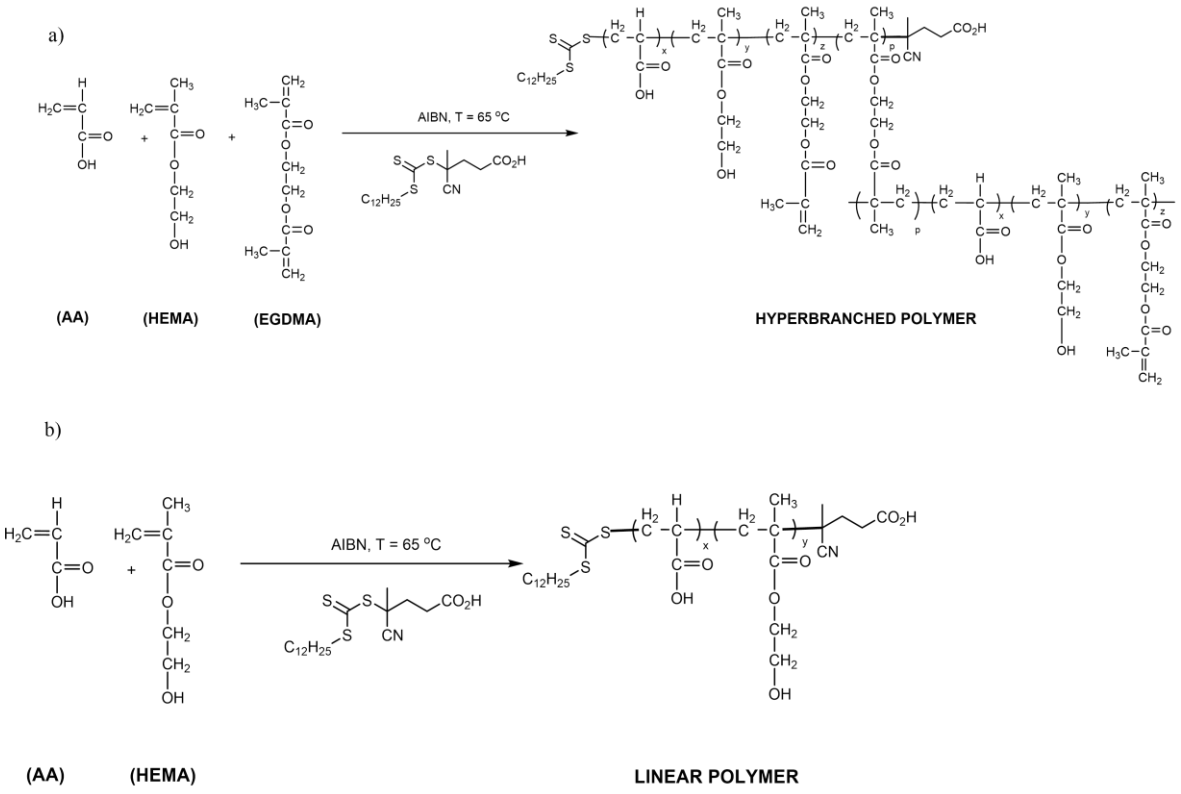
2.3.11.2. Reversible-Addition Fragmentation Chain Transfer polymerisation in solution

Solution polymerisations (in organic solvents) were conducted using similar procedures as in bulk polymerisations. In these cases calculated amounts of monomers, CTA and initiator were dissolved in solvent (DMF) under stirring at room temperature. Solutions were

degassed with nitrogen. After degassing, flasks were immersed in an oil bath at 65 ^{o}C, stirred (500 rpm). Aliquots (0.5 mL) were taken out for GPC analysis throughout the reaction.

2.3.11.3. Free Radical Polymerisation in bulk and in solution

Free radical polymerisation in bulk/ or in solution was conducted using similar procedures as for RAFT polymerisation mentioned in sections 2.3.11.1 and 2.3.11.2 but without the addition of RAFT agent.



Scheme 2-12: pH responsive copolymer synthesised by RAFT: a) AA-HEMA with EGDMA as a branching agent b) AA-HEMA linear polymer without branching agent.

2.4. Characterisation and Analysis Techniques

2.4.1. Characterisation of linear and hyperbranched polymers

The structure of all linear and hyperbranched polymers prepared during the studies were confirmed and characterised by a range of analytical methods and techniques. These included Gel Permeation Chromatography **(**GPC)/Size Exclusion Chromatography (SEC), Nuclear Magnetic Resonance Spectroscopy (NMR), Differential Scanning Calorimetry (DSC), Thermo Gravimetric Analysis (TGA), Scanning Electron Microscopy (SEM), Fourier Transform Infra-Red Spectroscopy (FTIR), Lower Critical Solution Temperature (LCST) measurements, photocrosslinking studies, and swelling and degradation studies.

2.4.2. Gel Permeation Chromatography /Size Exclusion Chromatography

Determination of the molecular weight and polydispersity is very important in polymer chemistry as the properties of a given polymer differ significantly depending on the molecular weight and its distribution. These parameters might provide a good indication of molecular size, viscosity and solubility of the polymer samples.[189,55] Different samples of the same polymer can have the same average chain length but very different distributions of chain lengths depending on the method of production.

One of the most commonly used method allowing the characterisation of molecular weight of polymers is Gel Permeation Chromatography also known as a Size Exclusion Chromatography.[190] This method is based on the behaviour of polymer molecules in solution. It is very reliable technique; polymers are dissolved in a suitable solvent and injected to the system of columns packed with porous beads (molecular sieves). There should be no interaction between the sample and the column packing. Analyzed sample of polymer in solution passes through columns and the polymer separation occurs according to molecular size, as the size of analyzed molecules determinate whether molecules can or cannot penetrate into the pores of the sieves.[191,192] Large molecules cannot pass through the pores within the sieves so they move quickly through the column through empty spaces between the sieves which implicate short retention time. Small molecules retain in the column for longer time, as they diffuse into the network of the pores. Consequently elution

occurs in the order of high molecular weight fractions first, followed by decreasing molecular weight fractions. A good balance between the solvent, sample and column material is important for a good separation mechanism. Important parameters to look at for a good GPC/SEC analysis include peak shape, stable baseline, tailing and exclusion limits.

The concentration required for sample preparation depends on the molecular weight of the polymer. Representative value commonly used as guidance for a polymer of molecular weight of approximately 100,000 is a concentration of 1 mg/mL (w/v).

The elution time of the polymer is related to molecular weight through the calibration of the columns with narrow polymer standards, of a known molecular weight. The column calibration should cover the full elution time region of the sample to avoid or to reduce the errors. The calibration curve describes how different size molecules elute from the column. However, this method does not yield a universal curve for all polymer samples, and might lead to limitations in the use of conventional GPC analysis and to errors.[193]

As it was mentioned the GPC instrument prior to use has to be calibrated. In this research, an injection of multiple standards was performed. Calibrations were accomplished at 40 ^{o}C using polystyrene and poly(methyl metacrylate) narrow standards as calibrates in suitable mobile phase. The examples of the calibration data used and obtained curves are shown in Table 2-1, Figure 2-1 and Table 2-2, Figure 2-2, p.61-62.

The error of the GPC calibration in range of our interest is not high and is less than 10% (peaks 2 to 9 Table 2-1 and Table 2-2). Majority of the data presented in this research thesis were run once while analysing the samples. For randomly selected PMMA samples produced in this research by FRP and RAFT polymerisation, GPC measurement was performed three times to investigate the error in data run on the system used in the laboratory conditions.

Table 2-1: Calibration data for the GPC measurement (PMMA standards in THF mobile phase).

Peaks	Retention Time (mins)	M_W (g/mol)	Log M_W	Percent Error
1	11.08	1944000	6.29	2.14
2	11.83	790000	5.90	-9.67
3	12.40	467400	5.67	-2.32
4	12.93	271400	5.43	-0.73
5	13.55	144000	5.16	0.56
6	14.12	79250	4.90	0.26
7	14.92	35300	4.55	3.23
8	15.90	13300	4.12	8.41
9	16.52	7100	3.85	10.13
10	17.70	1960	3.29	5.88
11	18.25	1020	3.01	-1.60
12	18.47	690	2.84	-19.66

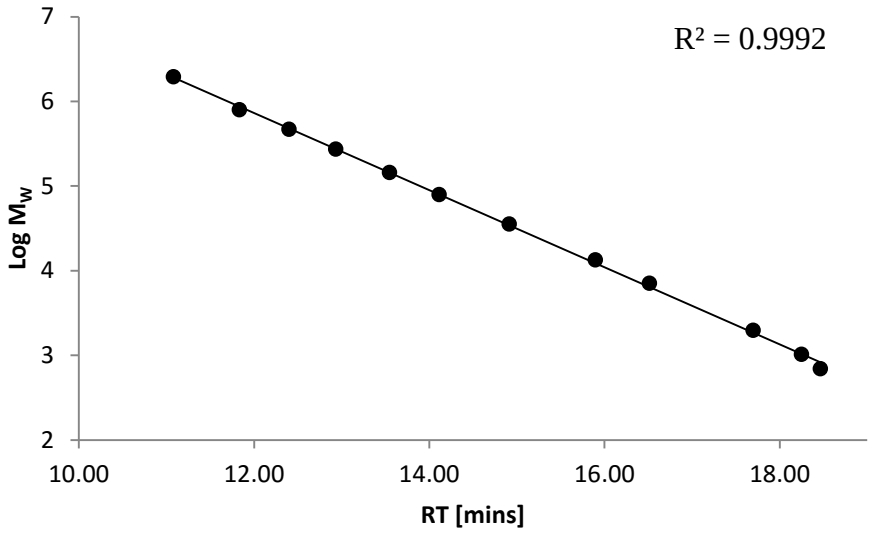


Figure 2-1: The calibration curve for GPC measurement (PMMA standards in THF mobile phase).

Table 2-2: Calibration data for the GPC measurement (PSt standards in DMF mobile phase).

Peaks	Retention Time (mins)	M_W (g/mol)	Log M_W	Percent Error
1	11.78	371100	5.57	-17.74
2	12.25	238700	5.38	-8.28
3	13.23	91800	4.96	6.87
4	13.95	46500	4.67	17.91
5	14.48	24600	4.39	14.84
6	15.22	10110	4.00	9.20
7	15.75	4910	3.69	-2.61
8	16.27	2590	3.41	-8.77
9	16.75	1570	3.20	-4.17
10	17.32	780	2.89	-10.83
11	17.90	370	2.57	-21.21
12	18.95	162	2.21	15.05

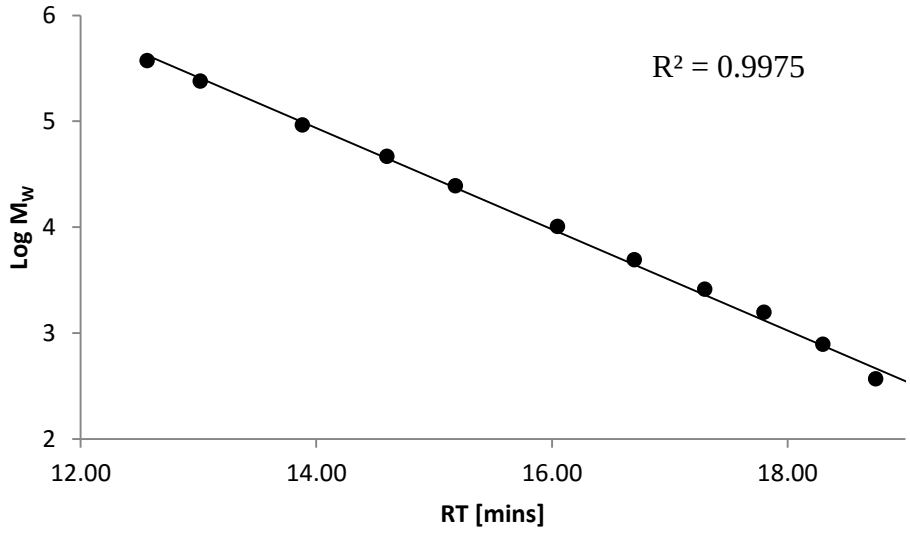


Figure 2-2: The calibration curve for GPC measurement (PSt standards in DMF mobile phase).

It is important to remember that the molecular weight of a polymer is not a single value but it is a distribution of mass (see Figure 2-3, p.64) which depends on the way the polymer is synthesized. Samples of synthetic polymers always contain polymer chains with a range of chain lengths.

The molecular weight of polymers is most usually reported as the number-average molar mass (*Mn*) or the weight-average molar mass (*Mw*).[55] The ratio of weight-average molecular weight and number-average molecular weight is called polydispersity index (PDI) and measures the distribution of molecular weights in the sample. As the molecular weight distribution narrows PDI is getting closer to 1, the polymer chains in the sample are considered to be almost the same length at PDI = 1. PDI values greater than 1.5 are to be found in less controlled polymerisations such as free radical or in HBP polymers synthesis.[70]

As it is already described in literature,[189] the number-average molecular weight can be calculated as:

$$\boldsymbol{Mn = \frac{\sum NiMi}{\sum Ni}} \qquad \textbf{E.q: 2-1}$$

The weight-average molecular weight can be calculated as:

$$\boldsymbol{Mw = \frac{\sum NiM_i^2}{\sum NiMi}} \qquad \textbf{E.q: 2-2}$$

Polydispersity can be calculated as:

$$\boldsymbol{PDI = \frac{Mw}{Mn}} \qquad \textbf{E.q: 2-3}$$

N_i stands for number of polymer chains of fraction *i* and M_i is a mass of polymer chains of fraction *i*.

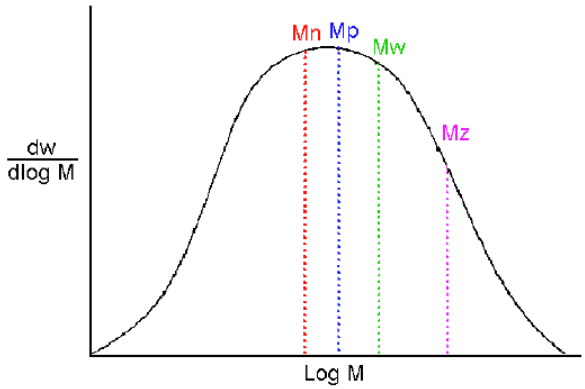


Figure 2-3: Illustration of the distribution of molecular weight in polymer sample. *Mp* is the characteristic value which is the peak maximum.

In this research, number-average molecular weight (*Mn*), weight-average molecular weight (*Mw*) and polydispersity (*Mw/Mn*) were obtained by GPC (PL-50, Polymer Labs) with a Refractive Index detector (RI) system manufactured by Agilent Technologies. The GPC analyses were undertaken at 40 ^{o}C with a flow rate 1 mL/min. Gel Permeation Chromatography (GPC) system used in majority of this study included guard column and two main columns used in series. Two combinations of columns were used. One of them included PolarGel - M guard column with two main PolarGel - M columns used in series, with dimethylformamide (DMF containing 0.01M LiBr) as an eluent. Adding LiBr into DMF eluent limits the interaction polymer-solvent-resin (e.g. limitation of the influence of ionic group like carboxyl group) which is often observed in chromatography of polymeric materials with ionic functionalities.[190] Interaction between polymer–solvent–resin might cause artificial shoulders to appear on the high molecular weight end of the distribution.[189,190] Second combination of columns used in this work had an arrangement of PolarGel - M guard column with two main PLgel Mixed-C columns connected in series, with tetrahydrofuran (THF) as an eluent.

Number-average molecular weight (*Mn*), weight-average molecular weight (*Mw*) and polydispersity (*Mw*/*Mn*) were also obtained by GPC (PL-120, Polymer Labs) with an Refractive Index detector (RI) and Multiangle Light Scattering (MALLS) detector (mini-Dawn) supplied by Wyatt Technology for hyperbranched polymer samples prepared according to section 2.3.8 and section 2.3.9 (p.53-54). In this case the columns (30 cm PLgel Mixed-C) were eluted by THF and calibrated with poly (methyl methacrylate) standards. All calibrations and analyses were performed at 40 °C and a flow rate of 1

mL/min. Absolute molecular weight data were obtained by GPC with MALLS detector and the average dn/dc value (0.076) of the copolymers was determined using a differential refractometer.

Differential refractometer detector (RI) was used to record the data; it is a concentration sensitive detector measuring the difference in refractive index between the solvent and the sample.[194] The organic solvents used in this project have following refractive indices: THF = 1.407, DMF = 1.430 at 20 ^{o}C. Refractive indices for calibration standards used: PMMA = 1.491 and PSt = 1.592.

2.4.2.1. Nuclear Magnetic Resonance Spectroscopy

All NMR spectra in this thesis, including ^{1}H NMR and ^{13}C NMR, were recorded on a 400MHz Bruker Spectrometer (Bruker, Germany). To analyse the spectra MestReNovaLITE processing software was used. Samples were pre-dissolved in a suitable deuterated solvent and the chemical shifts were referenced to tetramethylsilane (TMS). A concentration of 5 mg/mL in the solvent was used for ^{1}H NMR, whilst a concentration of 50 mg/mL for ^{13}C NMR as ^{13}C NMR is less sensitive than ^{1}H NMR. Usually 16 scans were collected for ^{1}H NMR spectra, whilst 1024 scans for ^{13}C NMR. The higher number of scans was needed to improve the signal to noise ratio and obtain a smooth baseline and an adequate spectrum of the relatively weak carbon NMR signals.

2.4.2.2. Differential Scanning Calorimetry /Thermo Gravimetric Analysis

To study thermal properties of polymer samples, Differential Scanning Calorimetry and Thermo Gravimetric Analysis techniques were used. When a polymer is heated/cooled through a specified temperature range, the measurement on its transitions due to morphological or chemical changes (or both) can be recorded. DSC is a technique frequently used in polymer science to study thermal transitions, such as the glass transition temperature (Tg) or melting temperature (Tm) in a particular sample.[189]

In typical DSC analysis, a small amount of the sample (typically 10 ~ 20 mg) was placed in a pan made of inert material (e.g. aluminium) and placed in the DSC heating block. At the same time a reference pan was placed alongside the sample at the appropriate point in the heating block (head). Reference pan generally is an empty sample pan made of inert material. The head was flushed with nitrogen to prevent condensation of water and to remove any atmospheric damp. The cell was cooled to a temperature below any expected thermal transitions in the sample and left to equilibrate. After the adjustments, the temperature of both the sample pan and reference pan was slowly increased at a consistent rate. With the temperature increasing, any thermal transitions of analysed samples become apparent. The instrument ought to be calibrated. Calibration involves using a standard with a known enthalpy of melting and a sharply defined melting point. In an exothermic transition, heat is absorbed by the sample and for this reason temperature of particular analysed sample increases compared to the reference pan, and is recorded as a peak in the thermogram. In this method the change in the energy input is measured when a temperature change is detected. The energy input (enthalpy) is proportional to the area under the peak and allows for quantitative measurement of the process (provided the instrument has been calibrated).

Thermo Gravimetric Analysis involves the observation of changes in the weight of a sample as it is heated. Similarly to DSC, in TGA both sample pan and reference pans are placed in a responsive thermo-balance inside a furnace, and heated at a consistent rate (in an inner atmosphere of nitrogen). The instrument constantly monitors the weight of the sample and the resultant thermogram is a representation of weight against given temperature range. Weight loss might occur as a result of evaporation of any residual solvents or moisture. There is also a possibility that at higher temperature decomposition of the polymer can take place and weight loss in analysed samples is recorded.

SDT Q600 V 4.1 Build 59 Module DSC-TGA was used to measure the thermostability and thermal phase transition behaviour of the pH responsive polymers prepared according to section 2.3.11, p.57. The weight loss of the hydrogel samples with different composition was measured under a nitrogen atmosphere. The samples were heated in a temperature range of 20 - 800 ^{o}C at the heating rate of 10 ^{o}C/minute and the weight loss was recorded. Sample sizes of approximately 15 mg were used.

In case of thermoresponsive biodegradable polymers prepared through conventional RAFT copolymerisation of PEGMEMA, PPGMA and DSDA (synthesized according to protocol in section 2.3.10, p.55-56) measurement of glass transition was performed at temperature range of -70 to 200 ^{o}C.

2.4.2.3. Measurement of Lower Critical Solution Temperature

Lower critical solution temperatures of the copolymer solutions can be determined by UV visible Spectrophotometry. Determination of LCST aims to find the lowest temperature below which the polymer stays soluble and above which gelation occurs. In the temperature above LCST of the polymer sample will fall out of solution. The solubility of polymers is temperature dependant. In general, increase of the temperature increases solubility of the sample, but the opposite can be also observed for certain synthetic polymers. Having in mind *in-situ* forming hydrogels this studies aimed to achieve an LCST at about of human body temperature. To determine the LCST, the polymer needs to be dissolved in de-ionized water, phosphate buffered saline (PBS pH = 7.4) or cell culture media. Solutions become cloudy above the LCST and clear below. The selected samples were scanned by the UV spectrometer at a fixed wavelength in various temperatures.

The LCSTs of the selected hyperbranched polymers synthesized according to protocols in section 2.3.8 and 2.3.9 (p.53-54) were achieved on temperature-controlled spectrometer during following analysis. 0.03% w/v deionised water was quantified by measuring their absorbance at 530 nm from 12 to 40 ^{o}C (heating rate = 0.5 ^{o}C/sec) with a Beckman DU-640 spectrophotometer. The data was collected every 2 seconds. The Malvern Nano Zetasizer was used to measure the hydrodynamic diameter and the distributions of polymer samples in water solutions. Polymer solutions (0.03% w/v) were prepared in deionised water and filtered prior to measurements using a 0.45 μm disposable filter into a 12.5×12.5 mm polystyrene disposable cuvette.

Lower critical solution temperatures for the biodegradable thermoresponsive hyperbranched polymers prepared according to section 2.3.10 were measured *via* visual observation in the first place for concentrations of 10 mg/ 1mL H_2O in temperature range from 0 to 70 ^{o}C and then by DSC. The values from both methods corresponded well.

2.4.2.4. Swelling and Degradation Studies

Ability of gels to swell was investigated after preparing solution of hyperbanched polymer in a suitable medium and preheating it to the LCST. Gels were then allowed to swell at that temperature. At regular times the incubation buffer were removed and the weight of the gels measured.

To study the swelling of hydrogels fabricated according to section 2.3.10 (p.55) using thermoresponsive biodegradable copolymers of PEGMEMA-PPGMA-DSDA, the selected samples of hydrogels were lyophilised, weighted individually and immersed in 2 mL of phosphate buffer (pH = 7.4). The excess solvent was removed and then the samples were weighed at regular time intervals. Measurements were performed in triplicates; the weight of the swollen sample was recorded on a digital balance as a function of time.

To study the swelling of pH responsive linear and dendric copolymers of AA-HEMA (prepared according to section 2.3.11, p.57), the selected samples were dried, weighted individually and immersed in 3 mL of the solvent in different pH for the time required (pH = 4; pH = 6.8 deionised water; pH = 7.4 phosphate buffer). The excess solvent was removed and then the samples were weighed at regular time intervals. Measurements were performed in triplicates; the weight of the swollen sample was recorded on a digital balance as a function of time.

The mean values were calculated, and then were used in the following equations:

$$\textbf{Swelling ratio} = \frac{\mathbf{Ws - Wo}}{\mathbf{Wo}} \times \mathbf{100}\% \qquad \textbf{E.q: 2-4}$$

Where, W_s is the weight of swelled hydrogel at certain time point and W_o is the weight of dry hydrogel.

The hyperbranched polymers presented in this thesis in section 2.3.10 (p.55) are designed to undergo degradation, both acid/base and enzymatic degradation. Chemical degradation studies (cleavage) of the disulfide-containing branched polymers/hydrogels fabricated from synthesized thermoresponsive biodegradable copolymers of PEGMEMA-PPGMA-DSDA

described in section 2.3.10 were performed by reduction with Dithiothreitol (DTT), also known as Clelands Reagent. It is an unusually strong reducing agent frequently used to reduce the disulfide bonds of proteins.

In typical chemical degradation studies of the above mentioned hydrogels fabricated from thermoresponsive biodegradable copolymers of PEGMEMA-PPGMA-DSDA, the required amount of samples (e.g. 6 mg) were weight into vials and dissolved in 2 mL THF. Fresh 1M solution of DTT was prepared by dissolving 7.7 mg into 50 µl of THF (fresh stock solution was prepared before the studies). The degradation test started when each of crosslinked polymers, dissolved in 2 mL THF were mixed with 50 µl of 1M DTT and incubated in oven at 50 ^{o}C for 5hrs. This harsh condition and degradation test at temperature above LCST of the analysed samples was used to check if complete degradation is possible. After 5h, samples were diluted with 2 mL THF and run GPC analysis. Following this harsh condition, the protocol was changed and mild conditions were used. The required amount of samples (e.g. 6 mg) were weight into vials and dissolved in 2 mL THF where the degradation test started when each crosslinked polymers, dissolved in THF were mixed with 50 µl of 1M DTT at room temperature and run GPC analysis immediately. At required time points GPC analysis were run to monitor the degradation kinetics. In following tests a different concentration of DTT were used in order to check if polymer structure can be tailored by adjusting concentration of DTT and to determine the extent of cleavage of the disulfide branch sites in the copolymer (data for each experiment can be found in the Chapter 4).

2.4.2.5. Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is an optical method used by researchers for over 50 years and it uses a beam of electrons to illuminate the specimen.[195]

Major problem associated with SEM imaging of polymers and hydrogel, is related to their poor conductivity of electricity. A charge builds up on a sample following the attack of electrons.[196] This can misrepresent the image. In order to avoid these issues the polymer/hydrogel sample must be covered with a layer of conductive coating such as carbon, palladium or gold. The electrons hit the sample, the polymer/hydrogel specimen

also emits electrons and due to coating they are able to flow. These are measured by the detector, which converts them to an electrical signal.[195,196]

SEM analysis was used in this research to view hydrogel surface of biodegradable hydrogels fabricated by means of Michael addition reaction from polymers prepared according to section 2.3.10 (p.55). The dry, lyophilised hydrogel samples were attached to an aluminium stub with an adhesive carbon tab and gold coated on Polaron E5000 SEM coating unit for 300 seconds. Images were obtained using a Hitachi S-520 Scanning Electron Microscope.

2.4.2.6. Fourier Transform Infra-Red Spectroscopy

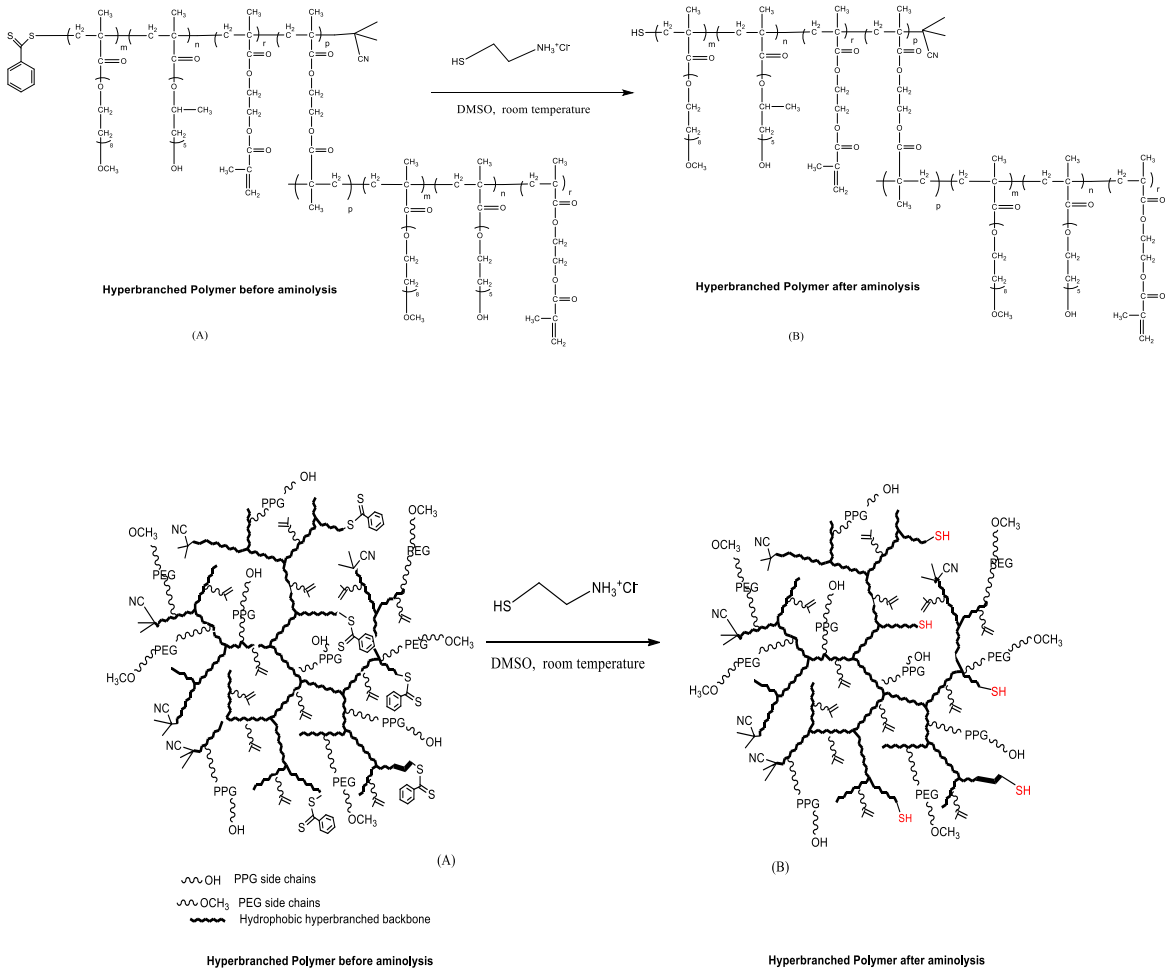
FTIR stands for Fourier Transform Infra-Red, and it is a method of infrared spectroscopy. An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the analysed material. Some of the infrared radiation is absorbed by the sample and some of it is transmitted through. The measured signal is digitized and sent to the computer where the Fourier transformation (mathematical calculations) takes place. Background spectrum must also be measured and this is normally a measurement with no sample in the beam (baseline). The final spectrum of analysed sample is then accessible to the user for any further manipulation and for interpretation.

Fourier transform infra-red spectroscopy (FTIR) was used in this research to detect the functional groups of pH responsive polymers of AA-HEMA and AA-HEMA-EGDMA synthesized according to section 2.3.11 and spectra were taken using PerkinElmer spectrum 100 instrument (KBr pellets containing 1% of the sample by weight were prepared and a scan range of 450 to 4000 cm^{-1} was used for analysis). To prepare KBr pellets containing the sample the following protocol was used: [197] a small quantity of sample representing approximately 1% was added to the powdered KBr (dry IR-grade). The mixture was ground until it was uniformly distributed throughout the KBr (by visual observation). The press body and anvils (a block with a hard surface) were thoroughly cleaned and dried and then loaded with mixture of sample/KBr. Pressure was applied by means of wrenches to the bolt style anvils simultaneously while compressed air was removed under vacuum. Pressure was

applied for about one to two minutes, then remove bolts to eject disc which is then placed in a holder in the path of the FTIR beam. The samples were scanned three times.

2.4.2.7. Aminolysis of hyperbranched polymers

In order to conduct the aminolysis of thermoresponsive HBPs (PEGMEMA-PPGMA-EGDMA) prepared by RAFT Polymerisation described in section 2.3.9, cysteamine hydrochloride (1.00 g) was added to the polymer solution (1.00 g of polymer in 10 mL DMSO). The reaction mixture was allowed to stir overnight at room temperature. The colour of the reaction mixture changed into colourless solution. As a next step, the product was further dialysed (MWCO 8,000 kDa) agaist fresh deioinised water for 24 hours and than freeze dried.



Scheme 2-13: Hyperbranched polymer synthesized *via in-situ* RAFT polymerisation before (A) and after aminolysis (B).[111]

2.5. Fabrication of Hydrogels

2.5.1. Thermal gelation, self-assembly and photo-crosslinking

Hydrogels from pH responsive polymers of AA-HEMA and AA-HEMA-EGDMA synthesized according to section 2.3.11 (p.57) were prepared *via* self-assembly.

Selected thermoresponsive polymers (100-300 mg) prepared according to protocols described in section 2.3.8, 2.3.9 and section 2.3.10 were dissolved in 1 mL deionised water at 4 ^{o}C and then incubated in vacuum oven at 37 ^{o}C for 5 to 30 minutes. Gel concentration was determined by visual observation, as no flow upon inversion of the vial within 10 seconds.

The hydrogels were also prepared through photopolymerisation by reacting 0.03% w/v of polymer sample, with 0.01% Irgacure 2959 which was used as a photoinitiator and by exposure to UV light sources (BluePoint lamp 4, 350–450 nm, Honle UV technology, light intensity of 50 mW/cm^2). In course of this work it was observed that the PEGMEMA-PPGMA-EGDMA hyperbranched polymers when exposed to UV light sources undergo photopolymerysation and crosslinking occurred due to the existence of multiple methacrylate functional groups within them.

2.5.2. Michael addition reaction

In this thesis, chemically crosslinked hydrogel system was developed from thermoresponsive biodegradable copolymers of PEGMEMA-PPGMA-DSDA synthesized according to section 2.3.10, p.55. Before the hydrogel was prepared, the number of free vinyl functional groups was determined by ^{1}H NMR characterisation. Free multiacrylate functional groups of the polymer reacted with pentaerythritol tetrakis (3-mercaptopropionate), QT, a thiol functional crosslinker (Figure 2-4). The compound was added at 25% of the required amount for equal molar ratio of vinyl group. A number of reactions were performed in 2 mL eppendorf safe-lock tubes, the mixing of the copolymers solutions of PEGMEMA-PPGMA-DSDA in PBS buffer (pH = 7.4) and QT was carried by reversing the tubes up and down (mild mixing) and then samples were left to incubate in vacuum oven, at 23 ^{o}C (room temperature) and 37 ^{o}C for 24 hours.

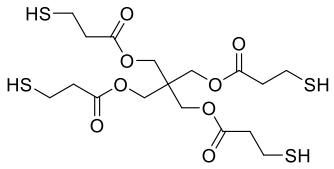


Figure 2-4: Pentaerythritol tetrakis (3-mercaptopropionate).

Chapter 3 Results and discussion on in-situ Reversible Addition-Fragmentation Chain Transfer Polymerisation approach

Parts of this chapter have been published in:

H. Tai, **A. Tochwin**, and W. Wang, Thermoresponsive Hyperbranched Polymers *via in-situ* RAFT Copolymerisation of PEG based Monomethacrylate and Dimethacrylate Monomers, *Polym. Chem.*, 2013, **51**, 3751–376, DOI: 10.1002/pola.26779.

3.1. Development of an *in-situ* Reversible-Addition Fragmentation Chain Transfer polymerisation approach

3.1.1. Introduction

The kinetics of the RAFT reaction, particularly where dithiobenzoates with a phenyl group (C_6H_5-C(=S)S-R) act as RAFT agent, are still under investigation and are not yet fully understood, despite the wide use and the success of RAFT polymerisation.[74,198,199,200] The synthesis of disulfide based RAFT agent involves preparation of dithiobenzoic acid (DTBA), which is an unstable liquid and should be stored at low temperatures (below –20 °C) or used immediately after acidification as a chain transfer agent (CTA). Therefore, it is usually converted into bis(thiobenzoyl)disulphide (section 2.3.1, p.45),[184] which is a stable solid and free from odour at the room temperature. It was envisioned that *in-situ* formation of RAFT agent using bis(thiobenzoyl)disulfide can simplify the polymerisation process, i.e. the disulphide is used as the precursor of the RAFT agent.[201] 2-Cyanoprop-2-yl dithiobenzoate (CPDB, section 2.3.2, p.46) is frequently employed as a RAFT agent in polymer synthesis. Dithiobenzoic acid demonstrated good controllability as a CTA in the polymerisations of methacrylate, methyl methacrylate and styrene[202] but showed difficulty in handling as it was stored as a solution of PhC(=S)SNa in distilled water, and acidified prior to use. Bis(thiobenzoyl)disulphide is much easier to work with and is used not only

for the synthesis of CPDB, but also for the syntheses of many different RAFT agents.[83,203] Another research group has previously reported simplification of the standard synthesis of CPDB[186] where they omitted the recrystallisation step of bis(thiobenzoyl)disulphide in order to avoid the loss of the material in this purification step.[186] They proved that the yield of CPDB prepared in the simplified synthesis increased by a factor of four comparing to the literature[204] where purified precursor was used. Moreover, the CPDB synthesized by the simplified procedure provided good control of the polymerisation of MMA, yielding PMMA samples with narrow molar mass distributions (polydispersity index, PDI $\leq$ 1.2), and behaved in the same manner as the CPDB synthesized by the standard method.[186]

Polymerisation *via* the RAFT method can be affected by the reaction temperature, the solvent, the nature of monomers and ratios of monomer/CTA and CTA/initiator.[75,205,187] It has been widely reported that 'living' controlled radical polymerisation should fulfil various experimental requirements.[5,2,3] It is clear that in order to obtain the desired structures, polymerisation parameters have to be optimised. Therefore, looking at different reaction parameters, while keeping the rest of the conditions constant, is crucial for understanding the reaction kinetics. Ability to predict molecular weight of resultant polymers, the relatively linear relationship between an increase in molecular weight and the conversion of monomer, and narrow polydispersity are the main criteria to be considered when justifying controllability of polymerisation.

As described in chapter 1 conventional RAFT polymerisation comprises five steps: initiation, chain transfer, reinitiation, chain equilibration and termination (Figure 1-11, p.20). It is known that initiation and termination occurs in the same way as in conventional radical polymerisation. In the early stages of the polymerisation, addition of the thiocarbonylthio compound [RSC(Z)=S] (also known as a RAFT agent) to the propagating radical ($P_n^{\bullet}$) is followed by fragmentation of the intermediate radical and provides a polymeric thiocarbonylthio compound [$P_nSC(Z)=S$] and a new radical ($R^{\bullet}$). Reaction of this radical ($R^{\bullet}$) with monomer forms a new propagating radical ($P_m^{\bullet}$). Rapid equilibrium between the active propagating radicals and the dormant polymeric thiocarbonylthio compounds provides equal probability for all chains to grow and allows for the production of narrow polydispersity polymers.

Using bis(thiobenzoyl)disulfide as the precursor of 2-cyanoprop-2-yl dithiobenzoate, and azobisisobutyronitrile as a initiator, gives the possibility to conduct conventional RAFT

process by creating a RAFT agent in the reaction mixture (*in-situ*). This method was used previously in the bulk synthesis of polystyrene where oligomers were prepared, using ratio of [AIBN] : [Styrene] as [1.5] : [1].[201] By changing the reaction time the degree of polymerisation was varied and the resultants polymer had PDI below 1.4. Recently we reported the data on the synthesis of PEG-based novel hyperbranched polymers where the *in-situ* approach was successfully adopted for the copolymerisation of poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, *Mn = 475*), poly(propylene glycol) methacrylate (PPGMA, *Mn = 375*) and ethylene glycol dimethacrylate (EGDMA).[111] This data is also presented and discussed in following section 3.2. The advantages of an *in-situ* RAFT approach make it a very attractive method for the preparation of polymers with well-defined structures for wide applications, for example coatings and adhesives. In particular, *in-situ* RAFT could be very appealing for an industry production process.

The successful polymerisation of methacrylate and its derivatives in the presence of CPDB have been well documented.[206,207,208,112] However, dedicated studies on the kinetics of *in-situ* RAFT polymerisations of MMA and Styrene have not been reported in the literature. This work aimed to study the kinetics of *in-situ* RAFT polymerisations of MMA and Styrene in comparison to conventional RAFT method. Different reaction conditions were used to study the effects of solvent, reaction temperature and ratios of the reactants.

Bis(thiobenzoyl)disulfide was used as a precursor of CTA in the homopolymerisations of methyl methacrylate and styrene, where 2-cyanoprop-2-yl dithiobenzoate was formed *in-situ* through a one-pot and two-stage approach (Scheme 2-9, p.52, Table 3-3 and Table 3-4, p.79-80). For the comparison, conventional RAFT homopolymerisations of the MMA and Styrene at 65 ^{o}C were conducted using 2-cyanoprop-2-yl dithiobenzoate as the RAFT agent (Scheme 2-8, p.51, Table 3-1 and Table 3-2, p.78). To compare conventional and *in-situ* RAFT polymerisations, experiments were conducted at a range of reaction conditions including changing the solvent and reaction temperature.

In section 3.1 and section 3.2, solid experimental results on the development of *in-situ* RAFT approach have been presented and the preparation of novel hyperbranched polymer from this developed *in-situ* RAFT approach is described.

3.1.2. Tailoring functionality by varying initiator and Reversible-Addition Fragment Chain Transfer agent

Modification of functional groups can tailor the properties of polymer solubility, compatibility, adhesion to various surfaces, self-assembly and chemical recognition.[116,166] As previously mentioned, RAFT polymerisation can be affected by the reaction temperature, the solvent, the nature of monomers and ratios of monomer/CTA and CTA/initiator.[209] By altering the ratio of the initiator to monomer, monomer conversion and other reaction conditions, the numbers of terminal functional groups within linear and hyperbranched polymers can be varied. In this thesis, during RAFT polymerisation, vinyl functionality was introduced by copolymerisation with a divinyl monomer, and additionally end functional groups were incorporated into the linear and hyperbranched polymers, e.g. initiator radical end group. These moieties can be varied and functionality of the polymers can be tailored. Moreover branching degree and shape of the HBP polymer can differ. A detailed study on the effects of initiator to monomer ratio and monomer conversion was carried out. Here, the results are discussed below.

3.1.3. Comparison of polymerisations using conventional and *in-situ* Reversible-Addition Fragmentation Chain Transfer approach to prepare Poly(methyl methacrylate) and Polystyrene

Experimental data on conventional RAFT and *in-situ* RAFT polymerisations of methyl methacrylate and styrene are presented in Table 3-1, Table 3-2, Table 3-3, and Table 3-4 respectively.

Table 3-1: Reaction conditions and molecular weight characteristics of PMMA homopolymers prepared *via* conventional RAFT method using 2-cyanoprop-2-yl dithiobenzoate as the RAFT agent.

Entry	Solvent	[MMA]:[CPDB]:[AIBN]	Mn^{a}, GPC (kg/mol)	Mw^{a}, GPC (kg/mol)	PDI[a]	Conv[a] (%)	Mn^{b}, NMR (kg/mol)	Mn^{c}, theory (kg/mol)
1	Ethyl acetate	50:0.5:0.2 Cr	9.2	12.2	1.3	96	8.5	9.8
2	Ethyl acetate	50:0.5:0.2 P	9.1	10.7	1.2	92	5.6	9.4
3	n-butanone	50:0.5:0.2 Cr	9.6	12.5	1.3	90	7.5	9.2
4	n-butanone	50:0.5:0.2 P	8.0	10.1	1.3	88	8.1	9.0

Reactions run at 65 ^{o}C. Solvent:Monomer volume ratio is 1:1. [a] Monomer conversion, Polydispersity and Number-average molecular weight and Weight-average molecular weight estimated by GPC; [b] Number-average molecular weight estimated by NMR; [c] calculated by E.q: 1-1, p.21, where: [M] and [RAFT] are initial moles concentration of monomer and CTA, *Conv* is monomer conversion, 221 is molecular weight of CPDB; crude (Cr, 70%), pure (P, 95%) determined by ^{1}H NMR. Reaction time = 9hrs.

Table 3-2: Reaction conditions and molecular weight characteristics of styrene homopolymers prepared *via* conventional RAFT method using 2-cyanoprop-2-yl dithiobenzoate as the RAFT agent.

Entry	Solvent	[St]:[CPDB]:[AIBN]	Mn^{a}, GPC (kg/mol)	Mw^{a}, GPC (kg/mol)	PDI[a]	Conv[a] (%)
1	n-butanone	100:1:1.4 Cr	1.7	2.1	1.2	28
2	n-butanone	100:1:1 Cr	6.3	9.6	1.5	65
3	Bulk	100:1:1 Cr	5.7	8.0	1.4	64

Reactions run at 65 ^{o}C. Solvent:Monomer volume ratio is 1:1. [a] Monomer conversion, Polydispersity, Number-average molecular weight and Weight-average molecular weight estimated by GPC; Purity: crude (Cr, 70%) determined by ^{1}H NMR. Reaction time = 5hrs.

Table 3-3: Reaction conditions and molecular weight characteristics of PMMA homopolymers prepared *via in-situ* RAFT approach.

Entry	Solvent	[MMA]:[CPDB]:[AIBN]	Reaction Time (h)		Mn^{a}, GPC (kg/mol)	Mw^{a}, GPC (kg/mol)	PDI[a]	Conv[a] (%)	Mn^{b}, NMR (kg/mol)	Mn^{c}, theory (kg/mol)
			80 °C	65 °C						
1	Ethyl acetate	50:0.5:0.7	4	5	5.5	7.3	1.3	95	4.2	5.0
2	Bulk	50:0.5:0.7	4	5	1.1	1.3	1.1	17	6.6	1.0
3	Bulk	50:0.5:0.7	2	7	2.9	3.6	1.2	69	4.3	3.8
4	Ethyl acetate	50:0.5:0.7	2	7	6.7	8.2	1.2	96	5.1	5.0
5	n-butanone	50:0.5:0.7	2	7	5.8	7.4	1.3	92	5.1	4.8
6	n-butanone	50:0.5:0.7	4	5	6.0	7.3	1.2	95	3.8	5.0
7	Bulk	50:0.5:0.7	0	3	7.0	9.1	1.3	73	9.4	3.9
8	Ethyl acetate	50:0.5:0.7	0	30	7.0	8.8	1.3	92	5.0	4.8
9	n-butanone	50:0.5:0.7	0	30	5.7	6.7	1.2	90	3.4	4.7
10	Ethyl acetate	50:0.5:0.7	9	0	6.8	8.3	1.3	92	4.9	4.8
11	n-butanone	50:0.5:0.7	10	0	6.2	8.1	1.3	95	5.7	5.0
12	n-butanone	50:0.5:5	3.5	0	4.3	5.8	1.4	94	2.5	4.9
13	n-butanone	50:2.5:3.5	4	44	2.6	3.3	1.3	83	1.7	1.1
14	n-butanone	50:0:0.2	0	5	30.8	62.3	2.0	84	-	-

Where solvent is used monomer volume ratio is 1:1. [a] Monomer conversion and Polydispersity estimated by GPC; [a] Number-average molecular weight estimated by GPC; [b] Number-average molecular weight estimated by NMR; [c] calculated by E.q: 1-1, p.21, where initial moles concentration of monomer and CTA, *Conv* monomer conversion, 221 is molecular weight of created *in-situ* CPDB.

Table 3-4: Reaction conditions and molecular weight characteristics of styrene homopolymers prepared *via in-situ* RAFT approach.

Solvent	[St]:[CPDB]:[AIBN]	Reaction Time (h)		Mn^{a}, GPC (kg/mol)	Mw^{a}, GPC (kg/mol)	PDI[a]	Conv[a] (%)
		80 °C	65 °C				
n-butanone	100:1:1.4	2	10	5.5	7.3	1.4	62
n-butanone	100:0.5:1.5	2	22	1.1	1.3	1.6	59
n-butanone	100:0.5:0.7	2	22	3.0	3.6	1.4	50
n-butanone	200:0.5:0.7	2	22	6.9	8.2	1.2	24
Bulk	100:0.5:0.7	2	22	5.8	7.4	1.4	60
n-butanone	100:0.5:0.7	0	42	6.0	7.3	1.4	58
n-butanone	100:0:1	0	5	7.0	9.1	1.7	11
Bulk	100:0:1	0	5	7.0	8.9	3.2	19

Where solvent is used monomer volume ratio is 1:1. [a] Monomer conversion and Polydispersity estimated by GPC; [a] Number-average molecular weight and Weight-average molecular weight estimated by GPC.

3.1.4. Effect of the purity of the Reversible-Addition Fragmentation Chain Transfer agent

The controllability of MMA homopolymerisation using crude 2-cyanoprop-2-yl dithiobenzoate (the purity of 70% determined by ^{1}H NMR) as the RAFT agent (entry 1, 3 in Table 3-1, p.78) was compared with that using purified 2-cyanoprop-2-yl dithiobenzoate (by chromatographic column) (the purity of 95% determined by ^{1}H NMR) (Entry 2, 4 in Table 3-1). The experimental data shows that there was no significant difference in the resultant molecular weight of polymers and their PDI's (Table 3-1). This result indicated that the RAFT agent with purity between 70-95% could yield similar controllability for the polymerisation. PMMA produced by employing crude RAFT agent had an average molecular weight slightly higher following higher conversion, but the controllability of the process was very similar because of the similar values of PDIs. For styrene conventional RAFT homopolymerisation, the crude CPDB was used.

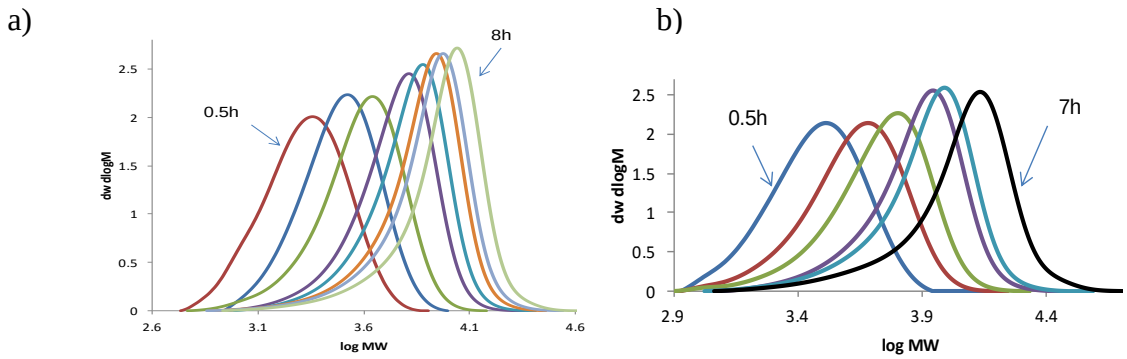


Figure 3-1: GPC data, effect of reaction time on molecular weight distribution for linear PMMA polymers prepared by conventional RAFT (entry 3 (a), entry 4 (b), [MMA]:[CPDB]:[AIBN] – a) 50:0.5:0.2 Cr and b) 50:0.5:0.2 P, Table 3-1).

Progression of molecular weight and kinetics of chosen samples are shown in Figure 3-1, Figure 3-2, Figure 3-3, Figure 3-4 and Figure 3-5. Those figures also demonstrated the similar kinetic controls when using RAFT agent with its purity of 70% and 95%.

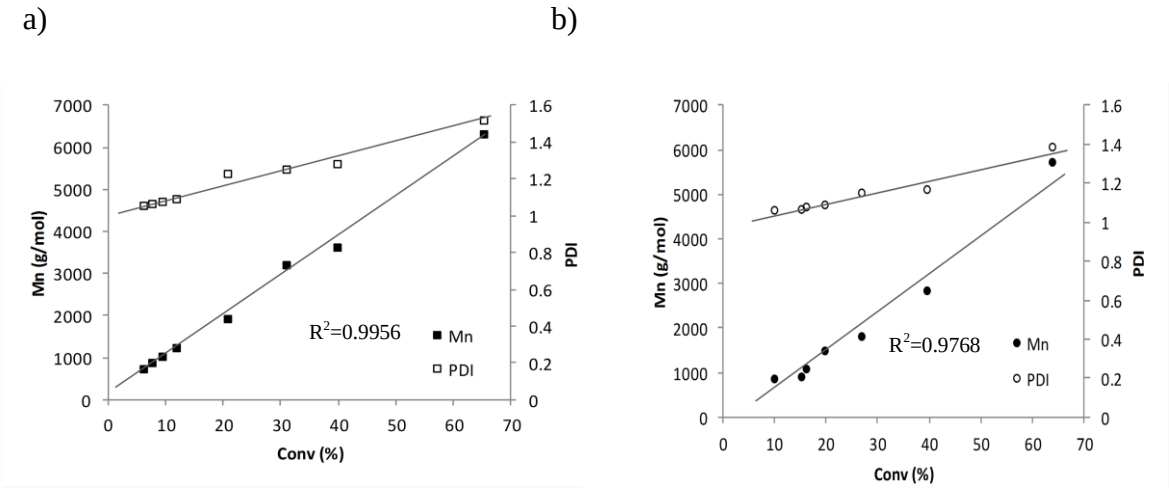


Figure 3-2: Kinetic curves for conventional RAFT polymerisation of St at 65 ^{o}C in n-butanone entry 2 (a) and in bulk entry 3 (b) ([St]:[CPDB]:[AIBN] – 100:1:1) in Table 3-2.

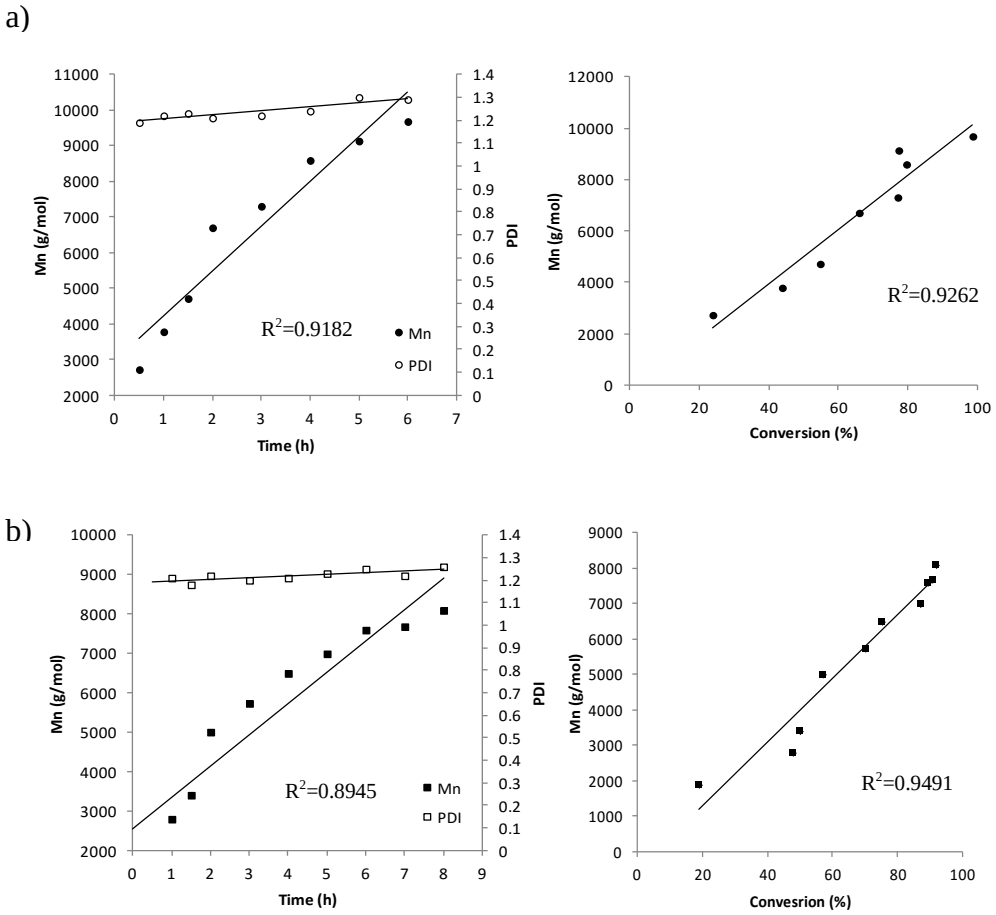


Figure 3-3: Kinetic curves for conventional RAFT polymerisation of MMA at 65 ^{o}C in n-butanone: number average molecular weight/PDI vs time and conversion for entry 3 (a) and entry 4 (b) in Table 3-1.

3.1.5. Effect of reaction temperature

The effects of the temperature on the RAFT polymerisations are not widely reported. An increase in temperature accelerates the rates of fragmentation and polymerisation in RAFT process; in addition it might also affect the rates of termination reactions, therefore it is expected to have some broadening of the molecular mass distribution at higher temperatures.[184] However, it has been reported that with dithiobenzoates narrower PDI can be attained at higher temperatures and the retardation is reduced.[74]

Bis(thiobenzoyl)disulfide (A in Scheme 2-9, p.52) in the polymerisation mixture was firstly employed to form the RAFT agent 2-cyanoprop-2-yl dithiobenzoate (B in Scheme 2-9) *in-situ* by reacting with initiator (AIBN) at 80 ^{o}C and then polymerisation occurred according to general RAFT mechanism at 65 ^{o}C (Scheme 2-8, p.51). It was observed that during the first phase, polymerisation of monomers was not detected by GPC. This can be explained by the competition of two reactions where the reactivity of the reactants involved towards AIBN is crucial. This include the reaction (1) where AIBN initiates the polymerisation of monomers and the reaction (2) where AIBN reacts with bis(thiobenzoyl)disulfide (A in Scheme 2-9) to form 2-cyanoprop-2-yl dithiobenzoate (B in Scheme 2-9). As disulfide is more reactive and easily to be reduced using AIBN, the second reaction is more favoured than the first one. In addition, it also explains why in the presence of disulphide the free radical polymerisation of vinyl monomers initiated by AIBN is inhibited. Data presented here also proves that it is possible to convert disulphide into RAFT agent at 65 ^{o}C but the reaction time and *in-situ* synthesis of CPDB is very long and it takes approximately 18 hrs (entries 7-9 in Table 3-3, entry 6 in Table 3-4, p.79-80) under the reported conditions, then polymerisation occurs in controlled manner.

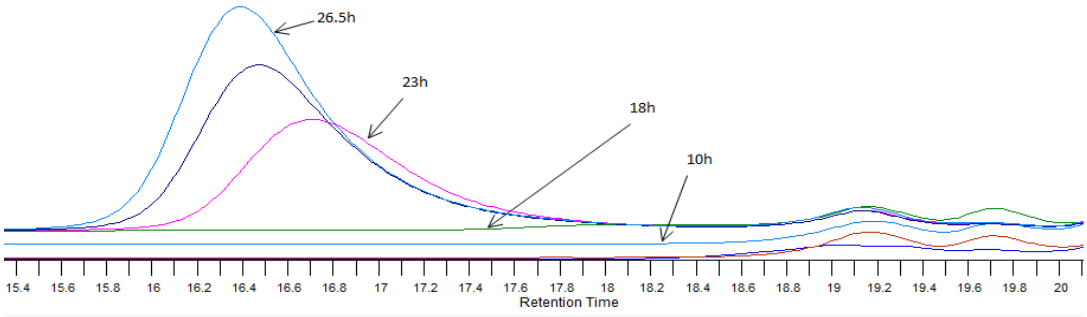


Figure 3-4: GPC traces for *in-situ* RAFT polymerisation of MMA, in n-butanone, at 65 ^{o}C. Entry 9 ([MMA]:[CPDB]:[AIBN]- 50:0.5:0.7, Table 3-3) shows 18 hrs delay time and controllability of reaction, advancement molecular weight with time.

Figure 3-4 (entry 9 in Table 3-3) demonstrate a 18 hrs delay time and reasonably good controllability of reaction after this time point. By increasing the temperature to 80 ^{o}C at the beginning (entries 1-6, 10-13 in Table 3-3; entries 1-5 in Table 3-4) the process

significantly speeds up, reducing time and cost of the reaction as AIBN decomposes quicker at higher polymerisation temperature and then begins the synthesis of CPDB.

The evolution of the molecular weight distribution follows the behaviour for a living polymerisation after creation of RAFT agent *in-situ.* In all cases the polydispersities of the PMMA polymers obtained by *in-situ* RAFT were relatively low (1.1 to 1.4, Table 3-3, p.79). The styrene polymers prepared by this method had polydispersities higher (1.2 to 1.6, Table 3-4, p.80). The same pattern was seen in the case of conventional RAFT polymerisation of these monomers (Table 3-1, Table 3-2, p.78).

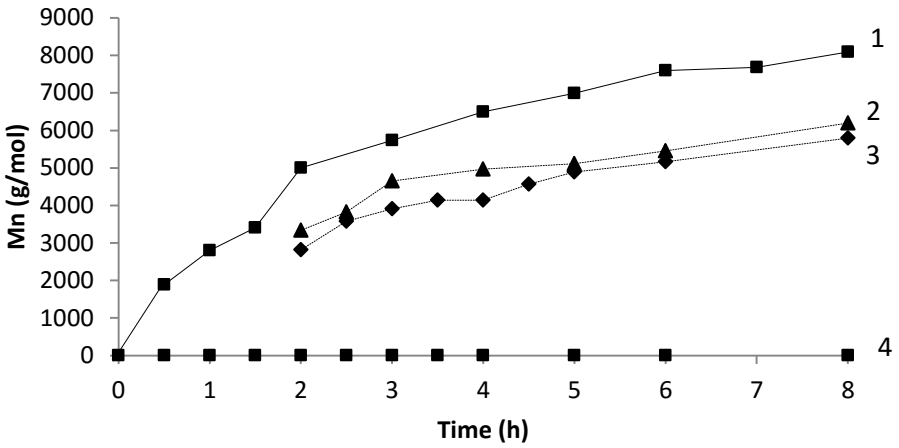


Figure 3-5: Evolution of number average molecular weight against reaction time up to 8hrs. Comparison of selected polymerisations shows relatively linear relationship between average molecular weight and time for PMMA (1 – conventional RAFT at 65 ^{o}C, PDI = 1.3 (entry 4, Table 3-1); 2 – *in-situ* RAFT at 80 ^{o}C, PDI = 1.3 (entry 11, Table 3-3); 3 - *in-situ* RAFT 80/65 ^{o}C, PDI = 1.3 (entry 5, Table 3-3); 4 – *in-situ* RAFT 65 ^{o}C, PDI = 1.2 (entry 9, Table 3-3); 2 produces polymers with similar controllability to 3 where the temperature was reduced to 65 ^{o}C after creation of RAFT *in-situ*. Decreasing the temperature has industrial advantages. It can reduce the heating costs and increase conversion if large scale batches of polymers are prepared.

Keeping the temperature constant at 80 ^{o}C can produce polymers with similar controllability to reactions in which the temperature was reduced to 65 ^{o}C after creation of RAFT *in-situ* (Table 3-3, Table 3-4). Decreasing the temperature to 65 ^{o}C, so that RAFT polymerisation could progress for a desired time has industrial advantages.

It can reduce the heating costs and increase conversion if larger scale batches of polymers are prepared (entries 2 and 3, Figure 3-5). The experimental observations demonstrated that bis(thiobenzoyl)disulphide can be fully converted into RAFT agent within 1.5 up to 2 hours at 80 ^{o}C in the presence of methyl methacrylate (as seen in Figure 3-6) or styrene.

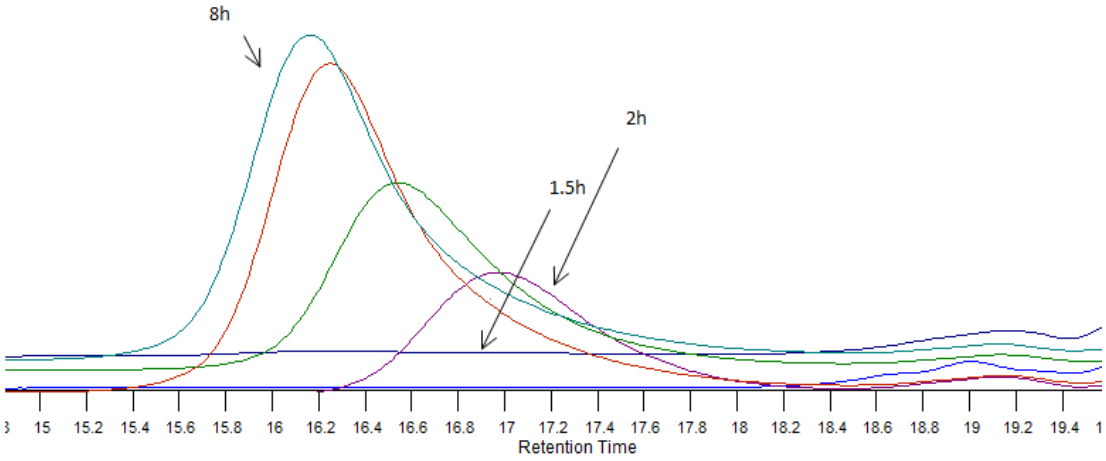


Figure 3-6: GPC trace for *in-situ* RAFT polymerisation of MMA in ethyl acetate, reaction at 80 ^{o}C for 2 hrs, and then at 65 ^{o}C. Entry 4 ([MMA]:[CPDB]:[AIBN] – 50:0.5:0.7, Table 3-3) shows 2 hrs delay time and controllability of reaction, advancement of molecular weight with time.

The relatively low PDI value for the prepared *in-situ* polymers demonstrated the controlled chain growth. It is important to remember that polymers with the broadness of the distribution can decrease the strength and toughness of the polymer, so PDI is an important factor to control.

3.1.6. Effect of the solvent and the ratio of initiator to chain transfer agent

At 65 ^{o}C, it was observed (Table 3-1, Table 3-2, p.78) that good control was obtained for the solution polymerisation by conventional RAFT reactions. Molecular weights, monomer conversion and kinetic are presented for selected samples (Figure 3-1, Figure 3-2, and Figure 3-3, p.82-83) and were run in n-butanone. A near a linear relationship was obtained for the evolution of molecular weight with monomer conversion; resultant polymers have

narrow PDI's below 1.3 for methyl methacrylate homopolymerisations in n-butanone and ethyl acetate, while for styrene the controllability of PDI was not as good and reached up to 1.5 in n-butane, even though the molecular weight of the polymers increased linearly with time and conversion. The results suggest that a reasonably good living character is achieved, and the radical concentrations were constant during the reactions, the polymerisations progressed in a controlled manner. For the effect of the solvent, it was estimated that ethyl acetate could provide similar control as n-butanone in terms of conversions, polydispersities of polymers and molecular weights.

To identify optimised conditions for the preparation of well-defined polymers by the use of *in-situ* RAFT method a set of reactions were run in bulk and in solution. In reactions where ratio of [MMA]:[disulfide]:[AIBN] = 50:0.5:0.7 was used, in a one-pot reaction mixture, in most cases 2 hours were enough to react bis(thiobenzoyl)disulfide *in-situ* with AIBN at 80 ^{o}C and create CPDB which could then mediate RAFT synthesis of polymer at 65 ^{o}C (Figure 3-6 (p.86), Figure 3-9 b). For styrene, using the ratio [St]:[disulfide]:[AIBN] = 100:0.5:0.7, 1.5 hour was sufficient to create RAFT agent *in-situ* (Figure 3-8, p.88). The temperature was varied for the bulk (entries 2, 3, 7 in Table 3-3, p.79; entry 5 in Table 3-4, p.80), in the presence of solvents such as ethyl acetate (entries 1, 4, 8, 10 in Table 3-3) and n-butanone (entries 5, 6, 9, 11 in Table 3-3; entries 1-4, 6 in Table 3-4). Bulk polymerisations were not easy to control and a few of the trials ended with lack of controllability where it was clear that reaction did not progress in a controlled fashion, there was a short delay time to start with but then polymerisation occurred similar to free radical polymerisation with sudden increase in molecular weight and solidification. These anomalies were not a surprise as it was previously reported and discussed in literature for similar problems with dithiobenzoates RAFT mediated reactions where phenyl group was present as a stabilisation group.[74,198,210] In entry 14 (Table 3-3) and entries 7- 8 (Table 3-4), free radical polymerisations (FRP) of MMA (in n-butanone) and St (in n-butanone, bulk) are presented. There was no control over polymerisation; resultant polymers had PDI's greater than 1.7 up to 3.2, with sudden increase and higher molecular weight than polymers prepared by RAFT methods.

For *in-situ* solution polymerisation of MMA, where a higher ratio of AIBN (RAFT:AIBN = 1:10) was used (entry 12, Table 3-3) during an inhibition time, no polymerisation was visible up to first 1.5 hour and then the reaction progressed very fast and suddenly solidified at 3 hours (entry 12, Figure 3-7). Resultant polymers had low molecular weight, high conversion and PDI = 1.4. For entry 13 (Table 3-3), the delay time of the polymerisation was much longer due to higher concentration of precursor of RAFT agent, reaction between monomer and radical started after 8 hours, and reached almost 60% conversion in 10 hours, with a low molecular weight less than 2000 g/mol. Reaching up 24 hours of the reaction time, molecular weight increased slightly with conversion 85% (entry 13, Figure 3-7). Reaction was stopped after 48 hours, solidification did not occur, yielded polymer had low average molecular weight and PDI below 1.3. Increasing concentration of RAFT agent in system decreases a molecular weight of resultant polymers.

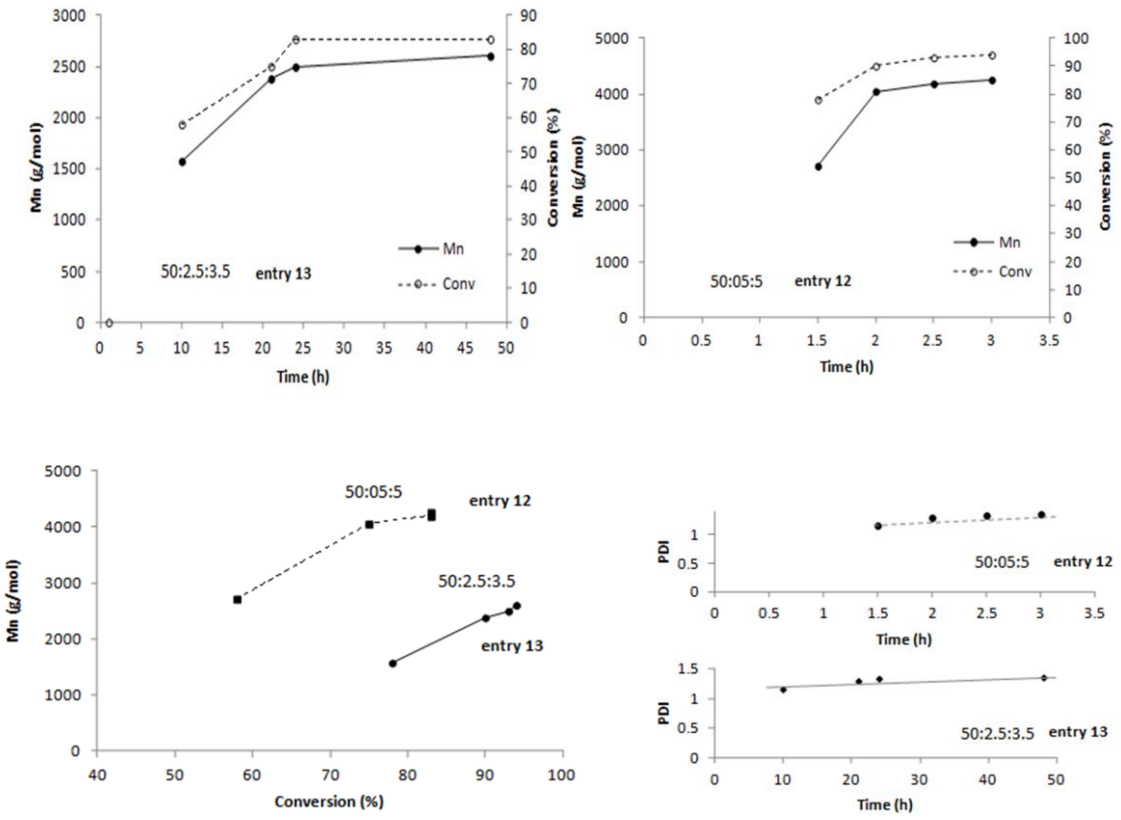


Figure 3-7: Kinetic curves for *in-situ* RAFT homopolymerisation of MMA. Entry 12 (50:0.5:5) and entry 13 (50:2.5:3.5), Table 3-3.

a) b)

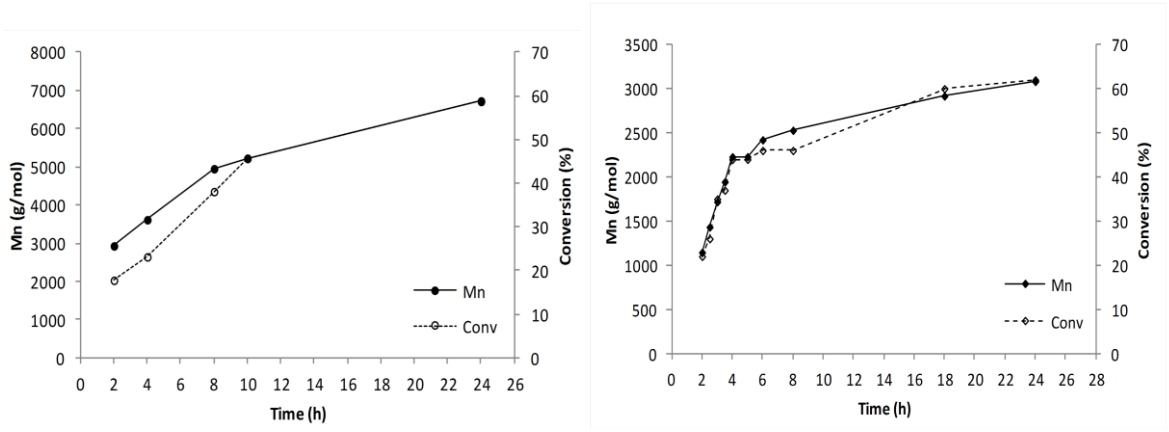


Figure 3-8: Kinetic curves for *in-situ* RAFT of St. Entry 1 (a), Entry 2 (b), Table 3-4.

a) b)

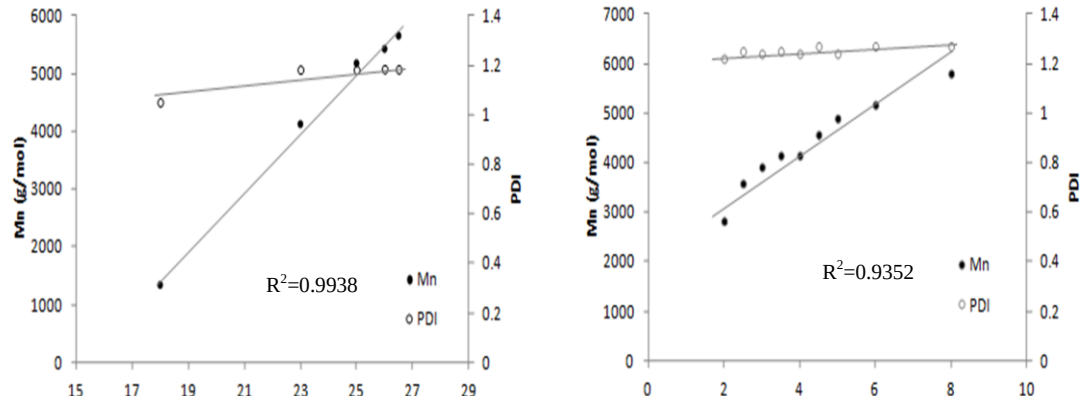


Figure 3-9 Kinetic curves for *in-situ* RAFT of MMA in n-butanone. Entry 9 (a), entry 5 (b), Table 3-3.

3.1.7. Nuclear Magnetic Resonance characterisation

The proton NMR spectra indicate that RAFT agents were incorporated into the polymer chains (typical ^{1}H NMR for resultant polymers are presented in Figures 3-10 and Figure 3-11, p.90). The signals at 7.3-8 ppm correspond to protons which belong to aromatic part of RAFT agent, which remain in the structure of resultant polymers. Number-average molecular weight (*Mn*) for polymers can be calculated by the use of integration of proton peaks and end-group analysis. Ability to detect end-group protons and sensitivity of the NMR instrument determines the upper limit permitting accurate measurement of *Mn*, which can be calculated when ratio of protons on the end-groups to protons on the polymer chain can be determined. It is important that polymer signals does not overlap with the end group, also that the end group signal is well resolved and the integration is reliable. By proper allocation of the end-group proton signals (approximately 7.4, 7.5, 7.9 ppm and 0.8-1.5 ppm) integral per proton can be calculated as a sum of end group proton integrals divided by number of protons in the two end groups.

Number of repeating monomer units (n) can be counted in next step and when determination of the number of repeating units is done, calculation of *Mn* can be estimated by the summation of the formula weight of the polymer.

$$\boldsymbol{Mn = (Fw\ end\ groups) + (Fw\ repeating\ unit) * (n)} \qquad \textbf{E.q: 3-1}$$

Fw stands for formula weight and n for number of repeating units.

In RAFT polymerisation, not all polymer chains are terminated with RAFT end group.[211,212] This could have an effect on the usage of prepared polymer as a macro-RAFT chain transfer agent in the next step. The leaving and stabilisation groups of the RAFT agent are able to control the polymerisation of all monomers used in the synthesis. If one of the groups is inappropriate for the control over the preparation of macro-RAFT-CTA or the control over further chain extension, the properties of the resultant polymer might be lost as a consequence of inefficient macro-RAFT chain transfer agent.[3]

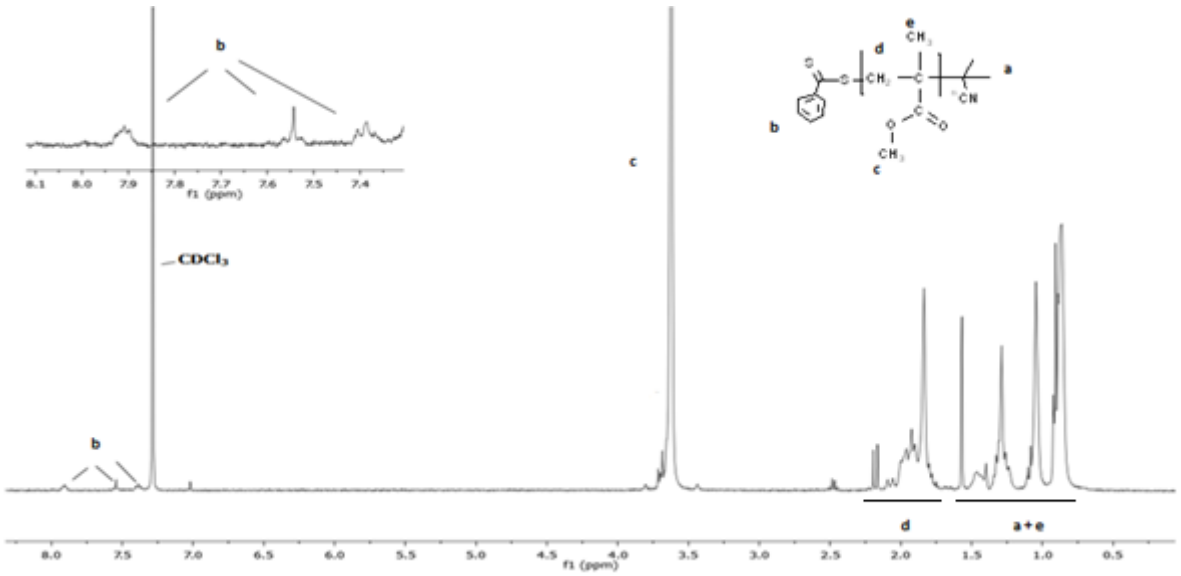


Figure 3-10: Typical ^{1}H NMR spectrum of PMMA prepared *via in-situ* RAFT process (entry 5, Table 3-3).

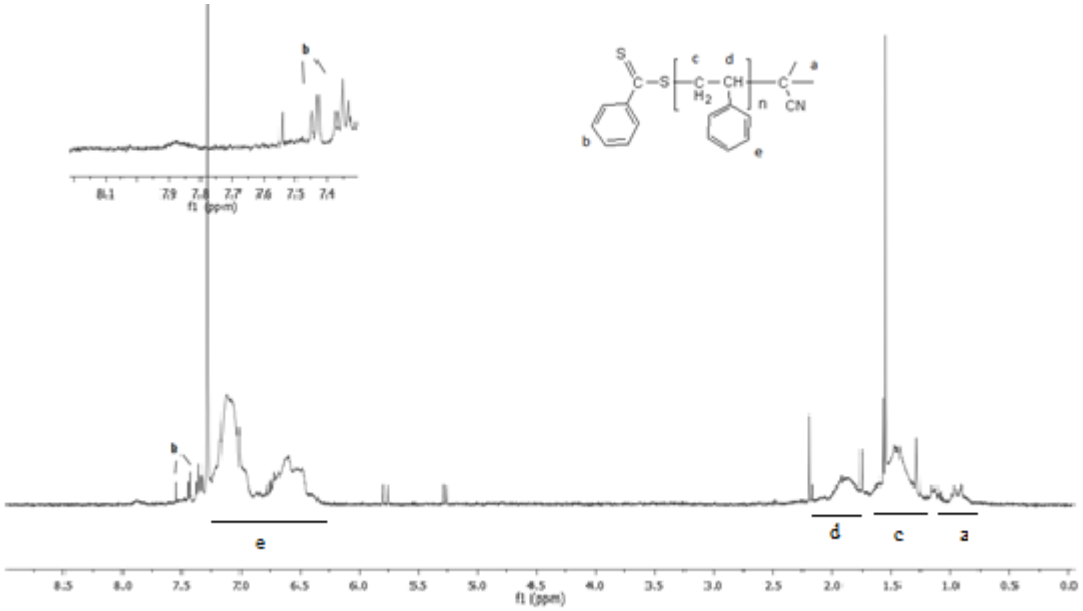


Figure 3-11: Typical ^{1}H NMR spectrum of PSt prepared *via in-situ* RAFT process (entry 3, Table 3-4).

3.1.8. Chain extension using Poly(methyl methacrylate) and Polystyrene macro Reversible-Addition Fragmentation Chain Transfer agents

Because the resultant polymers prepared in this study possess RAFT moieties in the structure, they have a potential to be used as a macro-RAFT agent and can aid the preparation of block copolymers through the polymerisation with a second monomer.

The macro-RAFT agent plays very similar role to the normal RAFT agent used for homopolymerisation described in previous section. In block polymerisation, macro-RAFT agent contains same Z- group as normal RAFT agent and leaving R- group which in fact is the polymeric chain. The ability of the leaving group to be released to the system and then further restart polymerisation is vital for successful block polymerisation with use of a macro-RAFT agent. Styrene was polymerised in the present of a PMMA macro-CTA prepared by *in-situ* and conventional RAFT methods (Table 3-5). A clear increase of molecular weight was observed after a few hours in each entry, this indicated that the chain extension had occurred. It is known that RAFT method does not stop the formation of dead chains and this is one of the explanations why we can see some shoulders on the GPC traces. The mechanism of the RAFT process explains the formation of small defects during the synthesis of the block copolymers and this process is very difficult to avoid. The dead polymer chains, initiator-derived block copolymer or initiator-derived homopolymer and dead homopolymer can be created during the chain extension reaction.[3] Adjustment of the reaction conditions could decrease the formation of defects so that they would not affect the performance of RAFT polymerisation where macro RAFT-CTA is used.

Table 3-5: Chain extension results using PMMA as a macro-CTA (reaction conditions and molecular weight characteristics).

Entry	T (h)	Solvent	[St]:[PMMAmacro-RAFT]:[AIBN]	Mn^{a}, GPC (kg/mol) *Macro-RAFT*	Mn^{a}, GPC (kg/mol)	Mw^{a}, GPC (kg/mol	PDI	Conv (%)
1	52	n-butanone	100:1:0.2	6.2	9.4	14.7	1.6	60
2	52	n-butanone	100:1:0.2	4.5	9.5	12.9	1.4	60
3	53	n-butanone	50:1:0.2	4.5	7.0	11.2	1.6	85
4	38	n-butanone	100:1:0.2	8.3	13.6	19.2	1.4	61
5	53	n-butanone	100:5:0.2	8.3	17.4	32.0	1.8	75

Where solvent is used monomer volume ratio is 1:1. [a] Number-average molecular weight and Weight-average molecular weight estimated by GPC. Monomer conversion and Polydispersity estimated by GPC; In entries 1, 2, 3, the PMMA prepared by *in-situ* RAFT method as a macro RAFT-CTA, in entries 4, 5 PMMA macro RAFT agents were prepared from conventional RAFT polymerisation. Reactions were conducted at 65 ^{o}C.

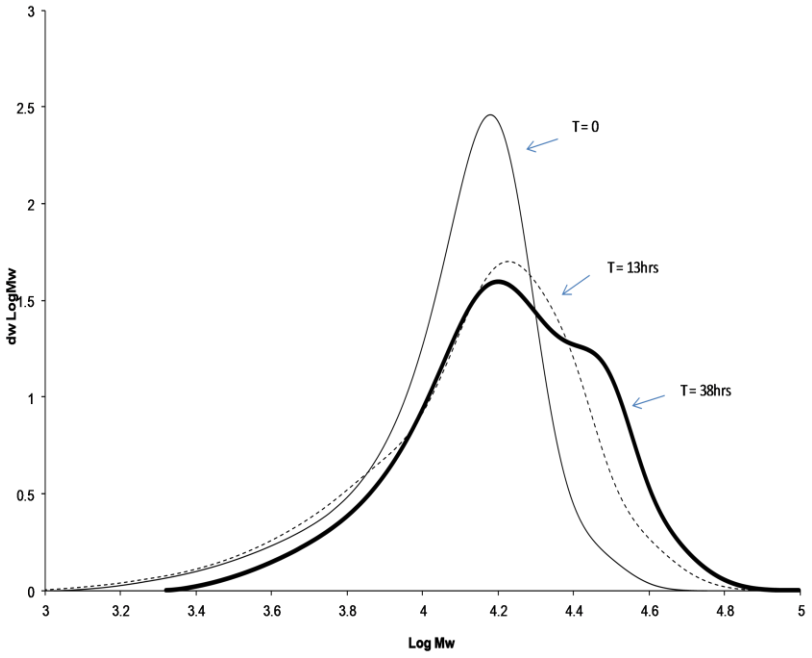
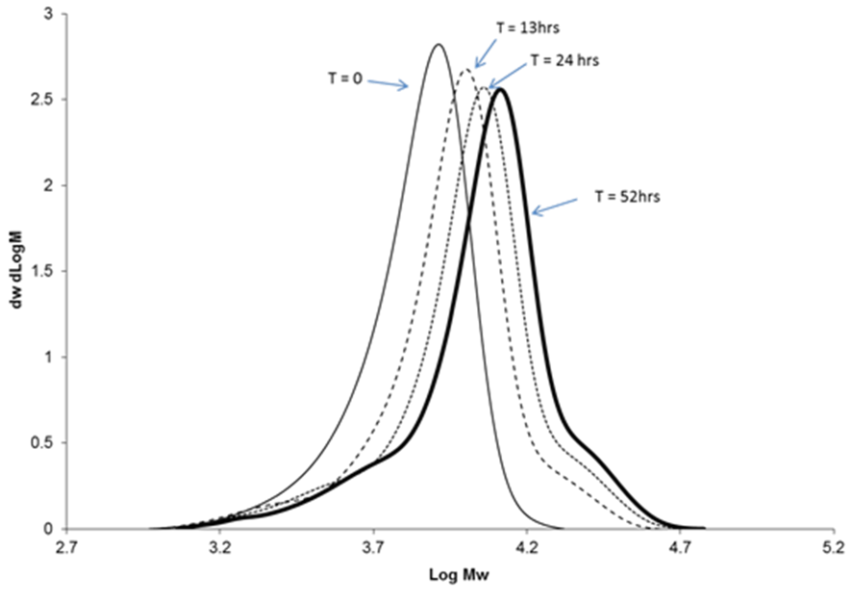


Figure 3-12: Chain extension at 60% conversion using PMMA macro-RAFT agent prepared by conventional RAFT, PMMA –b- Styren t = 38 hrs, Mn = 13.6, PDI = 1.4. (Feed ratio: [St]:[PMMAmacro-RAFT]:[AIBN] - 100:1:0.2, entry 4, Table 3-5)

a)



b)

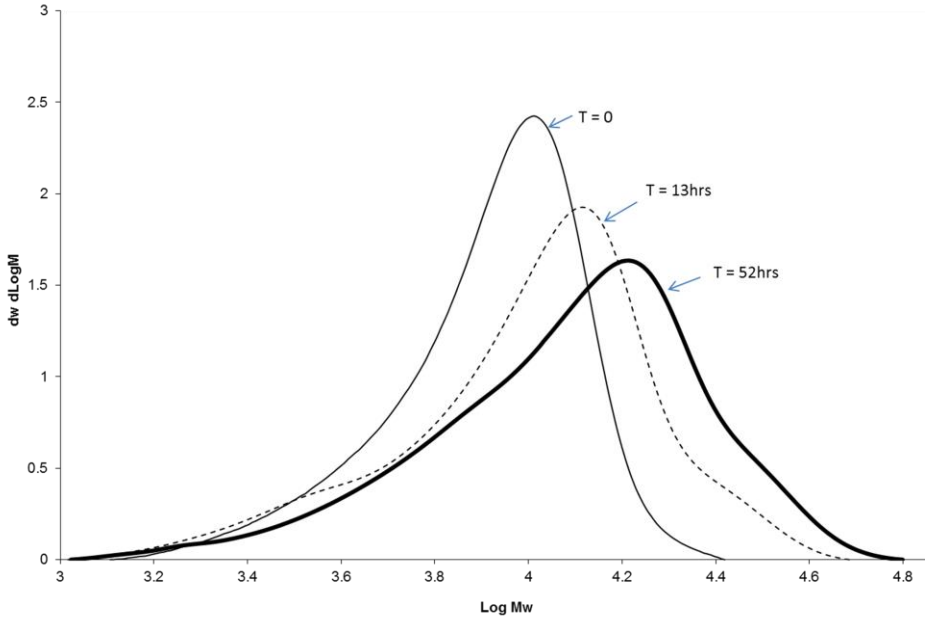


Figure 3-13: Chain extension at 60% conversion using PMMA macro-RAFT agent prepared by *in-situ* RAFT. (a) (entry 1, Table 3-5) PMMA –b- Styrene t = 52 hrs, Mn = 94.4, PDI = 1.6; (b) (entry 2, Table 3-5) PMMA –b- Styrene t = 52 hrs, Mn = 95.1, PDI = 1.4. (Feed ratio: [St]:[PMMAmacro-RAFT]:[AIBN] - 100:1:0.2)

The kinetics of one-pot and two-step *in-situ* RAFT polymerisations of MMA and Styrene were studied in comparison of conventional RAFT approach in different solvents and under different reaction temperatures. The results presented in section 3.1 showed a similar controllability of these two approaches, i.e. entry 1 in Table 3-1 (p.78) and entries 1 and 11 in Table 3-3 (p.79), where PDI and conversion of monomers in same conditions have no significant difference, although polymers prepared by *in-situ* methods had slightly lower molecular weights. The deviations of *Mn* value as to the difference between theoretical prediction and experimental measurement in Table 3-3 could be due to the systematic errors in the GPC calibration. The initial deviation of molecular weight could be also due to the AIBN fragmentation and reaction with monomers, which lead to short dead chains. However, we can conclude that this study has shown that the controllability of *in-situ* RAFT polymerisation remains at the similar level as the conventional RAFT approach.

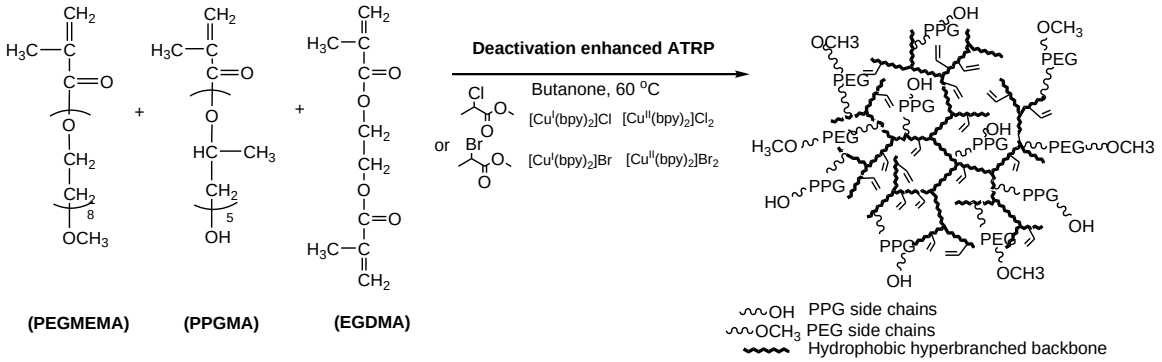
This *in-situ* route may be successfully applied in synthesis and produce polymers with relatively well controlled molecular weights. Importantly, this synthetic pathway is valuable but dithiobenzoate-mediated polymerisation presented in this section cannot be generalized for all RAFT processes without further tests as results might differ and will depend on initiators used and the leaving group (-R) of created *in-situ* RAFT agent.

3.2. Synthesis, characterisation and property evaluations of thermoresponsive hyperbranched polymers from *in-situ* Reversible-Addition Fragment Chain Transfer polymerisation

3.2.1. Introduction

Ethylene glycol dimethacrylate is classified as a multifunctional vinyl monomer (MVM) and in synthetic polymer chemistry is usually used to prepare crosslinked network structures. In EGDMA one ethylene glycol (OCH_2CH_2) is functionalized with 2 methacrylate groups. In a free radical polymerisation, the addition of only small amounts of MVM often leads to a crosslinked network.[89,88] In order to manage the control over the reaction so that the molecular weight and branched structure of the subsequent polymers can be achieved, controlled/living free radical polymerisations such as ATRP and RAFT were adopted for the synthesis of hyperbranched copolymers by copolymerisations of multifunctional monomers and vinyl monomers.[90,91] As mentioned in chapter 1, applying MVMs as branching agents to prepare controlled complex hyperbranched architectures was reported for a first time by Sherrington et.al..[93,94,95,96] Catalytic chain transfer polymerisation (CCTP) was used to homopolymerise or copolymerise EGDMA to form dendritic/hyperbranched polymers.[213] Soluble dendritic and single cyclized knot polymer were also reported recently and achieved through successful homopolymerisations of MVM by deactivation enhanced atom transfer radical polymerisation (de-ATRP) and RAFT polymerisation.[107, 108, 109, 110,214]

The successful copolymerisation of PEGMEMA, PPGMA and EGDMA was carried by the use of a one-step de-ATRP approach where hydrophilic PEGMEMA and hydrophobic PPGMA were used as the vinyl monomers, hydrophobic EGDMA as the branching agent.[23,161] These hyperbranched PEGMEMA-PPGMA-EGDMA copolymers have thermosensitive and photocrosslinkable properties and have demonstrated promising potentials for tissue engineering and drug delivery applications.



Scheme 3-1: Thermoresponsive hyperbranched polymers synthesized *via* de-ATRP of monovinyl monomers (PEGMEMA and PPGMA) and divinyl monomer (EGDMA).[23]

Lower critical solution temperature (LCST) of the copolymers prepared by de-ATRP were in the range from 20 to 40 ^{o}C and demonstrated high levels of branching (30-50 mol %) and vinyl functionality (5-25 mol %). The photocrosslinking property of the materials has been investigated using a UV system attached to the rheometer which was used to evaluate mechanical properties. Cytotoxicity assessments (Live/Dead staining and the Alamar Blue cell metabolism assay) were done using mouse C2C12 myoblast cells at concentrations less than 1 mg/mL and confirmed their cytocompatibility in vitro.[161] Deactivation enhanced ATRP was further developed for the preparation of thermoresponsive and photocrosslinkable hyperbranched polymers in work of Dong et.al. by the copolymerisation of PEGMEMA, 2-(2-methoxyethoxy) ethyl methacrylate and EGDMA, where Cu^{II}/Ligand and a small amount of reducing agent L-ascorbic acid were used to generate Cu^{I} *in-situ*.[215] Hyperbranched 2-(dimethylamino) ethyl methacrylate (DMAEMA)/EGDMA copolymer as a highly effective gene delivery vector was also prepared through this newly developed *in-situ* de-ATRP.[216] Moreover, new i*n-situ* formed hydrogel from PEG based multifunctional hyperbranched copolymers of polyethylene glycol diacrylate (PEGDA) and polyethylene glycol methyl ether methacryale (PEGMEMA) was also recently developed by conventional RAFT polymerisation approach[112] using 2-cyanoprop-2-yl dithiobenzoate as a RAFT agent, where high degree of multi-acrylate functional groups were incorporated into the hyperbranched structure and potentially could be used as attachment sites to which bioactive motifs could be conjugated. Moreover, the high degree of free vinyl functional groups (22% molar ratio) and branching degree (of 24%) result in the copolymer being easily crosslinkable with a thiol-functional crosslinker. The amount of vinyl groups and

branching degree can be easily tailored by changing the molar ratio of PEGDA and PEGMEMA in polymer synthesis.[112]

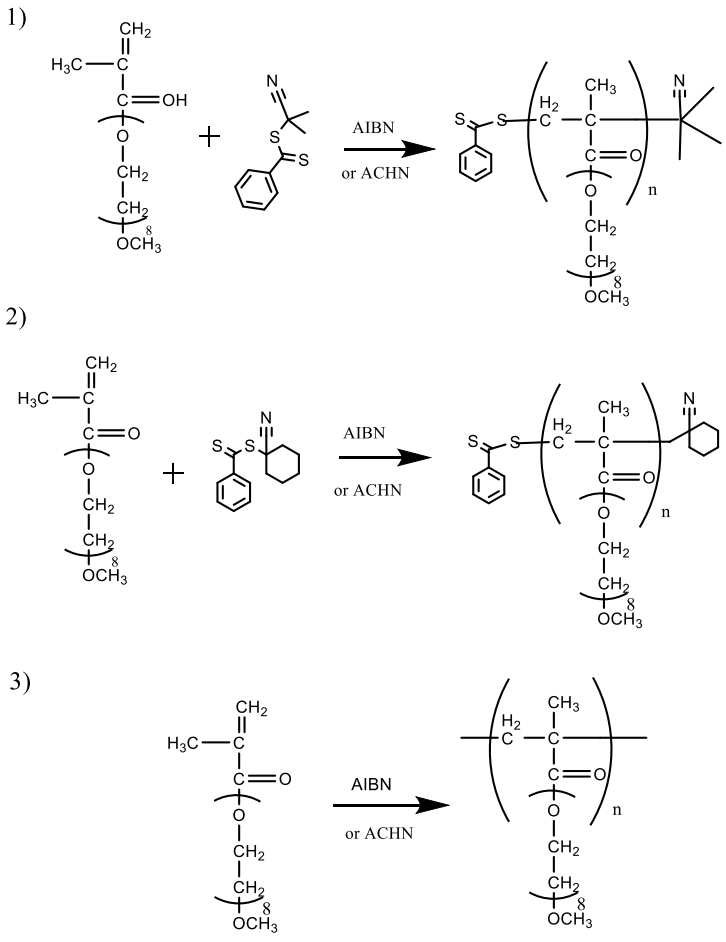
In this section, the synthetic method for thermoresponsive hyperbranched polymers composed of PEGMEMA-PPGMA-EGDMA was extended from ATRP to RAFT and *in-situ* approach. RAFT polymerisation is more versatile than ATRP approach because it does not require metal catalysts and is applicable to a wider range of vinyl monomers. Moreover, the RAFT agent segments in the resultant structure can be readily reduced to thiols, which are very useful functional groups for further post functionalisation through thiol-ene click chemsitry.[217,218]

3.2.2. Evaluation of Reversible-Addition Fragment Chain Transfer agents and initiators - initial study

As an initial study for the preparation of thermoresponsive hyperbranched PEG based polymers, we examined the performance of two radical initiators 1,1'-azobis-cyclohexane-carbonitrile (ACHN) and 2,2'-azobis(isobutyronitrile) (AIBN) in connection with two disulfide based RAFT agents (CPDB and ACBN, synthesized in house according to sections 2.3.2-3, chapter 2). To our knowledge, RAFT agent (1-cyano-1-cyclohexyl dithiobenzoate, ACBN) is not commonly used in the literature, while 2-cyanoprop-2-yl dithiobenzoate (CPDB) is often employed. For the needs of this study the materials were tested on homopolymerisation of poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, *Mn* = 475). RAFT and FRP of PEGMEMA were conducted in n-butanone; syntheses were carried as parallel reactions.

Four conventional RAFT reactions and two FRPs (Scheme 3-2, entries 1 to 6, respectively) were tested under different reaction conditions:

1) PEGMEMA and AIBN with CPDB
2) PEGMEMA and ACHN with ACBN
3) PEGMEMA and AIBN with ACBN
4) PEGMEMA and ACHN with CPDB
5) PEGMEMA and AIBN
6) PEGMEMA and ACHN



Scheme 3-2: Conventional RAFT reactions (1-2) and two FRPs (3) of PEGMEMA. Radical initiators ACHN and AIBN in connection with two disulfide based RAFT agents CPDB and ACBN.

The aliquots taken for GPC analysis were dissolved in THF (carrier solvent), filtrated and injected into the column with a continuous flow of THF. Analysed samples (in solution) passed through columns. As explained (section 2.4.1.1, chapter 2) the size of analysed molecules determinate whether molecules can or cannot penetrate into the pores of the sieves. Large molecules cannot pass through the pores so they move quickly through the columns through empty spaces between the sieves. Small molecules retain on the columns for longer time, as they diffuse into the network of the pores. Due to above, we expected to see the evidence of PEGMEMA polymerisation on GPC trace.

In the first trial all the experiments mentioned above (entries 1 to 6 in Table 3-6, p.101) were run at 60 ^{o}C. Free radical polymerisation (entries 5 and 6 in Table 3-6) showed visually evidence of polymerisation just after 3 hours in both cases when solutions became more viscous and difficult to aliquot. Reaction mixture became very sticky in 3.5 hrs and rapidly gelled in case of FRP homopolymerisation of PEGMEMA in the presence of AIBN, and gelled in 4 hrs, in case of homopolymerisation of PEGMEMA in the presence of ACHN (from the start point). This longer gelation time was expected for the reaction with ACHN as an initiator.

Parallel RAFT polymerisations running under the same conditions were much slower (amount of radical initiator, 1%). The visible evidence of homopolymerisation was noticed in 23 hrs for the RAFT reaction of PEGMEMA and AIBN with CPDB, 27 hrs for PEGMEMA and ACHN with ACBN, where reaction mixtures started to change the viscosity and aliquots were more difficult to take. RAFT reactions in this experiments set up gelled at 48 and 52 hrs respectively (from the start point). Although gelation occurred, evidence of this homopolymerisation was not confirmed on the GPC trace. Samples taken in selected times e.g. zero T = 0 and then 1 h, 3.5 hrs, 4hrs, 17hrs, 27 hrs, 41 hrs and 52 hrs did not cause a signal able to be detected and recorded on GPC trace.

In all presented experimental conditions, just macromer of PEGMEMA (*Mn* = 475) was recorded. Conversion of macromer to polymer was not recorded. Due to these unaccepted results both reactions of FRP and RAFT homopolymerisation were set up again with a slight change of the conditions: amount of radical initiator (5% this time) and increase of the temperature from 60 ^{o}C (entry 1-6 in Table 3-6) to 70 ^{o}C (entry 7-12 in Table 3-6). In this attempt we focused on the synthesis technique and on the GPC sample preparation

method to scrupulously avoiding any oxygen contamination. GPC samples were taken very accurately and after introducing air, thickened by evaporating solvent (n-butanone), than diluted with THF. The temperature and amount of initiator increased, so reaction expected to be even faster.

As a result, in FRP synthesis, visual change of viscosity and stickiness was observed after 1 h and material completely gelled in 1.5 and 2 hrs, as expected gelation occurred quicker, in shorter time. RAFT reaction gelled in 17 hrs in case of PEGMEMA and AIBN with CPDB and in 20 hrs in case of PEGMEMA and ACHN with ACBN. As expected, reactions occurred faster than in the previous experiments at 60 ^{o}C. Importantly, we noticed that in reactions (entry 1-2 and 7-8 in Table 3-6, p.101) in which used initiator was the same as the initiator used to create RAFT agent prior to polymerisation, run faster than in case of reactions (entry 3-4 and 9-10 in Table 3-6) wherein the initiator used for preparation of RAFT agent was different than the one used in homopolymerisation. Moreover, in repeated experiments, GPC also did not show evidence of PEGMEMA (*Mn* = 475) conversion to higher molecular weight polymer. High molecular weight Poly(PEGMEMA) was not detectable on employed GPC system (e.g. Figure 3-14 , Figure 3-15, Table 3-6).

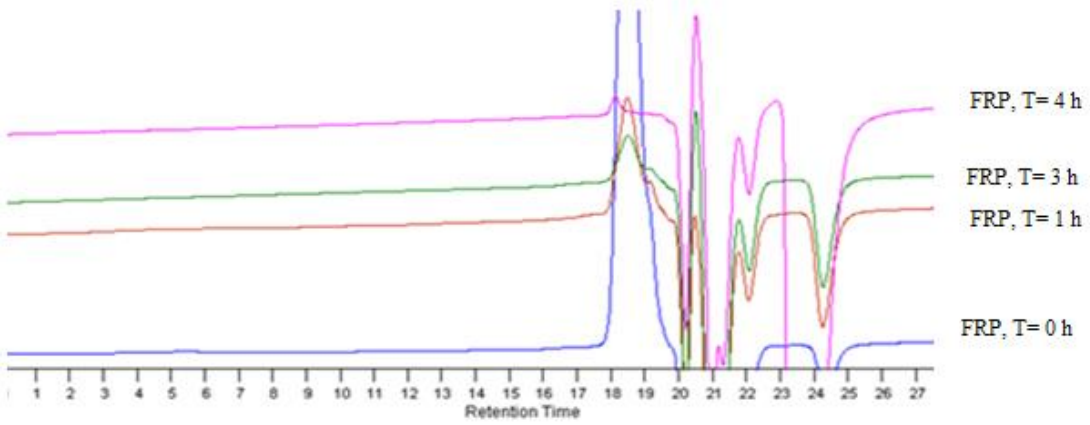


Figure 3-14: GPC Overlay of chromatograms for FRP homopolymerisation of PEGMEMA with AIBN, solvent n-butanone, no advancement of molecular weight.

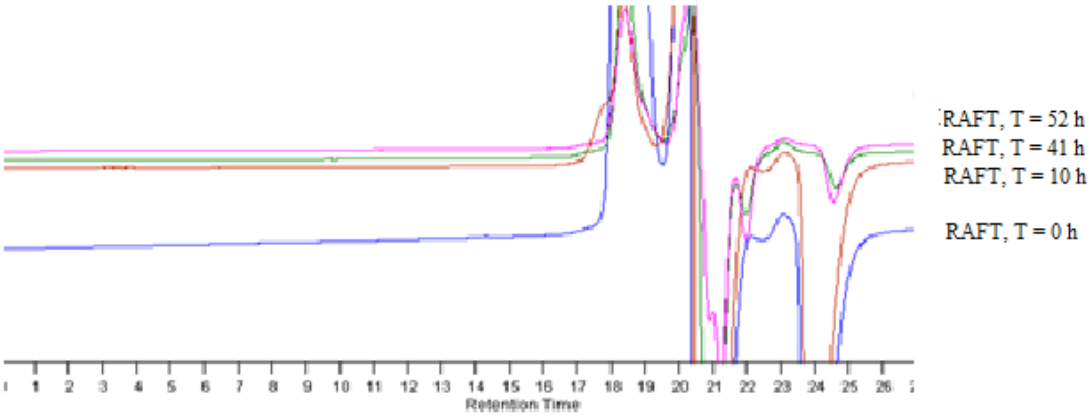


Figure 3-15: GPC Overlay of Chromatograms for RAFT homopolymerisation of PEGMEMA and ACHN with CPDB, in solvent n-butanone, no advancement of molecular weight.

Table 3-6: Homopolymerisation of PEGMEMA: RAFT reactions (1-4, 7-10) and FRP (5-6, 11-2) tested at 60 °C (entry 1-6) and 70 °C (entry 7-12).

Entry	[M] : [I] : [R][a]	SR[b] (v/v)	Cov[c] (%)	GPC RI		G[f] (min)
				Mw[d] (kg/mol)	PDI[e]	
1	100:1:1	1:1	-	-	-	1380
2	100:1:1	1:1	-	-	-	1620
3	100:1:1	1:1	-	-	-	2460
4	100:1:1	1:1	-	-	-	3120
5	100:1:0	1:1	-	-	-	210
6	100:1:0	1:1	-	-	-	240
7	100:5:1	1:1	-	-	-	1020
8	100:5:1	1:1	-	-	-	1200
9	100:5:1	1:1	-	-	-	1500
10	100:5:1	1:1	-	-	-	2760
11	100:5:0	1:1	-	-	-	90
12	100:5:0	1:1	-	-	-	120

Entry no is associated with experiments 1-6 discussed in this section; [a] Macromer molar ratio (PEGMEMA) : Initiator (I): RAFT agent; [b] Volume ratio of monomer and solvent (n-butanon) (v/v); [c] Monomer conversion estimated by GPC; [d] Weight-average molecular weight; [e] Polydispersity index (M_w/M_n); [f] Gel time, determined by visual observation;

Due to above reported issues with the detection of Poly(PEGMEMA), PEGMEMA was substituted with MMA and evaluation of RAFT agents with relevant initiators was continued as follows. Four conventional RAFT reaction systems and two FRP were tested at 60 ^{o}C (data is presented in Table 3-7, entries 1-6 respectively):

1) MMA and AIBN with CPDB
2) MMA and ACHN with ACBN
3) MMA and AIBN with ACBN
4) MMA and ACHN with CPDB
5) MMA and AIBN
6) MMA and ACHN

Table 3-7: Homopolymerisation of MMA: RAFT reactions (1-4) and FRP (5-6) tested at 60 ^{o}C.

Entry	[M] : [I] : [R][a]	SR[b] (v/v)	Cov[c] (%)	GPC RI		G[f] (min)
				Mw[d] (kg/mol)	PDI[e]	
1	100:1:1	1:1	88	10	1.2	420
2	100:1:1	1:1	60	40	1.1	720
3	100:1:1	1:1	98	15	1.7	480
4	100:1:1	1:1	55	30	1.6	760
5	100:1:0	1:1	85	300	1.4	210
6	100:1:0	1:1	70	50	2.1	300

Entry no is associated with experiments 1-6 discussed in this section; [a] Monomer molar ratio (MMA) : Initiator (I): RAFT agent; [b] Volume ratio of monomer and solvent (n-butanon) (v/v); [c] Monomer conversion estimated by GPC; [d] Weight-average molecular weight; [e] Polydispersity index (M_w/M_n); [f] Gel time, determined by visual observation; reaction temperature 60 ^{o}C.

The GPC data from FRP of MMA (entries 5 and 6 in Table 3-7) demonstrated that monomer converted to PMMA in the presence of AIBN was very fast and conversion reached over 62% in the first hour. Reaction was stopped at 3.5 hrs, with 85% conversion, PDI = 1.4. Whereas in the FRP where ACHN was used as an initiator a visual change of viscosity was observed in the second hour and reaction was stopped at 5 hrs, with 70% conversion, PDI = 2.1. Free radical homopolymerisation of MMA at 60 ^{o}C resulted with

higher conversion, shorter reaction time and lower PDI in the presence of AIBN. Homopolymers synthesized by FRP in the presence of ACHN resulted in polymers with a wider PDI, longer reaction times and lower molecular weights, and lower conversions.

The RAFT reactions (entries 2 and 3 in Table 3-7, p.102) where ACBN was used as a RAFT agent and ACHN and AIBN as an initiators resulted in polymers with lower molecular weight compared to polymers from free radical polymerisations. The GPC results showed that PMMA was obtained from RAFT polymerisation (entry 2 in Table 3-7), using ACBN as a RAFT agent and ACHN as a initiator, with reasonable control of the molecular weight (40 K), 60% conversion and a narrow PDI = 1.1. PMMA from RAFT polymerisation using ACBN as a RAFT agent and AIBN as an initiator (entry 3 in Table 3-7), resulted in polymer of PDI = 1.7, 98% conversion, molecular weight (15 K), the reaction was reasonably controllable through time progression. Reaction represented by entry 2 (Table 3-7) took longer than reaction represented by entry 3 (Table 3-7). The RAFT reactions (entries 1 and 4 in Table 3-7) where CPDB was used as a RAFT agent and AIBN and ACHN as an initiators delivered information that use of AIBN allowed polymer with lower PDI then ACHN (1.2 and 1.6, respectively). Moreover use of CPDB in connection with AIBN allowed shorter synthesis time, with higher conversion.

RAFT agent ACBN with AIBN as an initiator (entry 3 in Table 3-7) did not control the reaction as good as with ACHN as an initiator (entry 2 in Table 3-7) in polymerisation of MMA. Likewise CPDB with ACHN (entry 4 in Table 3-7) did not control the reaction as good as with AIBN (entry 1 in Table 3-7).

Above results indicated that PMMA was successfully synthesized with convincingly good control by each of the RAFT reactions set up (1-4, represented by entries 1-4, Table 3-7). However using same initiator in polymerisation process as in the RAFT agent synthesis provided better control over reaction and resulted polymers with lower PDI and higher molecular weights (entries 1 and 2, Table 3-7).

3.2.3. Synthesis and characterisation of thermoresponsive hyperbranched polymers *via in-situ* Reversible-Addition Fragmentation Chain Transfer copolymerisation

The thermoresponsive hyperbranched polymers of PEGMEMA-PPGMEMA-EGDMA with multiple methacrylate groups and RAFT agent residues were prepared by two methods: conventional and *in-situ* RAFT polymerisation approaches. In the conventional method, 2-cyanoprop-2-yl dithiobenzoate (CPDB, RAFT agent) was prepared in advance by multistep synthesis, then purified by column chromatography (section 2.3.1 and 2.3.2, p.45, chapter 2) and finally used in copolymerisation. The *in-situ* method was developed as a one-pot and two-stage reaction with the vinyl monomers as described in section 3.1, and aimed to simplify the RAFT copolymerisation of novel polymers. The procedure for the *in-situ* synthesis of hyperbranched copolymers of PEGMEMA-PPGMEMA-EGDMA is described in chapter 2, section 2.3.9. The reactions were carried out in n-butanone; the resultant PEGMEMA-PPGMEMA-EGDMA samples were purified by precipitation in hexane and dialysis in water. The polymers were well characterized and the thermoresponsive behaviour was studied. The main aim of this work was to use the *in-situ* RAFT process for the synthesis of PEGMEMA-PPGMEMA-EGDMA.

A series of reactions by the use of developed *in-situ* (Scheme 2-10, p.55; entries 1-6 in Table 3-8) method were run and as a result hyperbranched PEGMEMA-PPGMA-EGDMA polymers were successfully prepared. To compare, a set of conventional RAFT reactions (B in Scheme 2-10) were conducted exactly under the same conditions as entries 4-6 in Table 3-8. The experimental data demonstrated that polymerisations and further chain extension polymerisation were well controlled.

Free radical polymerisations of PEGMEMA-PPGMA-EGDMA with AIBN and ACBN as initiators were also conducted for comparison. In first case, reaction gelled within first 15 min, where in second case this process took average 60 min to gel. In initial studies, AIBN and CPDB were selected; this initiator and RAFT agent was used in the thermoresponsive hyperbranched polymer synthesis.

Table 3-8: Reaction conditions and properties of thermoresponsive hyperbranched polymers from *in-situ* RAFT copolymerisations of PEGMEMA, PPGMA and EGDMA.

Entry	f[a]	Cov[b] %	GPC RI		GPC MALLS			^{1}H NMR		
			Mw[c] kg/mol	PDI[d]	Mw[c] kg/mol	PDI[d]	Plot[e]	DOB[f] mol %	DBC[g] mol %	F[h]
1	25/65/10	51	49.0	2.59	170.0	1.77	0.39	3.7	15.3	30/51/19
2	30/40/30	60	57.5	2.89	159.7	2.53	0.37	17.7	22.2	32/28/40
3	25/45/30	60	78.4	3.53	288.1	3.62	0.28	17.4	21.5	28/33/39
4	35/35/30	18	7.7	1.69	18.7	1.45	-	34.5	23.1	26/16/58
5	35/35/30	47	24.5	2.47	29.6	1.70	-	25.4	27.2	28/19/53
6	35/35/30	61	58.9	3.32	405.9	2.68	0.34	21.6	21.0	36/22/42

[a] Monomer feed molar ratio PEGMEMA:PPGMA:EGDMA;[b] Monomer conversion estimated by GPC; [c] Weight-average molecular weight; [d] Polydispersity; [e] Slope of conformational plot; [f] Degree of Branching; [g] Double Bond Content, [h] Polymer Composition [PEGMEMA]:[PPGMA]:[EGDMA]= m:n:(r+p); Polymerisation conditions: 65 ^{o}C in n-butanone; solvent and monomer volume ratio is 1:1; the molar ratio of [total monomer]/[RAFT disufide]/[AIBN]=50/1/1.4.

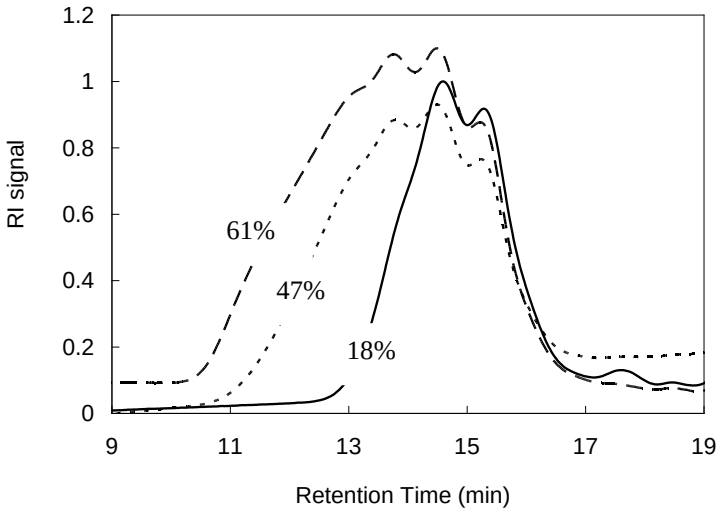


Figure 3-16: Copolymerisation of PEGMEMA, PPGMA and EGDMA by *in-situ* RAFT polymerisation (entries 4, 5, 6 in Table 3-8) at different monomer conversions. GPC traces for the signals from RI detector.

Results demonstrated that the *in-situ* RAFT polymerisation of PEGMEMA, PPGMA and EGDMA showed the similar controllability to the conventional RAFT copolymerisation approach as the GPC traces obtained for *in-situ* RAFT polymerisations were similar to those obtained for the conventional RAFT polymers with clear shift from the long retention time to the short retention time, which indicate increase of molecular weight with the monomer conversion (Figure 3-16, p.105).

As it was already described in section 3.1 using the example of methyl methacrylate and styrene, in the *in-situ* RAFT copolymerisation there is a competition between two reactions. The two reactions are (1) AIBN initiates the polymerisation of monomers and (2) AIBN reacts with bis(thiobenzoyl) disulfide to form 2-cyanoprop-2-yl. The same pattern was observed with the copolymerisation of PEGMEMA, PPGMA and EGDMA. In the presence of the studied monomers the disulphide was fully converted into RAFT agent within 5 hours at 80 ^{o}C, monitored by TLC (thin layer chromatography). Therefore, after this stage the reaction temperature was reduced to 65 ^{o}C to allow the RAFT polymerisation progress for a desired reaction time, while the reactions were continually monitored by GPC.

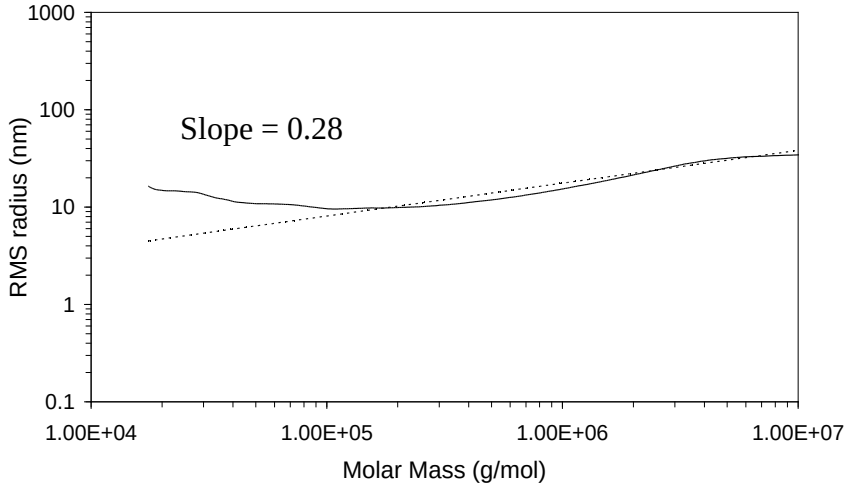


Figure 3-17: A typical conformational plot for the hyperbranched polymer (monomer feed molar ratio PEGMEMA:PPGMA:EGDMA - 25/45/30, entry 3 in Table 3-8).

The conformation plots (as seen in Table 3-8, p.105, and e.g. presented in Figure 3-17) of the copolymers have the slope values below 0.5, which indicates the hyperbranched

structure of polymers. The hyperbranched structures of the copolymers were also confirmed by ^{1}H NMR (Figure 3-18).

The composition of the copolymers (h, Table 3-8, p.105), represented by m, n, r and p values in the chemical structure, is calculated from the integral data of ^{1}H NMR. The reactivity of the monomers influences the final composition of the copolymer, which commonly differs from the initial feed composition of the monomers.

The characteristic peaks at chemical shifts of 6.1 and 5.6 ppm come from to the vinyl functional groups in the copolymer and the others are assigned as indicated in Figure 3-18.

The chemical shift between 7.4-7.8 ppm in ^{1}H NMR spectrum confirmed the existence of dithiobenzoyl functional groups within the polymer structure. Integrating m, n, r and p peaks allows us to determine copolymer composition.

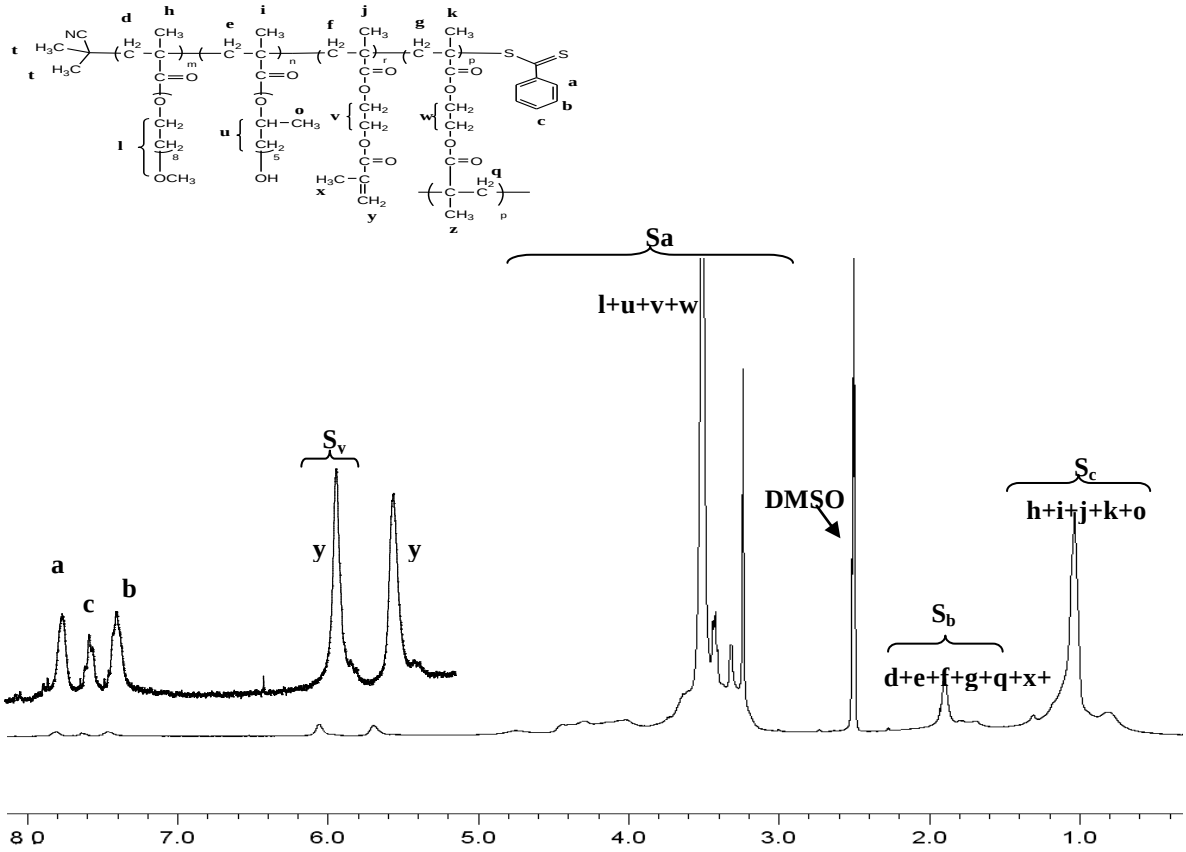


Figure 3-18: ^{1}H NMR of PEGMEMA-PPGMA-EGDMA copolymer (monomer feed ratio - PEGMEMA:PPGMA:EGDMA -30/40/30, entry 2 in Table 3-8) prepared *via in-situ* RAFT copolymerisation.

Equations (E.q: 3-1 to E.q: 3-4) outline the calculations based on polymers structure, number of protons and NMR integration (Figure 3-18):

$$\mathbf{r} = \boldsymbol{Sy} = \mathbf{1} \qquad \textbf{E.q: 3-1}$$

$$\boldsymbol{n} = (\mathbf{2}\boldsymbol{Sc} - \mathbf{3}\boldsymbol{Sb})/\mathbf{30} \qquad \textbf{E.q: 3-2}$$

$$\boldsymbol{m} = (\boldsymbol{Sa} - \boldsymbol{Sb} - \mathbf{13}\boldsymbol{n})/\mathbf{33} \qquad \textbf{E.q: 3-3}$$

$$\boldsymbol{p} = [\mathbf{3}\boldsymbol{Sb} - \mathbf{6}(\boldsymbol{m} + \boldsymbol{n})]/\mathbf{12} \qquad \textbf{E.q: 3-4}$$

The double bond content (DBC) and degree of branching (DOB) of the copolymers were calculated from the following equations:

$$\textbf{double bond content } \% = \frac{\boldsymbol{r}}{(\boldsymbol{m}+\boldsymbol{n}+\boldsymbol{r}+\boldsymbol{p})} \times \mathbf{100} \qquad \textbf{E.q: 3-5}$$

$$\textbf{branching degree } \% = \frac{\boldsymbol{p}}{(\boldsymbol{m}+\boldsymbol{n}+\boldsymbol{r}+\boldsymbol{p})} \times \mathbf{100} \qquad \textbf{E.q: 3-6}$$

The double bond content represents the mol percentage of EGDMA with free vinyl functional groups in the copolymer and the degree of branching represents the mol percentage of EGDMA as branching units in the copolymer. DOB is an important factor to characterize hyprebranched polymer. The degree of branching and double bond of the resultant hyperbranched copolymers can be tailored by changing the monomer feed composition.[111] From the data presented in Table 3-8, p.105, it is clear that the divinyl monomer EGDMA has a high reactivity in the conventional RAFT/*in-situ* RAFT copolymerisations which agrees with the results obtained from ATRP polymerisation of these monomers. As it is presented the water soluble hyperbranched copolymers of PEGMEMA, PPGMA and EGDMA with both high levels of branching (up to 34%) and vinyl functionality (up to 27%) were achieved by utilizing high concentrations of multifunctional vinyl monomer EGDMA (30% of the total feed monomers).

Multimodal molecular weight distributions were observed on GPC traces (Figure 3-16, p.105), and this could be explained by the mechanism of copolymerisation proposed for monovinyl monomer and divinyl branching agent (Figure 3-19). At the very early stage, the propagation of the copolymerisation of monovinyl monomer and divinyl branching agent, resulted in linear structures that subsequently formed branched and hyperbranched polymers. At the later stage of the polymerisation, the hyperbranched polymer growing chains combined in order to form large macromolecules and lead to a rapid increase in the molecular weight of the polymers resulting GPC traces with multimodal molecular weight distribution.

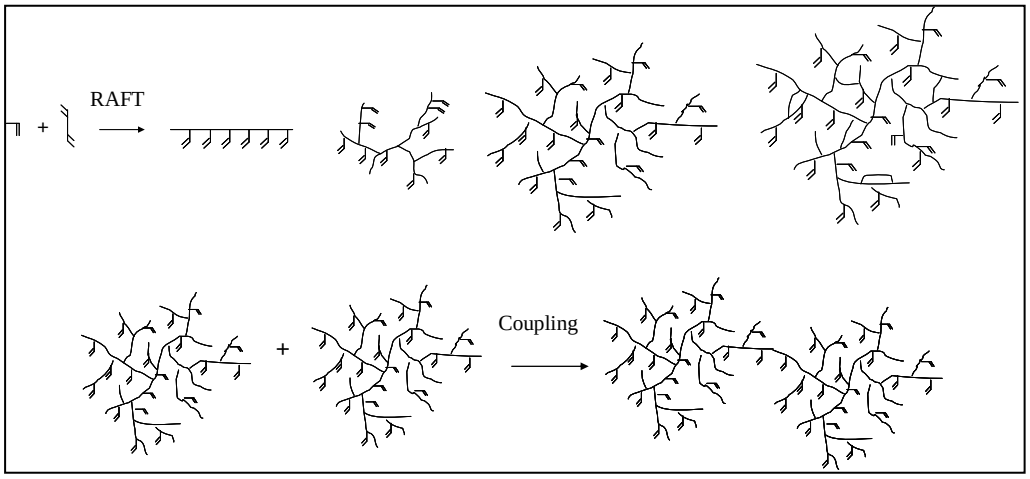


Figure 3-19: Proposed polymerisation mechanism for copolymerisation of monovinyl monomer and divinyl branching agent.[111]

The reversible equilibrium between growing chain radicals and RAFT agent intermediate radicals leads to a reduced chain radical concentration in the reaction mixture, subsequently leading to a decrease in the propagation rate and crosslinking rate. Free vinyl groups can be used for photopolymerisation or can be oxidized for further post functionalization. RAFT agent segments can be used for chain extension or be modified by aminolysis to introduce thiol functional groups.

3.2.4. Aminolysis of hyperbranched thermoresponsive polymers

The RAFT mechanism proceeds by inclusion of monomer units into the C-S bond of the RAFT agent in the presence of a radical initiator. When the process is completed there is an option to remove RAFT end groups.[219] A number of procedures are available to cleave thiocarbonylthio groups, the main methods of removal of the RAFT groups have been discussed many times in published reports.[220,219] One route is thru the reaction of thiocarbonythio groups with excess amine and results in the formation of a thiol end group that can be subsequently utilised in a number of reactions.[220,221] Both primary and secondary amines which act as nucleophiles can convert a thio- carbonylthio group to a thiol. The method has been adopted by various researches and used to cleave RAFT end groups from polymers.[220]

The confirmation that RAFT agent moieties have been cleaved from the molecular chains of polymer samples can be achieved by using traditional polymer characterisation methods such as NMR and GPC. One of the easiest ways used in the laboratories to confirm that the end group of RAFT agent incorporated into the polymers structure has successfully been removed is visual observation of the polymer samples before and after aminolysis. RAFT groups are often coloured and this colour disappears when the reaction of removal of the RAFT end group was successful, as removal groups are not coloured. The aminolysis route used to cleave thiocarbonylthio groups from thermoresponsive polymers discussed at this point is presented in section 2.4.2.7, p.71. Pink coloured samples of copolymer (PEGMEMA-PPGMA-EGDMA) prepared by *in-situ* RAFT lost its colour after aminolysis.

As it is shown on ^{1}H NMR spectrum Figure 3-20, the RAFT agent moieties have been cleaved from the molecular chains. The chemical shift between 7.4-7.8 ppm were visible in copolymer before aminolysis but they disappeared from the polymer sample after aminolysis. This confirmed that the removal of dithiobenzoyl functional groups from the polymer structure was successful.

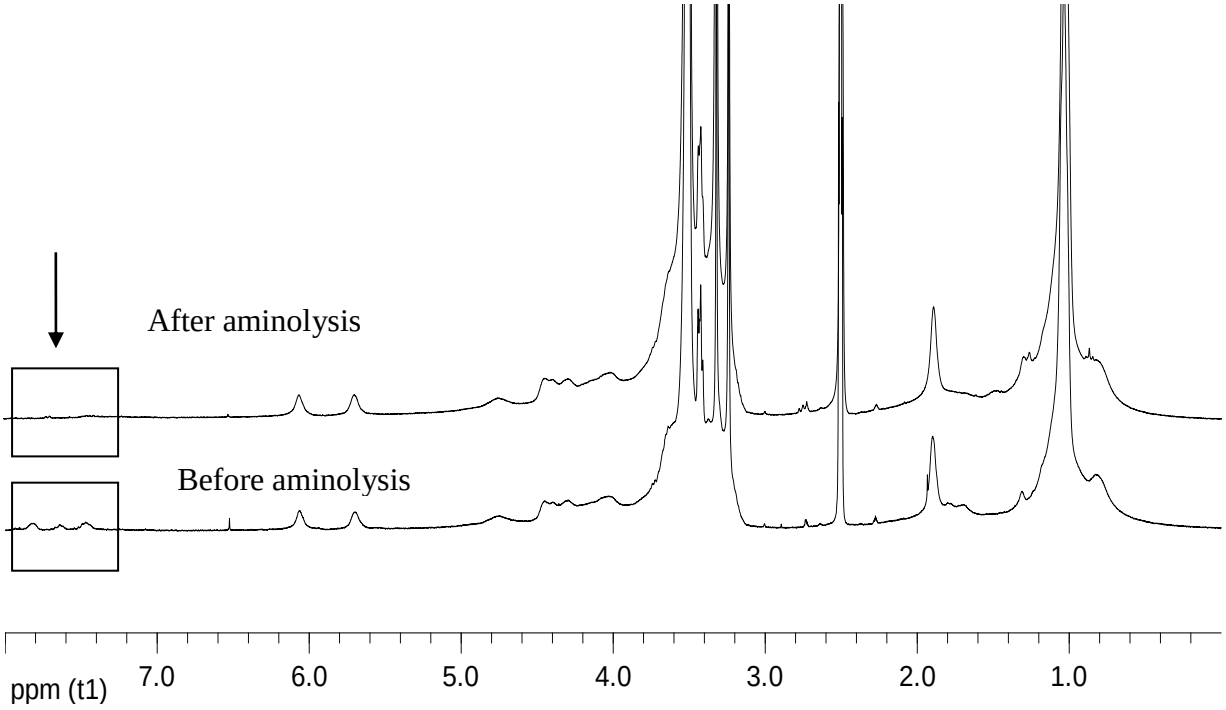


Figure 3-20: ^{1}H NMR of PEGMEMA-PPGMA-EGDMA (monomer feed ratio - PEGMEMA:PPGMA:EGDMA - 25/45/30, entry 3 in Table 3-8) before and after aminolysis.

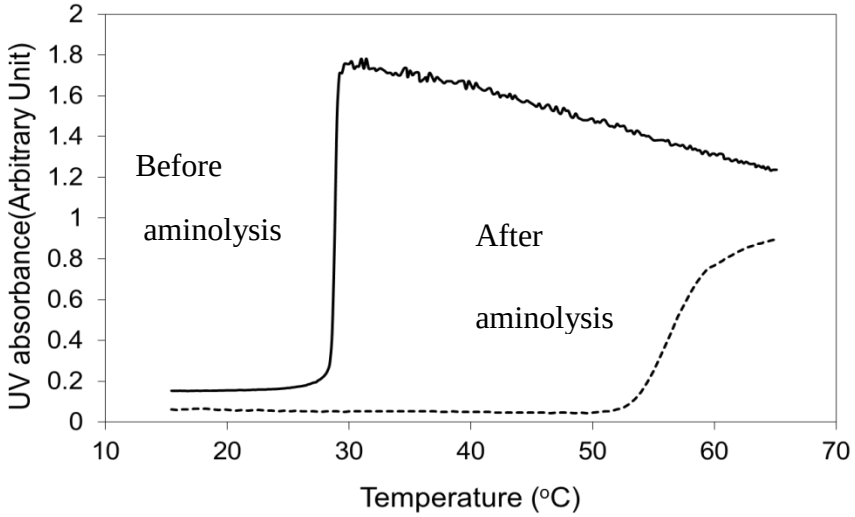


Figure 3-21: UV visible spectra (recorded on temperature-controlled spectrometer) for the copolymer (monomer feed ratio - PEGMEMA:PPGMA:EGDMA -30/40/30, entry 2 in Table 3-8) before and after aminolysis.

3.2.5. Studies on the properties of responsive and hyperbranched polymers synthesized *via in-situ* or conventional Reversible-Addition Fragment Chain Transfer polymerisation approach

3.2.5.1. Lower Critical Solution Temperature and particle sizes of the copolymers in dilute aqueous solutions

When it comes to the thermoresponsive hydrogels the challenges involve precise control over LCST and gelation kinetics, stability and mechanical properties as well as degradation profiles.[178,222] The LCST of thermoresponsive polymers depends on the composition and the molecular weight of the polymers. A high content of hydrophilic units in the copolymer and a lower molecular weight will lead to a higher LCST and the other way around. The overall final effect on LCSTs is the combination of these two factors, as well as the degree of branching. The PEGMEMA-PPGMA-EGDMA copolymers prepared in this section by changing the feed monomer ratio in the polymer synthesis and presented in Table 3-8 (p.105), demonstrated LCST's between 22 and 33 ^{o}C (Table 3-9).

Table 3-9: LCST of thermoresponsive hyperbranched polymers from *in-situ* and conventional RAFT copolymerisations of PEGMEMA, PPGMA and EGDMA

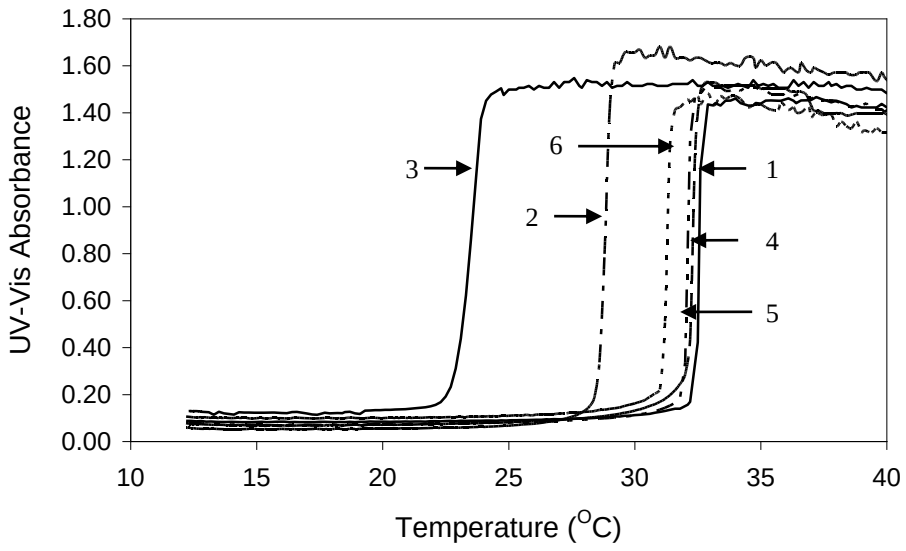
Entry	F[a]	Cov[b] %	LCST[c] ^{o}C
1	30/51/19	51	32.8
2	32/28/40	60	28.5
3	28/33/39	60	22.5
4	26/16/58	18	31.9
5	28/19/53	47	31.3
6	36/22/42	61	30.8

[a] Polymer Composition [PEGMEMA]:[PPGMA]:[EGDMA]= m:n:(r+p); [b] Monomer conversion estimated by GPC; [c] Lower critical solution temperature, obtained by UV visible. Sample code corresponds with Table 3-8.

As it is seen in entry 6 in Table 3-9, the polymer has a higher PEGMEMA content, but shows a lower LCST than the entries 4 and 5, which is due to its higher molecular weight. The effect of molecular weight presented in entries 4 to 6 for copolymers with the same composition was not large, and can be taken as not significant.[223] This is seen on LCST

data obtained by UV visible (Figure 3-22a) and DLS (Figure 3-22b). When changing the temperature from 10 to 40 ^{o}C the changes in particle sizes were recorded (Figure 3-22b). The particles aggregated when the temperature increased above LCST to the size of about 1000 nm, at the temperature below LCST they had size about 25 nm. When the temperatures increased from 33 to 40 ^{o}C a slight decrease in the UV visible absorbance was observed. This decrease was caused by the particle aggregation leading to a decrease in cloudiness of the milky solutions.

a)



b)

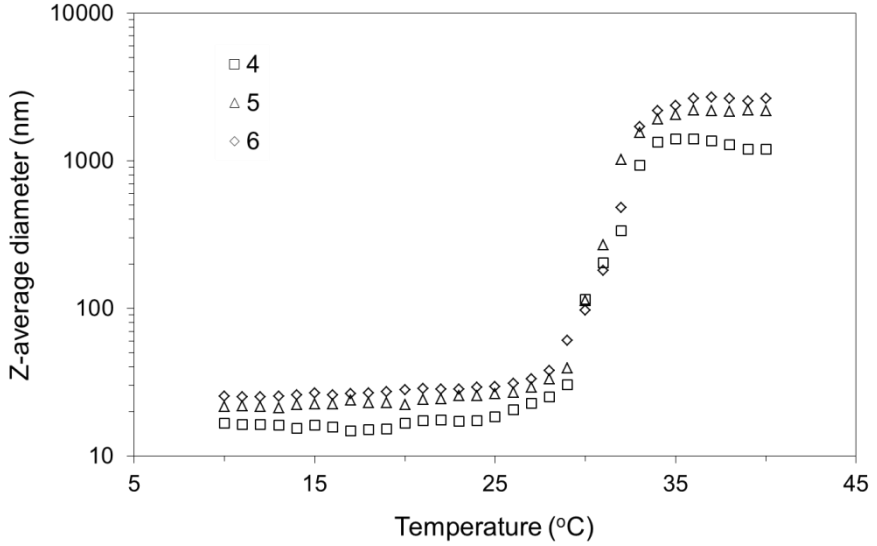


Figure 3-22: (a) LCST data for PEGMEMA-PPGMA-EGDMA copolymers (entries 1-6 in Table 3-8). (b) DLS data for PEGMEMA-PPGMA-EGDMA copolymers (entries 4-6 in Table 3-8).

It was noted that the removal of RAFT agent groups caused sligthly decrease in the molecular weight of the polymer (*Mw* from 288 KDa to 219 KDa), but the LCST increased dramatically from 28.5 ^{o}C to 55 ^{o}C (Figure 3-21, p.111) after the aminolysis. The higher LCST is evidently caused by the introduction of thiol functional groups (-SH). The polymer became more hydrophilic, therefore, it precipitated out of the solution at a higher temperature.

3.2.5.2. Photocrosslinking studies - hydrogels prepared through thermal gelation and photopolymerisation

Certain design parameters should be met when fabrication of hydrogels takes place, so they can be considered to be used in tissue engineering or drug delivery.[224,225,226] An absolutely critical parameter is the biocompatibility of hydrogels.[227,228] Moreover, classical physical parameters such as degradation and mechanics, as well as biological performance parameters such as cell adhesion are often well considered. Synthetic polymers can be prepared with controlled structures and functions; this allows manipulating the properties and is giving a range of choices to seek different materials.[229] Many synthetic hydrogels/polymers do not degrade under physiological conditions, in addition quite often toxic chemicals are used in their synthesis and their processing may require extensive purification steps. Therefore, it is good to understand the mechanism of gelling, which may include ionic or covalent crosslinking and phase transition behaviour. In all means, the reality is that no material will satisfy all design parameters in all applications, but a wide range of materials might find uses in various applications.[229,230,224,226]

The photocrosslinking occurred when the PEGMEMA-PPGMA-EGDMA hyperbranched polymers were exposed to UV sources due to the presence of free multiple methacrylate functional groups within them. The thermoresponsive polymers (100-300 mg) were dissolved in 1 mL deionised water at 4 ^{o}C and then placed at 37 ^{o}C for 5 minutes. Gel concentration was determined as no flow upon inversion of the vial within 10 seconds. It was found that gel points of these copolymers with *Mw* above 50 kg/mol (at 37 ^{o}C) were ca.15%. The copolymers with a low molecular weight showed precipitation, but no gels formed up to 30% concentration. It was observed that the sizes of the copolymer chains in

dilute aqueous solutions decreased after exposure to UV sources (for 30 minutes at 20 ^{o}C) indicating the formation of microgels (Figure 3-23).

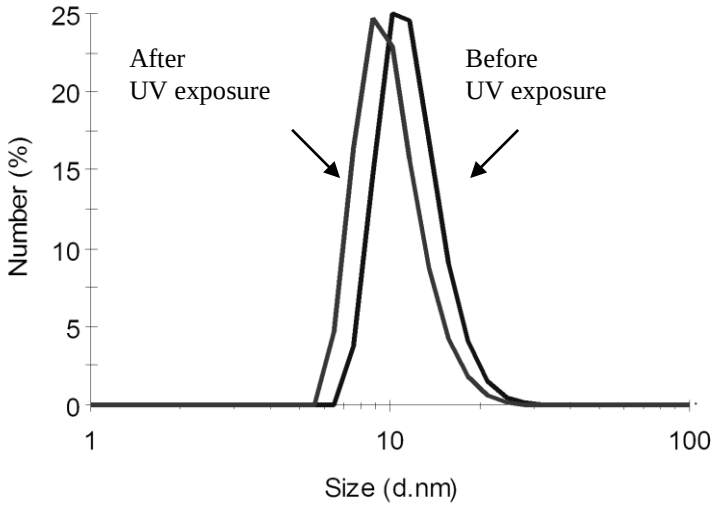


Figure 3-23: Particle size distributions recorded by DLS for the copolymer dilute solutions (0.03 % w/v) before and after exposure UV for 30 minutes at 20 ^{o}C (copolymer 2 in Table 3-8, monomer feed ratio - PEGMEMA:PPGMA:EGDMA- 30/40/30).

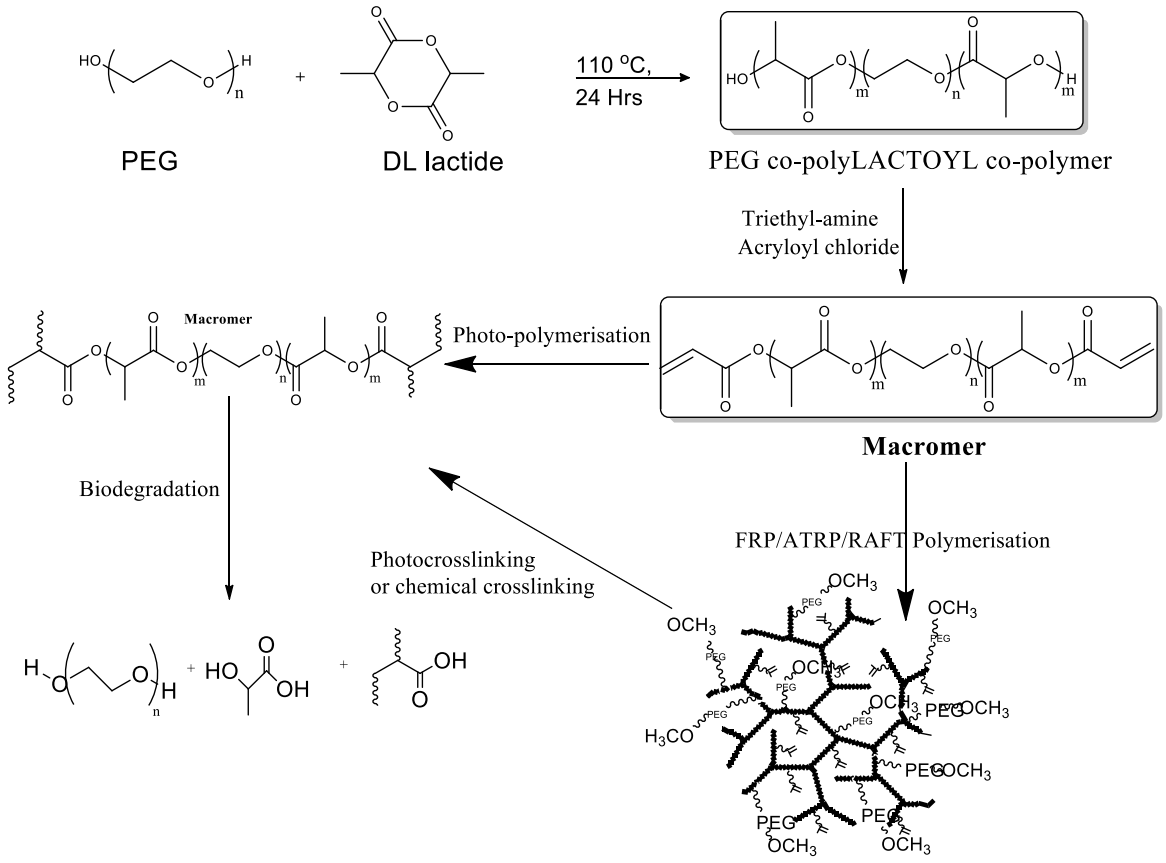
Chapter 4 Results and discussion on synthesis, characterisation and property evaluations of degradable and thermoresponsive copolymers prepared by Reversible-Addition Fragment Chain Transfer copolymerisation

4.1. Introduction

In chapter 3, section 3.2, hyperbranched photocrosslinkable and thermoresponsive polymers were successfully synthesized through controlled radical polymerisations of PEGMEA, PPGMA and EGDMA.[111,112,23,161,69] These polymeric materials despite many advantages were non degradable under physiological conditions. To advance the system, different approaches were undertaken in order to introduce biodegradability. One of the approach involved the use of biodegradable macromer as an alternative to EGDMA.[231] A series of PEG based telechelic copolymers (polymers with both ends of the same functionality) were prepared through Ring Opening Polymerisation (ROP). The macromers were designed to have three structural domains, a water-soluble central polymer domain with hydrolytically degradable polymer extensions at each end, both terminated with photo-polymerisable groups. In addition, PEG-PLA macromers were designed to be nontoxic and soluble in water.[231] The copolymerisations of PEG (M_w = 1000 g/mol) and D,L-lactide were conducted in the first step and then macromers containing poly(ethylene glycol)-co-poly(D,L-lactide) copolymers (P_{DL}LA-co-PEG-co-P_{DL}LA) were acrylated on each end, giving required vinyl functionality for their use as branching/crosslinking agent (Scheme 4-1). The water soluble macromers were successfully polymerised with PEGMEMA using FRP and conventional RAFT methods, producing hyperbranched polymers for use as biodegradable hydrogel systems.

Another approach to obtain degradable polymers is to replace EGDMA with disulfide-based diacrylate. Recently highly branched degradable poly(dimethylaminoethyl

methacrylate) (PDMAEMA) was synthesized by *in-situ* de-ATRP and used as alternative to its linear counterpart.[232] In this case degradation was observed with a faster reduction rate for hyperbranched structures in the presence of glutathione. It was confirmed that polymer with high degree of branching, containing shorter primary chains cleaves into smaller pieces.[232] The high branching was achieved by a high ratio of initiator/DMAEMA (1:8 – 1:32). The previous designs and attempts in the synthesis of PDMAEMA through ATRP and RAFT resulted in non-degradable structures.



Scheme 4-1: Introducing biodegradability by using $P_{DL}LA$-co-PEG-co-$P_{DL}LA$ diacrylate macromer as a branching agent (adapted from Ref.[231]).

4.2. Synthesis and characterisation of thermoresponsive, degradable copolymers

In this study PEGMEMA-PPGMA-DSDA hyperbranched copolymers were synthesized using conventional RAFT polymerisation approach (section 2.3.10, Scheme 2-11, p.56, chapter 2).

Disulfide-based diacrylate (DSDA) was used in the synthesis to introduce degradability due to presence of -S-S- groups. Disulfide bonds can be readily and selectively cleaved using various reducing reagents.[233,38] The copolymers were tailored in order that they could be readily cleavable under mild conditions, physically crosslinked at body temperature and moreover chemically crosslinked with thiol crosslinker (QT) by Michael addition type reaction. Moreover, the addition of DSDA in the synthesis of PEGMEMA-PPGMA copolymer should lead to branched structures.

Disulfide-based branching agents were previously introduced to polymers with low solubility and high molecular weights (synthesized through ATRP). The addition of disulfide-based branching allowed to prepare hydrogels that can undergo degradation (due to presence of disulphide bond) and produce soluble polymers at lower molecular weights.[233,39,37,234]

The reactions were monitored by GPC analysis and examples of GPC chromatograms obtained for the branched PEGMEMA-PPGMA copolymers using the DSDA as a branching agent are shown in Figure 4-1 and Figure 4-2. These data confirm the copolymer chains growth over time. The molecular weight and polydispersity data of the synthesized and tailored copolymers are presented in Table 4-1, p.120.

Effective preparation of hyperbanched polymer requires finding reaction conditions which allow higher monomer conversion. We aimed to achieve high conversions but avoiding gelation. This is very important in living polymerisations where the degree of polymerisation (dictated by the molar ratio of monomer/initiator) increases with monomer conversion. In FRP polymerisations, high molecular weight chains can be generated even at low monomer conversions but resultant copolymers are often insoluble crosslinked structures. While working on different copolymers by RAFT synthesis we concluded that monomer conversion is an important indicator in the preparation of soluble branched

copolymers. The relatively low molar ratio of initiator to vinyl monomer was maintained (1:50 to 1:100). In this study high conversions were achieved, with no gelation. Moreover, the relatively low PDI values for the synthesized structures demonstrated the controlled chain growth (Table 4-1).

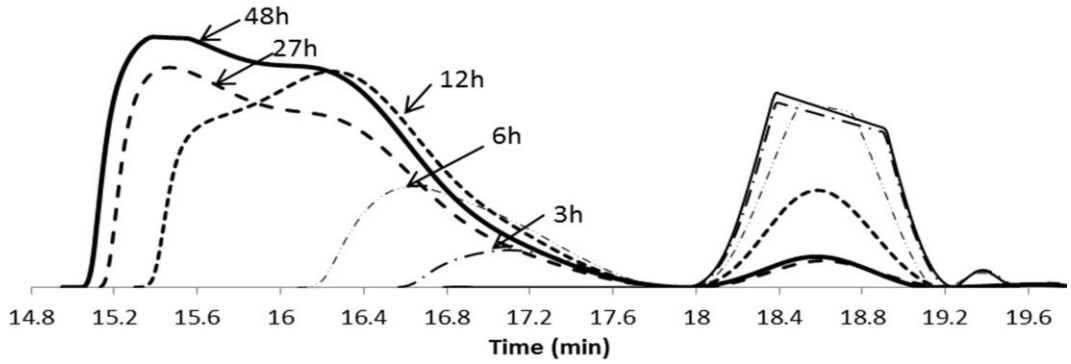


Figure 4-1: GPC traces from RI detector for entry 1 (Table 4-1) with final molecular weigh 12.7 kDa. Conversion against time: 3h = 16%, 6h = 44%, 12h = 83%, 27h = 94%, 48h = 95%.

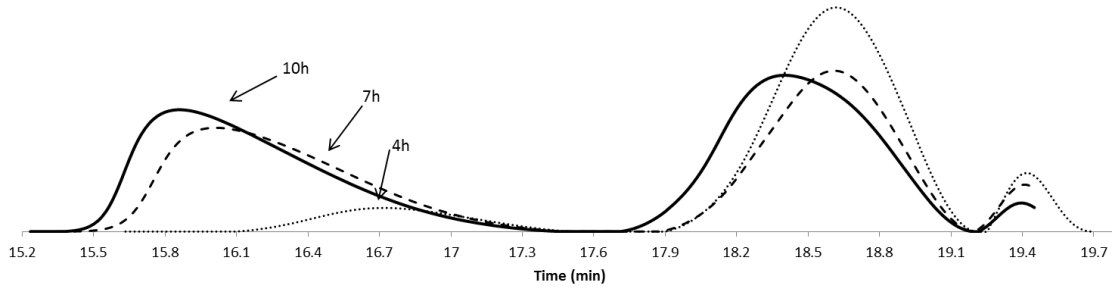


Figure 4-2: GPC traces from RI detector for entry 7 (Table 4-1) with final molecular weigh 10.2 kDa.

In the first stage, polymerisation demonstrated relatively slow and linear chain growth with narrow GPC traces, monomer peak were decreasing slowly, while at the later stage of the reaction the intermolecular crosslinking led to the slightly broader PDI values, tailing in low molecular weight side of the trace and ended with hyperbranched structures. The hyperbranched structures of the copolymer were confirmed by ^{1}H NMR.

Table 4-1: Reaction conditions and GPC data for degradable and thermoresponsive polymers from conventional RAFT copolymerisations of PEGMEMA, PPGMA and DSDA.

Entry	f[a]	R:I	RT (h)	Cov[b] %	GPC RI		
					Mw[c] kg/mol	*Mn*[d] kg/mol	PDI[e]
1	70 : 20 :10	5 : 1	48	95	12.7	9.0	1.40
2	50 : 40 : 10	2 : 0.4	24	80	7.3	6.6	1.12
3	50 : 40 : 10	1 : 0.2	24	64	11.3	9.8	1.15
4	20 : 70 : 10	1 : 0.2	29	54	12.4	10.1	1.22
5	15 : 65 : 20	1 : 0.2	25	58	11.2	8.9	1.26
6	20 : 70 : 10	1 : 0.2	17	65	13.4	11.4	1.17
7	20 : 70 : 10	0.5 : 0.1	11	46	10.2	8.7	1.16

[a] Monomer feed molar ratio PEGMEMA:PPGMA:DSDA; [b] Monomer conversion estimated by GPC; [c] Weight-average molecular weight; [d] Number-average molecular weight; [e] Polydispersity; Polymerisation conditions: 65 ^{o}C in n-butanone.

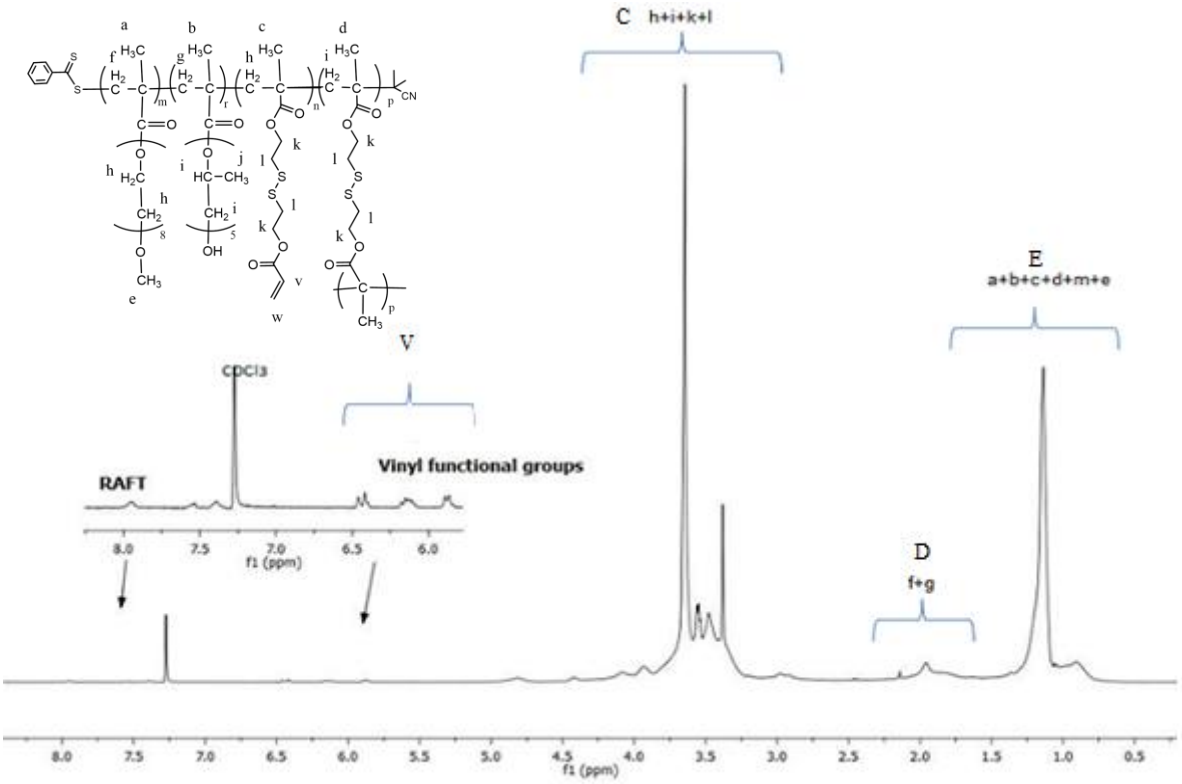


Figure 4-3: ^{1}H NMR of PEGMEMA-PPGMA-DSDA copolymer prepared by conventional RAFT polymerisation.

Polymer compositions (m, n, r, and p) were calculated using equations 4-1 to 4-5, from peak integrations according to ^{1}H NMR analysis. The examples are given below. Sample coding: R4 corresponds with entry 4 in Table 4-1 (p.120) and Table 4-6 (p.126), R7 corresponds with entry 7 respectively.

Equations (E.q: 4-1 to E.q: 4-4) outline the calculations:

$$\mathbf{3r} = (\mathbf{V}) \qquad \textbf{E.q: 4-1}$$

$$\mathbf{35m + 15n + 9r + 9p} = (\mathbf{C}) \qquad \textbf{E.q: 4-2}$$

$$\mathbf{2m + 2n + 2r + 2p} = (\mathbf{D}) \qquad \textbf{E.q: 4-3}$$

$$\mathbf{3m + 18n} = (\mathbf{E}) \qquad \textbf{E.q: 4-4}$$

$$\mathbf{r + m + n + p} = \mathbf{100} \qquad \textbf{E.q: 4-5}$$

Where: $m = PEGMEMA$; $n = PPGMA$; $r = DSDA$; $p = Hyperbranched\ DSDA$. Double bond content and branching degree were calculated according to E.q: 3-5 and E.q: 3-6, p.107, presented in section 3.2.3. Tables 4-2 and Table 4-4 include two sets of values. First set in a first row corresponds to values calculated according to above equations and set of values in second row is referred to actual % composition.

The double bond content represents the mol percentage of DSDA with free vinyl functional groups in the copolymer and the degree of branching represents the mol percentage of DSDA as branching units in the copolymer.

A high degree of branching (up to 31 mol %) was achieved in the synthesized copolymers without gelation, while low level of free vinyl groups was attained. However, this still allowed copolymers to react readily with thiol functional crosslinker (QT) through Michael addition to form chemically crosslinked network.

As presented (Table 4-3 and Table 4-5) the decrease in amount of RAFT agent and initiator (Table 4-1, p.120) altered the double bound content and branching degree in synthesized copolymers. Decreasing amount of R:I in half, caused increase in double bound content and in branching degree of the resultant copolymers.

Table 4-2: Peak integration for copolymer of PEGMEMA-PPGMA-DSDA based on ^{1}H NMR (PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2), entry 4 in Table 4-1).

Polymer	V	C	D	E	m (%)	n (%)	r (%)	p (%)
R4	1.03	158.53	43.65	134.97	21	43	1.03	22
					24.1	49.4	1.1	25.3

Table 4-3: Design *via* actual composition of PEGMEMA-PPGMA-DSDA copolymer: PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2), entry 4 in Table 4-1.

R4	PEGMEMA	PPGMA	DSDA		Double Bond (%)	Branching degree (%)
Design	**20**	**70**	**10**	100	1.1	25.3
Actual	24.1	49.4	26.4	99.9		

Table 4-4: Peak integration for copolymer of PEGMEMA-PPGMA-DSDA based on ^{1}H NMR (PEGMEMA-PPGMA-DSDA/R:I (20:70:10/0.5:0.1), entry 7 in Table 4-1).

Polymer	V	C	D	E	m (%)	n (%)	r (%)	p (%)
R7	1.12	140.45	26.63	113.83	12.95	26.66	1.116	18.23
					22.0	45.2	2.4	30.9

Table 4-5: Design *via* actual composition of PEGMEMA-PPGMA-DSDA copolymer: PEGMEMA-PPGMA-DSDA/R:I (20:70:10/0.5:0.1), entry 7 in Table 4-1.

R7	PEGMEMA	PPGMA	DSDA		Double Bond (%)	Branching degree (%)
Design	**20**	**70**	**10**	100	2.4	30.9
Actual	22	45.2	33.3	100.5		

4.3. Fabrication of hydrogel from Michael addition reaction using degradable and thermoresponsive copolymers

Chemical crosslinking used to form hydrogels through radical reactions of thiols, disulphide bond formation, sulfones and acrylate functional groups has proven to be attractive as it can enhance mechanical properties, encapsulate cells, and vary the crosslinking density of hydrogels.[112,235,236] Hydrogel fabrication through thiol-ene Michael addition reactions has offered versatility and improved workability, gelation time and *in-situ* gelling at physiological conditions.[237] Moreover this "click" reaction has limited equipment requirements, which is always a bonus.

Michael addition-type reaction between thiol and acrylate requires the presence of a base to act as a catalyst.[238,239,240] As the reaction is selective towards thiols in physiological conditions, the side reactions towards amines in the body are limited. Reaction precedes with formation of a triethylammonium cation and a thiolate anion, a powerful nucleophile.[241] This type of reaction typically delivers gelation in a few minutes up to tens of minutes at physiological pH.[242] The basic conditions in this work were supplied by the use of PBS buffer (pH 7.44). Nucleophilic addition of thiol and diacrylate has been

studied relatively well in last few years.[243] Nucleophilic attack at the activated free vinyl groups of the polymer generates a strong base, able to deprotonate thiols and consequently results in the formation of thiolate anions, which can participate in the rapid formation of thiol-Michael addition products by the hydrothiolation of activated vinyl groups.[244, 245, 246]

Degradable and thermoresponsive copolymers (synthesized according to section 2.3.10, p.55) due to the presence of the multifunctional vinyl monomer DSDA in the polymer structure have the potential to undergo the Michael addition-type reaction to form chemical gelation. This reaction is relatively easy to run, with no need for chemical initiator and often can result in rapid gelation.[247, 248, 249]

The vinyl groups in the copolymer structure were evidenced by ^{1}H NMR spectrum with the three chemical shifts between 5.8 and 6.4 ppm. Calculation of the level of free vinyl groups can be very complex in this copolymer; hence, it was approximated, with the assumption that each DSDA unit retains a single vinyl group. A number of Michael addition-type reactions were performed using QT (pentaerythritol tetrakis, thiol functional crosslinker).

The low degree of free vinyl functional groups (about 1% molar ratio) in prepared copolymers resulted in a very soft/mellow chemically crosslinked hydrogel. The pinkish color of the froth indicated presence of RAFT functional group in the hydrogel structure which could allow further modifications. Moreover, the amount of the free vinyl groups and branching degree could be further tailored by changing the molar ratio of PPGMA, DSDA and PEGMEMA in polymer synthesis. Increase of free vinyl groups can significantly improve the reactivity during the Michael addition; consequently change the substantial form of hydrogel.

The fabrication of chemically crosslinked hydrogel from PEGMEMA-PPGMA-DSDA copolymer was attempted several times, at 23 ^{o}C (room temperature) and 37 ^{o}C. In each trial, gel was defined by visual examination as unable to flow when eppendorf tubes were inverted (as seen on Figure 4-4, p.125).

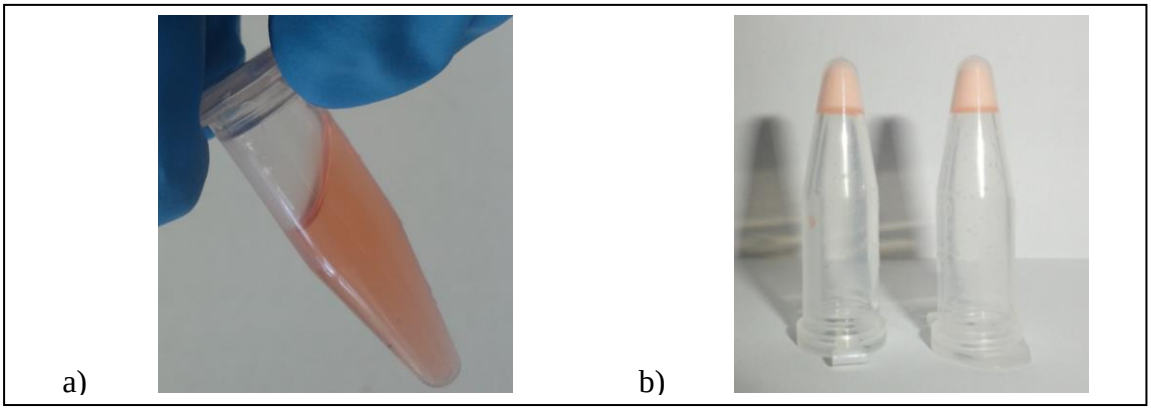


Figure 4-4: 20 wt % polymer solution in PBS buffer (PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2), entry 4, Table 4-1) undergo Michael addition-type reaction: a) 1 min after mixing with QT (1:1 vinyl group to SH); b) 0.5h after mixing with QT (1:1 vinyl group to SH) and incubated at 37 ^{o}C.

The reactions at room temperature (23 ^{o}C) using 20 wt % and 40 wt % of prepared and purified copolymer, resulted in milky solutions and a white precipitate (as seen on Figure 4-5). These results indicated that the reaction occurred.

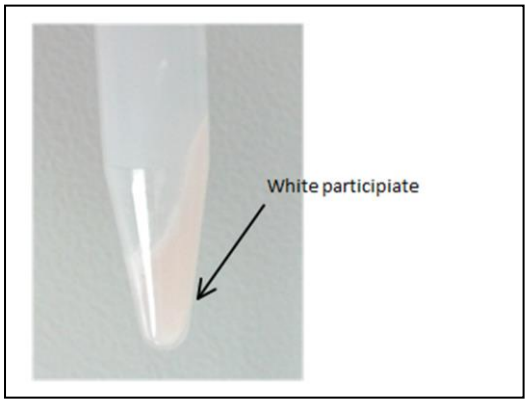


Figure 4-5: 20 wt % polymer solution in PBS buffer (PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2), entry 4, Table 4-1) - Michael addition-type reaction at room temperature (24 h after mixing with QT, 1:1 vinyl group to SH), white precipitate present in eppendorf tube.

Due to the low level of free vinyl functional groups, weak hydrogels were obtained. Thus further studies on gelation conditions using Michael addition-type reaction are required. Gels may be optimised for tissue engineering applications that require different softness, pore sizes and porosity.

4.4. Lower Critical Solution Temperature and Differential Scanning Calorimetry of the degradable and thermoresponsive copolymers

As stated in previous sections, phase transition temperatures for the copolymers studied in this thesis were tailored close to body temperature. We aimed to have copolymers which are soluble in water at room temperature, while forming a thermal gel close to body temperature. By altering ratios of hydrophobic/hydrophilic parts within the copolymer we can manipulate the phase transition temperature of the polymer composition. The more hydrophilic the copolymer is, the higher LCST will be observed.[69,250] The LCST of the PEGMEMA-PPGMA-DSDA copolymers synthesized according to the experimental procedure presented in Table 4-1 (p.120) were measured by two methods mentioned earlier in chapter 2. At the temperature above LCST for each sample the solutions reversibly became cloudy. The solutions were clear below this temperature. The values from both methods corresponded well and are listed in Table 4-6. For entry 6 in Table 4-6 two values were observable by DSC and only one by visual observation. This DSC data indicates that this polymer is partially miscible at certain temperature range. Below 17 ^{o}C and above 30 ^{o}C the sample is clear, signifying that components are miscible in all proportions, however in the interval from 17 ^{o}C to 30 ^{o}C sample is only partially miscible. This also explains different value of 28 ^{o}C given by visual observation. The human eye is less accurate than the instrument and clearly it was difficult in this case to assess visually the phase transition at this polymer concentration. By varying the monomer feed ratio in the copolymer synthesis (Table 4-1), the LCST's are tailored between 17 and 56 ^{o}C.

Table 4-6: LCST of PEGMEMA-PPGMA-DSDA copolymers from conventional RAFT copolymerisations.

Entry	LCST ^{o}C	
	LCST [1]	LCST [2]
1	-	-
2	55	56
3	57	57
4	28	28
5	22	23
6	28	17 & 30
7	18	17

LCST obtained by visual observation[1] and by DSC[2]; Sample code corresponds with Table 4-1, p120.

Due to thermal responsive properties, the copolymer solutions were found to form physical gels at the concentration about 20% w/v (and above) when the temperature was raised beyond their LCST. The polymers (100-500 mg) were dissolved in deionised water at 10 ^{o}C and then placed at 37 ^{o}C for 10 minutes. It is known that the physical thermal gelation is reversible and in addition displays weak mechanical properties which might hold back clinical application of hydrogel.[251,252,253] Therefore, the chemical crosslinking can be introduced and tailored to enhance the gel mechanical properties.[254]

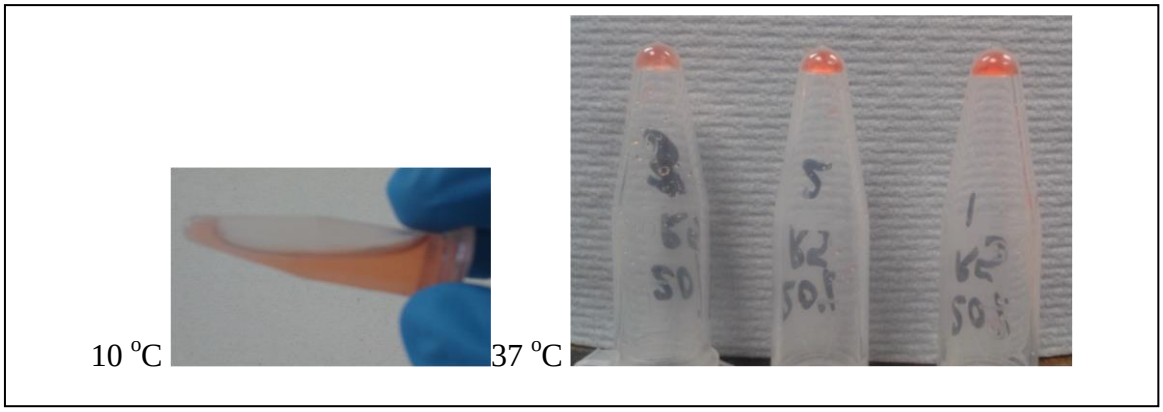


Figure 4-6: Thermally induced gelation from 20 % copolymer solution (PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2), entry 4, Table 4-1).

Figure 4-7, Figure 4-8 and Figure 4-9 (p.128-129) demonstrate the results of the temperature scans for the selected copolymer solutions recorded by the DSC. The method measured LCST [2] data listed in Table 4-6, p.126).

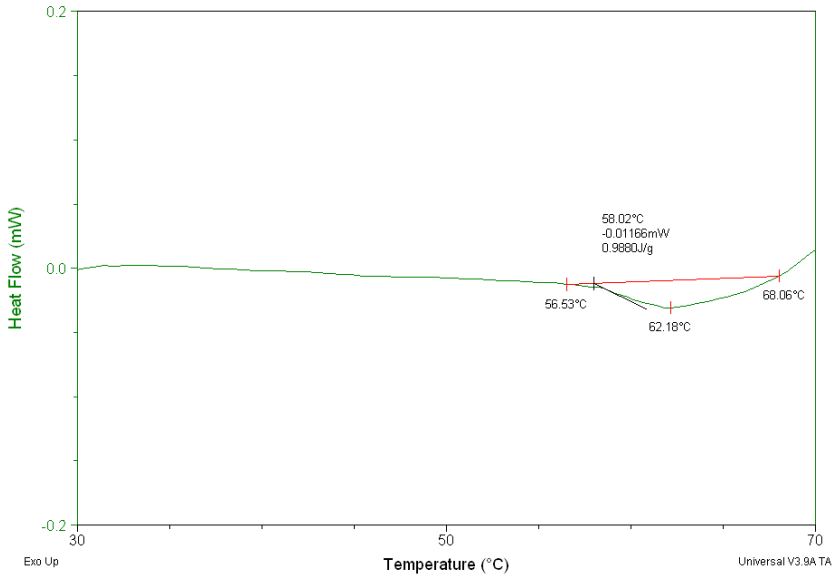


Figure 4-7: LCST – DSC measurement for thermoresponsive PEGMEMA-PPGMA-DSDA/R:I (50:40:10/2:0.4), entry 2 Table 4-6.

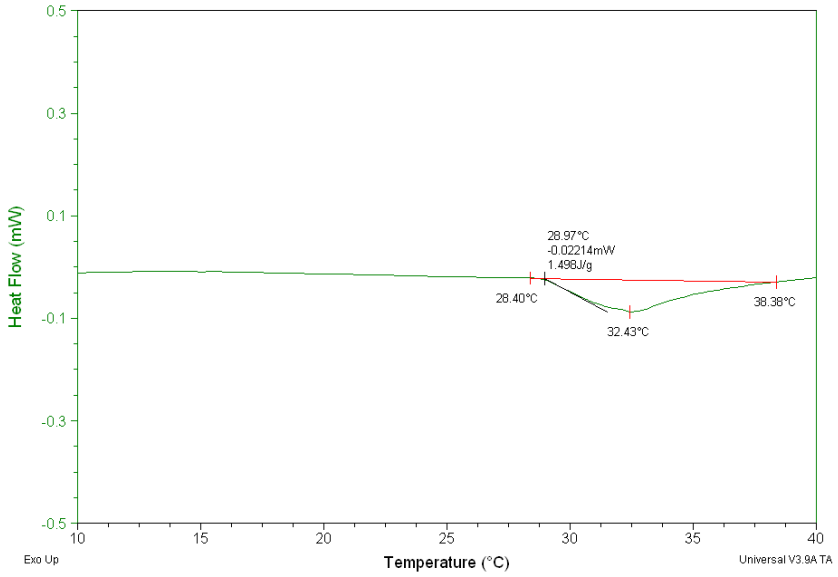


Figure 4-8: LCST – DSC measurement for thermoresponsive PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2) entry 4 in Table 4-6.

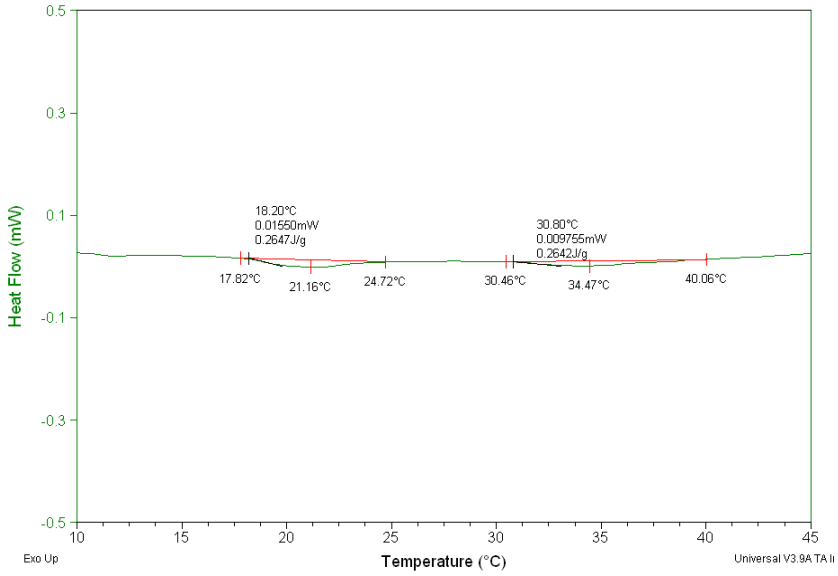


Figure 4-9: LCST – DSC measurement for thermoresponsive PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2), entry 6 in Table 4-6.

In this study, determination of the glass transition temperature (Tg) was completed by DSC measurements and the data are summarised in Table 4-7. Below this temperature, copolymer becomes hard and easy to break, like a glass. For the entry 7 Table 4-7 the Tg point was not found but the melting temperature was spotted which was a sign of crystalline polymer (Figure 4-11). Copolymer compositions represented by entries 1 to 6 were in amorphous state.

Table 4-7: Glass transition temperature of thermoresponsive PEGMEMA-PPGMA-DSDA copolymers.

Entry	Tg (^{o}C)	Melt temperature (^{o}C)
1	-59.30	
2	-59.41	
3	-74.59	
4	-49.58	
5	-42.46	
6	-44.48	
7	n/a	2.34 and 83.01

Sample code (entry) corresponds with Table 4-1 (p.120) and Table 4-6 (p.126).

Figure 4-10 and Figure 4-11 demonstrates the data for the selected copolymer solutions as well as Figure 4-12 shows overlay of Tg measurement.

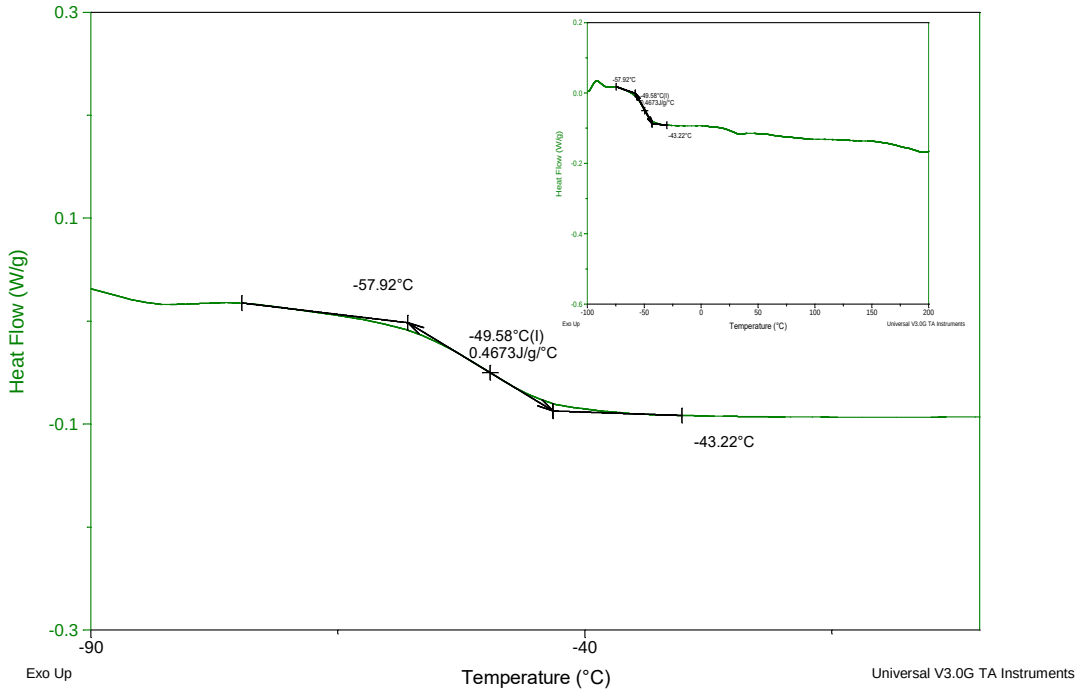


Figure 4-10: Tg measurement for thermoresponsive PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2), entry 4 in Table 4-7.

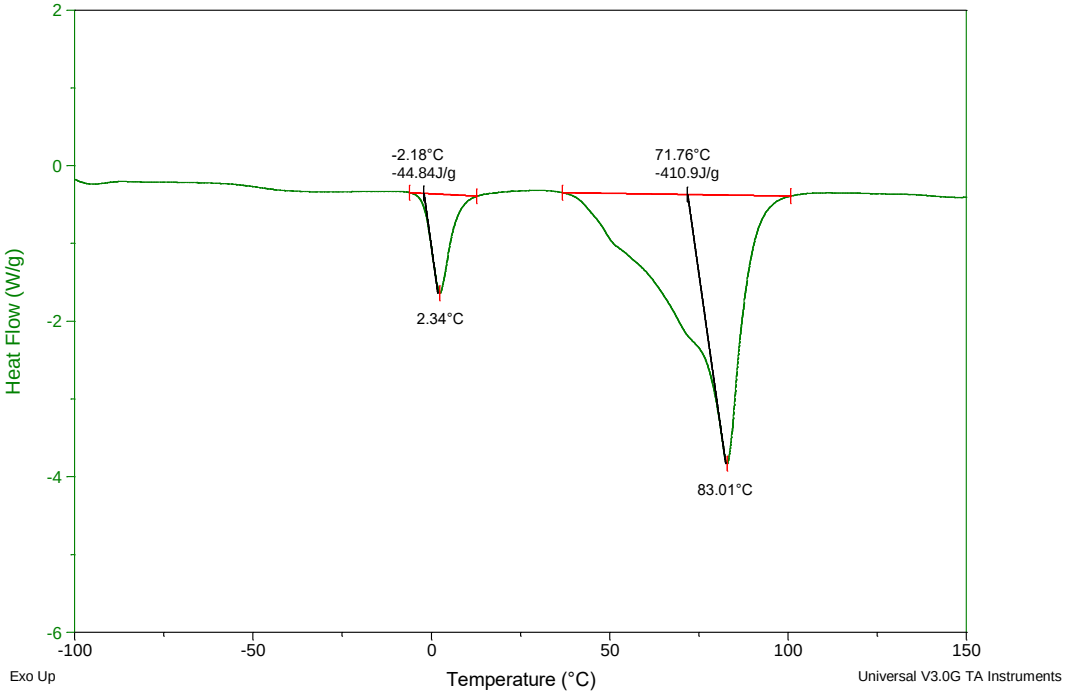


Figure 4-11: Melting temperature for thermoresponsive PEGMEMA-PPGMA-DSDA/R:I (20:70:10/0.5:0.1), entry 7 in Table 4-7.

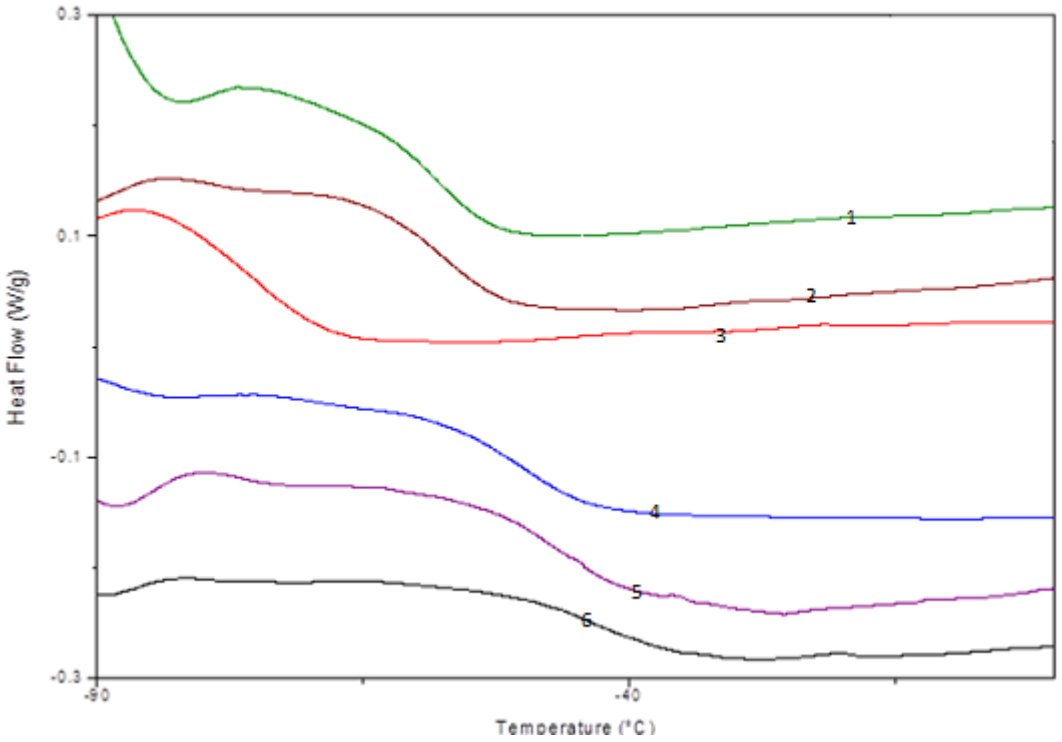


Figure 4-12: Overlay of Tg measurement for thermoresponsive PEGMEMA-PPGMA-DSDA (entries 1 to 6 in Table 4-7).

4.5. Scanning electron microscopy

The method was employed to observe the porous structure of lyophilised chemically crosslinked gels (polymer concentration: 20 wt% and 40 wt%, and a control sample of PEGMEMA-PPGMA-DSDA, Figure 4-13). The samples were mounted on an aluminium stub using an adhesive carbon tab and sputter coated with gold before images were obtained. It is important to note that this data are not conclusive. The morphology by SEM analysis did not show clear porous structure due to amorphous state and nature of the samples, and the limitation of the instrument.

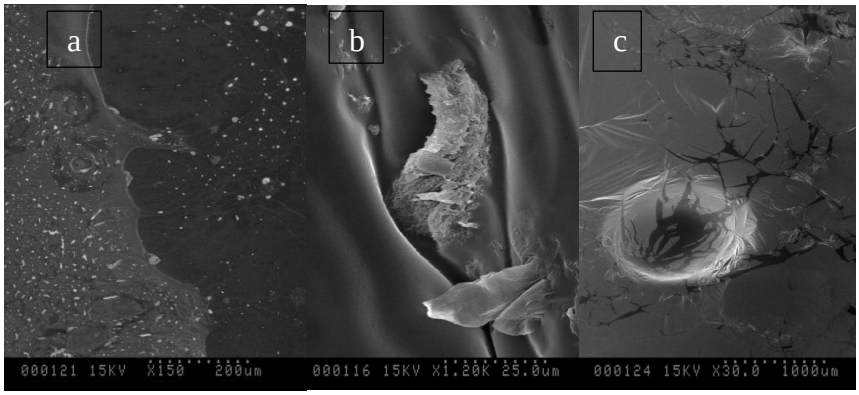


Figure 4-13: Exemplary SEM images for the lyophilised gel samples prepared from PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2) copolymers: (a) 20 wt%; (b) 40 wt% chemically crosslinked with QT, and (c) control sample.

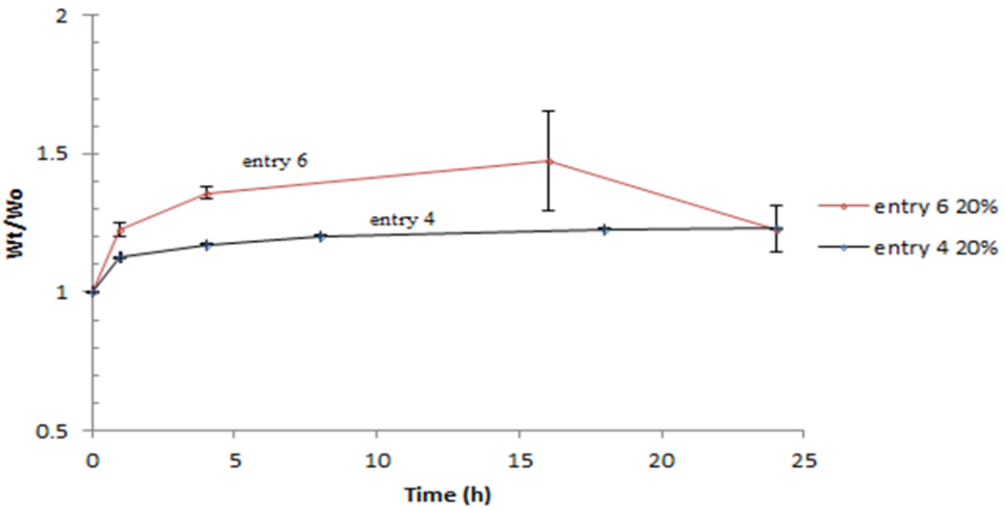
4.6. Swelling studies

The swelling profile of the thermoresponsive and degradable PEGMEMA-PPGMA-DSDA hydrogels was obtained by soaking selected samples in PBS buffer. Each data point was attained as an average value from three samples analysed under the same conditions, with error bars shown. Small volume changes were observed in all the samples studied for swelling at 37 °C in PBS. Typical gelation time range was from 10 to 30 minutes.

Figure 4-14 (a, b) shows the swelling tests of the 20% chemically crosslinked gels (entry 4 and entry 6 in Table 4-1, p.120) with QT at 37 ^{o}C.

Initially for the concentration of 20% copolymer in hydrogels, increase in the swelling within 1 to 15 hours was observed, however after 15 hours for entry 6 and after 24 hours for entry 4 the hydrogels reached maximum swelling stage in PBS (Figure 4-14, p.133). Studied samples had the same polymer composition and concentration, the difference was that polymer synthesis for entry 6 was stopped at higher conversion and had higher molecular weight than entry 4 with slightly lower PDI. It has been demonstrated that for up to 24 hours there was no weight loss and hydrogels were able to swell, then with time progression it appeared that samples started to dissolve and in 4 - 5 days almost half of the original weight was lost. This can be explained by weak stability and integrity of the 20% hydrogels due to low percentage of free vinyl groups in the polymer compositions.

a)



b)

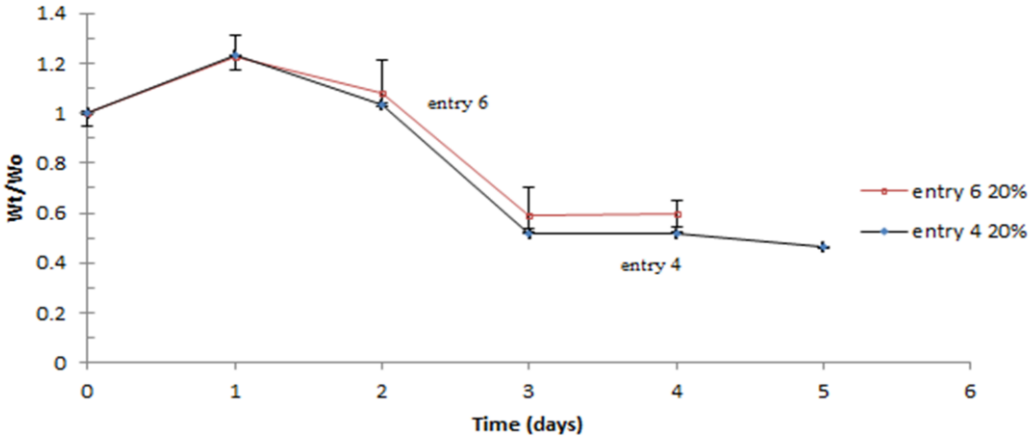
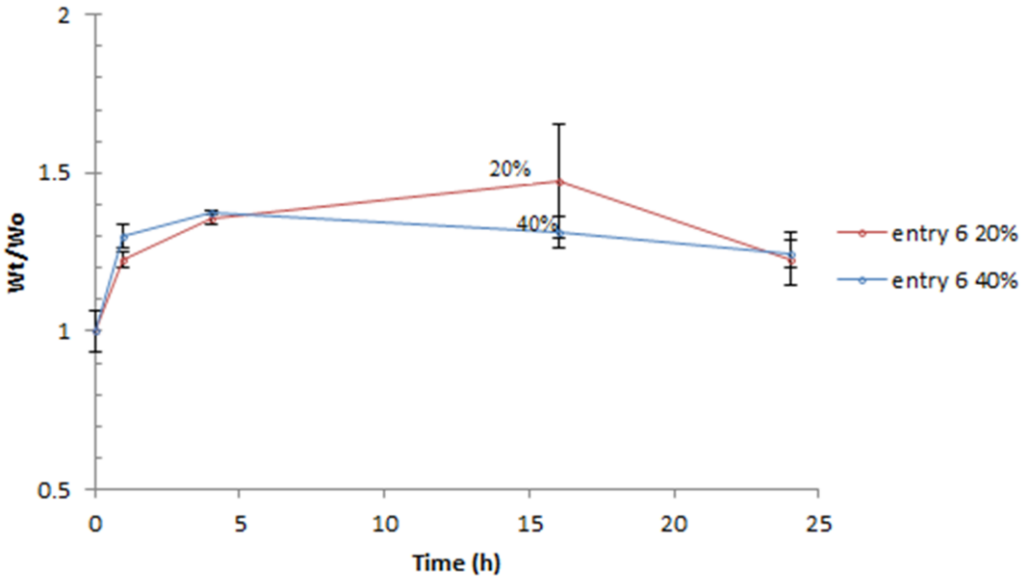


Figure 4-14: Swelling studies carried out on chemically crosslinked 20 % hydrogels (PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2), entry 4 and entry 6, Table 4-1) in PBS buffer (1M, pH 7.4) at 37 °C up to: a) 24h and b) 5 days.

As seen on Figure 4-15 (p.134) difference in swelling of the hydrogels prepared at different concentrations was observed. The swelling proportion for PEGMEMA-PPGMA-DSDA - QT hydrogel prepared using 20% polymer (entry 6 Table 4-1, p.120) reached a maximum of 1.48 after 16 hours in contrast to 40% gel which had similar maximum of swelling close to 1.40 at 5 hours (Figure 4-15).

a)



b)

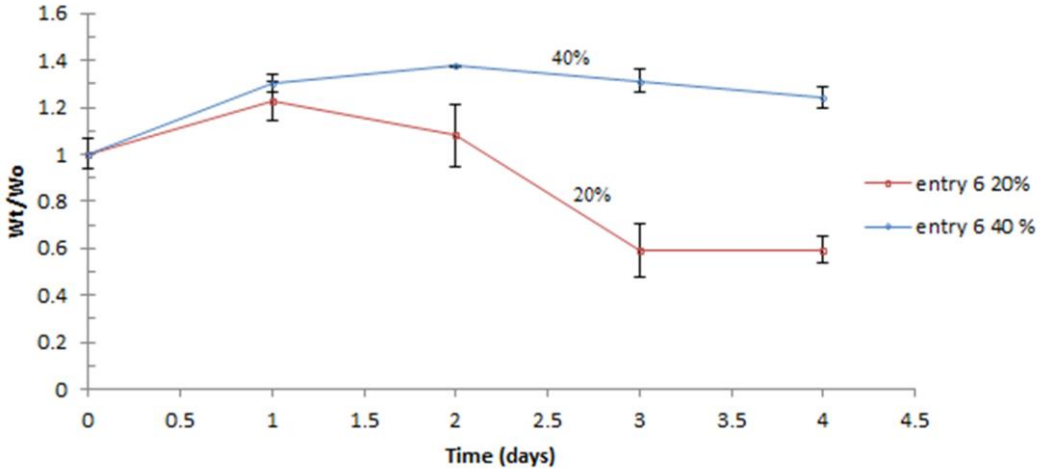


Figure 4-15: Swelling studies carried out on chemically crosslinked 20% and 40% hydrogels (PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2), entry 6, Table 4-1) in PBS buffer (1M, pH 7.4) at 37 °C after a) 24h and b) 4 days.

Higher polymer concentration produced more stable hydrogel. It has been demonstrated that the swelling and stability of the prepared gels is highly dependent on the polymer concentration used when preparing the hydrogels.

Error bars presented in Figure 4-14 and Figure 4-15 express potential error, an uncertainty in a reported measurement; a low standard deviation (SD) indicates that the data points tend to be very close to the mean of analysed samples, while high standard deviation point out that the data are spread out over a large range of values. Table 4-8 is stating standard deviation for representative samples at selected time points.

Table 4-8: SD errors for swelling studies carried out on chemically crosslinked 20% and 40% hydrogels presented in Figure 4-14 and Figure 4-15.

Entry 6 20%		Entry 6 40%		Entry 4 20%	
Time point (h)	SD	Time point (h)	SD	Time point (h)	SD
1	0.04582	1	0.11150	1	0.00420
4	0.03832	4	0.06321	4	0.00563
16	0.30969	16	0.01031	8	0.00456
24	0.14339	24	0.08828	18	0.00412
48	0.22714	48	0.07485	24	0.00416
72	0.19860	72	0.05994	40	0.00519
96	0.09339	96	0.01612	48	0.00520
		144	0.19401	72	0.00214
				96	0.00098
				120	0.00595

As mentioned earlier, the swelling profile was provided by soaking the samples for a period of time in a PBS buffer. After certain time point the excess solvent was removed (as much as it was possible) with syringe and needle, then the samples were weighted. Measurements were performed in triplicates; the error is hugely affected by the technique of solvent removal as well as by the nature and softness of created hydrogels.

4.7. Degradation

The degradation behaviour of randomly crosslinked network of thermoresponsive PEGMEMA-PPGMA-DSDA copolymers upon the addition of water-soluble DTT reducing agent (also known as Clelands Reagent) was monitored by GPC. The degradation studies were performed in two different solvents, i.e. water and tetrahydrofuran (THF). Samples tested for the reductive degradation were fully soluble in above solvents (at room temperature) as viscous solutions. In the first trial 0.1M solution of DTT in water was added into copolymers water solution to a final concentration of 0.001M DTT. All samples were well mixed, then aliquoted to separate vials (incubated in vacuum oven at 37 ^{o}C) and run on GPC at required time points. Small tailing and increase in PDI of analysed samples were recorded on GPC traces. This indicated that the process of reduction occurred. It was possible that final concentration of DTT in analysed copolymers was too weak to totally cleave disulfide bonds. In second trial, samples chosen for the degradation were dissolved in THF and 1M DTT was freshly prepared in THF. The degradation test started using each of the copolymer sample, dissolved in 1 mL THF, then mixed with 1M DTT to final concentration of 0.1M DTT, and incubated in oven at 50 ^{o}C for 5 hours. High temperature and harsh conditions were used to check if a complete degradation of the polymer is possible. GPC samples were run and as a result no polymer peak was observed (Figure 4-17, p.137). It indicated that PEGMEMA-PPGMA-DSDA copolymers in THF were readily cleaved in the presence of DTT. However, it was highly impossible by looking at copolymer composition that all polymers degraded into small molecule. It could undergo microgelation so that cleaved polymers were not detected by our GPC. Particle size measurement of the samples was recommended and would give the advantage of knowing what has happened in the polymer solutions after DTT treatment, but at the time of the particular study we did not have access to particle size analyser.

^{1}H NMR performed on the samples after treatment with DTT confirmed presence of low molecular weight polymer, therefore it is possible that the microgelation occurred but further investigation of the polymer behaviour after cleavage is required.

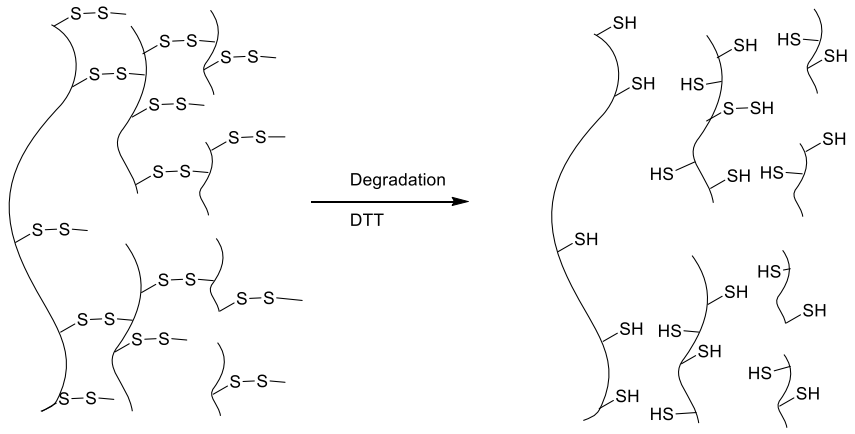
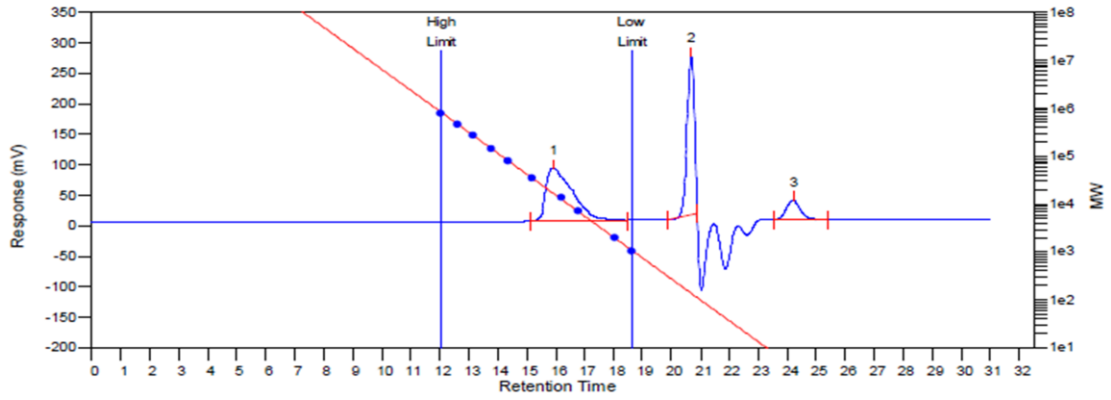


Figure 4-16: Schematic representation of the degradation PEGMEMA-PPGMA-DSDA polymer to primary chains by disulfide bond cleavage.

a)



b)

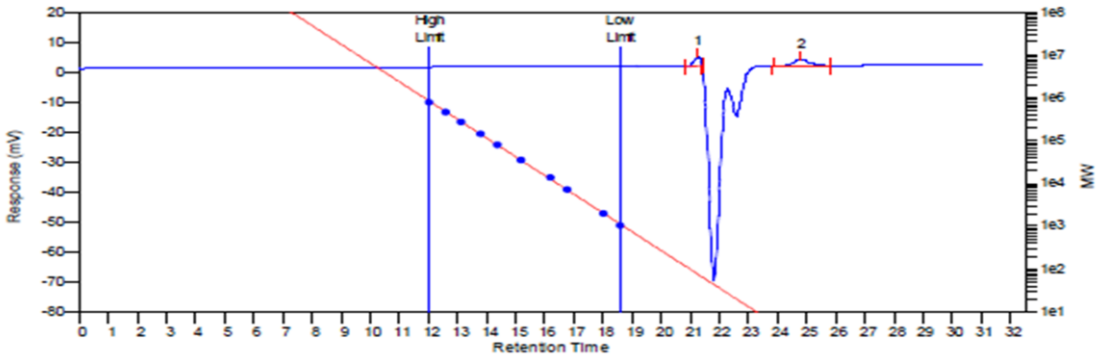


Figure 4-17: GPC traces recorded at time a) 0 h and b) 5 hrs during the reductive degradation of the branched copolymer (PEGMEMA-PPGMA-DSDA/R:I (20:70:10/1:0.2), entry 4 in Table 4-1) with 0.1M solution of DTT in THF at 50 ^{o}C.

In order to monitor the cleavage, selected samples were introduced to DTT at room temperature (20 ^{o}C) (0.1M DTT final concentration) and run at required time points to check if the kinetics of degradation can be monitored on the GPC system. Copolymers fast degraded into individual polymeric chains and with time finally disappeared as seen on Figure 4-18 (the GPC trace changed with reaction time, showing peaks with lower molecular weights and finally no peak of polymer was detected on the system); the chromatogram proved cleavage of the copolymer in the presence of DTT. The peak for the polymer on the GPC traces decreased with the time, and after 3 hours there was no sign of polymer in high molecular range.

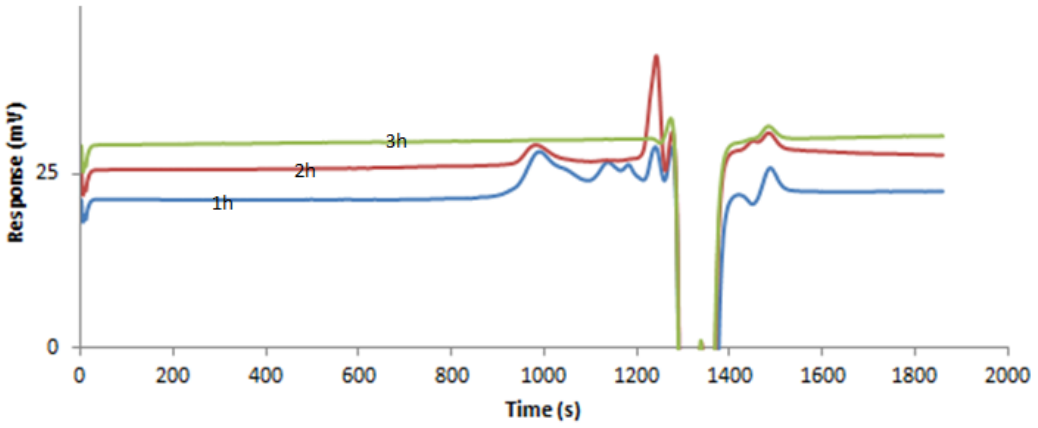


Figure 4-18: Overlaps of SEC traces recorded at various reaction time using the refractive index detector during the reductive degradation of the branched copolymer (PEGMEMA-PPGMA-DSDA/R:I, 20:70:10/1:0.2), entry 4, Table 4-1) with 0.1M solution of DTT in THF at 20 ^{o}C.

According to GPC data obtained for entry 3 Table 4-1 (p.120), copolymer was cleaved and was not detectable on GPC within 2 hours at room temperature. This was faster than for the copolymer sample represented by entry 4 Table 4-1 (at 3h there was no indication of high molecular weight on the GPC trace). Moreover, entry 1 Table 4-1 was cleaved faster than entry 3 and entry 4, since after 1.5 hours there was no sign of high molecular weight polymer on GPC trace.

If the linkages between primary chains are cleavable, the polymer will have potential to fragment into small pieces of oligomers. By looking on the GPC trace (Figure 4-18)

representing entry 4 Table 4-1, in the first hour, we could clearly see cleavage of disulfide (-S-S-) linkage. A noticeable difference in retention time for the starting polymer and its cleaved products was observed, confirming the cleavage of the branches/arms from the core by reduction of the -S-S- bonds using DTT. It is important to note that no change in molecular weight of copolymer sample measured by GPC in THF/or water was observed in the absence of DTT. The copolymers were very stable in the solution without DTT. In view of the fact that the redox potential of the disulphide bond depends on solvent polarity, the experiments proved that the reaction was more effective in THF than in water.

Chapter 5 Results and discussion on synthesis, characterisation and property evaluation of pH responsive copolymers

5.1. Introduction

Hydrogels have the ability to swell in an aqueous environment and can be used for controlled release and drug delivery.[255] As it was mentioned in chapter 1, responsive hydrogels with pH sensitive and/or thermo sensitive properties can be designed to respond to true physiological settings, becoming an ideal candidates for a drug delivery system.[22,256]

There are a high number of researches in this area, reporting highly, moderate and poorly swollen hydrogels. For instance poly(vinyl alcohol) (PVA) and various cellulose derivatives belong to a group of highly swollen hydrogels, where poly(hydroxyethyl methacrylate) (PHEMA) and many of its products classified as a moderate or poorly swollen hydrogel systems. As previously reported by others, 2-hydroxyethyl methacrylate (HEMA), a synthetic monomer commonly used for hydrogel construction due to its good biocompatibility[257] does not create a high swelling hydrogel if it is not combined with a more water soluble monomer. It is reported that hydrogels based on PHEMA demonstrate low swelling but have very strong mechanical properties, therefore these hydrogels can potentially offer little but stable swelling and they are often used in biomedical and pharmaceutical applications such as implants, contact lens as well as in drug delivery carriers.[258,259,260,261,15]

Hydrogels of PHEMA achieved from bulk or solution polymerisation have a nonporous structure which causes limits in their water content and mass transport.[262] To overcome this issue, the copolymerisation of 2-hydroxyethyl methacrylate with monomers containing ionic groups is highly recommended. Development of synthetic hydrogels based on PHEMA crosslinked with EGDMA began in 1960 and made a revolution in research

developing soft contact lenses.[15,263] These hydrogels reach stable swelling level in aqueous solutions that depends mainly on the crosslink density.[264] The poor oxygen transport and mechanical instability led to further development of PHEMA hydrogels.[263] In the dehydrated state most hydrogels are solid/hard, but as the polymer network in a hydrogel contains hydrophilic groups, it swells in water and causing it to become soft, and to take on elastic properties. It is common that a hydrophilic monomer can be polymerised with other less or more hydrophilic monomers to achieve desired swelling properties. For instance PHEMA was copolymerised with N-vinylpyrrolidone (NVP) of higher than HEMA hydrophilicity,[265] which improved oxygen permeability.

Methacrylic and acrylic monomers react randomly during a free radical polymerisation.[266] It is observed that radical copolymerisation of comonomers with different reactivity leads to compositional heterogeneity.[54] In the case of crosslinking acrylic acid (AA) and HEMA monomers, it is known that in addition to the compositional heterogeneity of created copolymers there will be a second level of heterogeneity of the final system which is linked to the crosslinking density and depends on the cross linker used.[267] The addition of another monomer or crosslinker with a different solubility nature[268,269] can change the swelling ratio of copolymer/hydrogel but on the other hand it can also cause undesirable structural heterogeneity in the end product. Moreover, high concentration of a more water soluble monomer can influence mechanical properties of HEMA. Therefore, aspects such as balance of hydrophilicity and hydrophobicity of the reacting mixture, a gelation time of the reactions, an equilibrium of swelling and mechanical properties need to be taken into account while designing and preparing copolymers.[270]

This work aimed to achieve a good balance of swelling of HEMA copolymers through producing linear and dendritic copolymers by the use of RAFT polymerisation. RAFT method has been used previously to synthesize polymers containing AA and HEMA or HEMA and EGDMA in different compositions/feed ratios and solvents also using different chain transfer agent (CTA).[271,272,273] To our knowledge, a dendritic copolymer containing the three monomers, i.e. AA, HEMA and EGDMA, prepared thru RAFT has not been reported yet.

5.2. Synthesis and characterisation of 2-hydroxyethyl methacrylate and acrylic acid copolymers in the presence or absence of branching agent

In this section RAFT polymerisation was adapted to produce linear and dendritic copolymers of AA and HEMA in the presence or absence of EGDMA as the branching agent. The co-polymer was designed to be biocompatible and non-toxic, so that it can be used as a drug delivery system. Ideally we should see thermo and pH sensitivity within, under physiological pH and temperature. The biodegrability would be a significant advantage but it can be introduced to the system in a later stage.

Reactions were carried using (4-cyano-4[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid, as a RAFT agent (prepared according to section 2.3.5, p.48) in bulk and in organic solvent. Moreover, the conventional free radical polymerisations were also conducted for the comparison. In this work, polymer chain growth was monitored using GPC analysis. A kinetic study of the reactions had been carried out; the aliquots taken for analysis were dissolved in DMF (carrier solvent), filtrated and injected into GPC system, than separated on the columns with a continuous flow of DMF. To analyse the influence of the solvent, initiator, RAFT agent and incorporation of branching agent, a series of reactions have been conducted (Table 5-1 and Table 5-2).

Table 5-1: Copolymerisation of AA and 2-hydroxyethyl methacrylate by RAFT and FRP approach - reaction conditions and properties.

Entry	[M] : [I] : [R][a]	SR[b] (v/v)	Cov[c] %	GPC RI *Mw*[d] kDa	GPC RI PDI[e]	G[f] min	polymer appearance	S[g]	S[h]
1	(20/80):1:0	0	72.6	21.0	2.07	10	whitecrystaline	-	Y
2	(20/80):1:1	0	99.2	35.1	1.81	20	yellow powder	-	Y
3	(20/80):1:1	1:1[B]	87.2	24.2	1.72	80	yellow powder	-	Y
4	(20/80):0.25:0.75	1:1[B]	82.8	27.7	1.93	90	yellow powder	-	Y
5	(20/80):1:1	1:1	93.7	26.3	1.73	180	yellow powder	Y>75^{o}C	Y
6	(20/80):0.25:0.75	1:1	93.2	31.7	1.59	300	yellow solid	-	Y

[a] Monomer feed molar ratio (AA:HEMA) : Initiator (I - AIBN): RAFT agent; [b] Volume ratio of monomer and solvent (DMF or [B] n-Butanone) (v/v); [c] Monomer conversion estimated by GPC; [d] Weight-average molecular weight (kDa – 1,000 g/mol); [e] Polydispersity index (M_w/M_n); [f] Gel time, determined by visual observation; [g] Solubility in water after gelation time; [h] Solubility in DMF after gelation time; linear resultants after purification are soluble in methanol; Polymer appearance were determined by visual observation; Reaction temperature = 65 ^{o}C.

Table 5-2: Copolymerisation of acrylic acid and 2-hydroxyethyl methacrylate by RAFT and FRP polymerisation in the presence of the divinyl monomer EGDMA - reaction conditions and properties.

Entry	[M] : [I] : [R][a]	SR[b] (v/v)	Cov[c] %	GPC RI *Mw*[d] kDa	GPC RI PDI[e]	G[f] min	polymer appearance	S[g]	S[h]
1	(10/80/10):1:1	1:1	37.7	20.4	1.54	65	yellowcrystaline	N	Y
2	(10/80/10):0.25:0.75	1:1	70.3	41.7	3.43	90	yellow powder	-	N
3	(10/80/10):1:1	5:1	88.0	155.2	9.21	270	yellow gel	N	Y
4	(10/80/10):1:1	5:1	83.9	30.4	3.29	160	yellowcrystaline	N	Y
5	(10/80/10):1:1	5:1	96.6	67.9	3.41	140	yellowcrystaline	N	Y
6	(15/80/5):1:1	1:1	77.2	124.5	8.39	85	yellowcrystaline	N	Y
7	(10/80/10):1:0	5:1	-	-	-	25	whitecrystaline	N	Y
8	(10/80/10):1:0	0	-	-	-	4	white hard solid	N	Y

[a] Monomer feed molar ratio (AA:HEMA:EGDMA) : Initiator (I - AIBN): RAFT agent; [b] Volume ratio of monomers and solvent (DMF) (v/v); [c] Monomer conversion estimated by GPC; [d] Weight-average molecular weight (kDa – 1,000 g/mol); [e] Polydispersity index (M_w/M_n); [f] Gel time, determined by visual observation; [h] Solubility in water after gelation time; Solubility in DMF before gelation time; Reaction temperature = 65 ^{o}C;

Firstly, free radical copolymerisation of AA and HEMA (entry 1 in Table 5-1, p.143) in bulk occurred rapidly as the reaction mixture gelled within 10 minutes. Poly dispersity index (PDI) reached 2.07 with conversion above 72% and weight average molecular weight over 21 kDa. After the addition of 1% of RAFT agent (entry 2 in Table 5-1), an increase in gelation time was clearly observed and the copolymer with higher *Mw* (35 kDa) and lower PDI (1.81) was formed at a higher monomer conversion (99.2%). The delayed gel point and the low PDI of the polymers formed were due to the use of RAFT agent which provided better control over the polymerisation comparing to conventional FRP.

The effect of the solvent, the ratio of RAFT agent and initiator

Secondly, the non-protic solvent (n-butanone) was used to conduct solution polymerisation (entry 3 in Table 5-1) to study the effect of a solvent on the controllability of the polymerisations, including the gel time, molecular weight and polydispersity of the polymers. The rest of the parameters were kept the same unless stated in Table 5-1. The use of n-butanone led to a decrease in polymerisation rate, demonstrated by an increased in the gelation time. The samples were taken at t = 0, 10, 20, 30, 60 and 80 minutes for GPC analysis (Figure 5-1 and Figure 5-2).

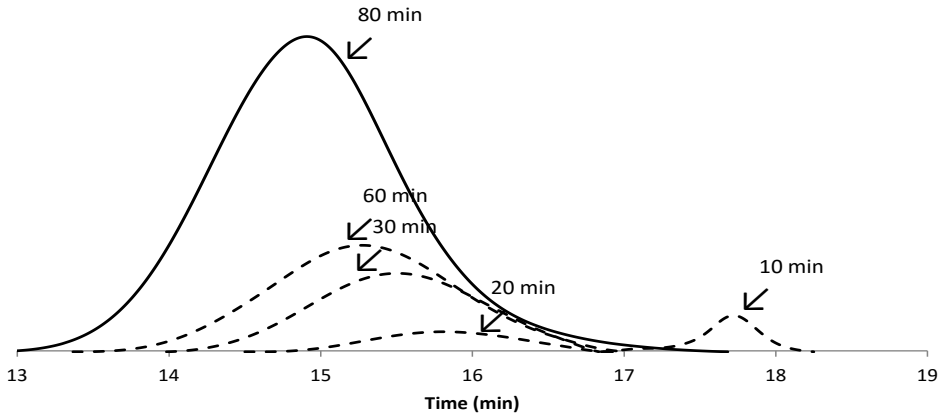


Figure 5-1: GPC traces from RI detector for (AA/HEMA):I:R - (20/80):1:1, entry 3 in Table 5-1) with final molecular weigh 24.2 kDa, a polydispersity 1.7 after 80 min. The reaction was conducted in n-butanone.

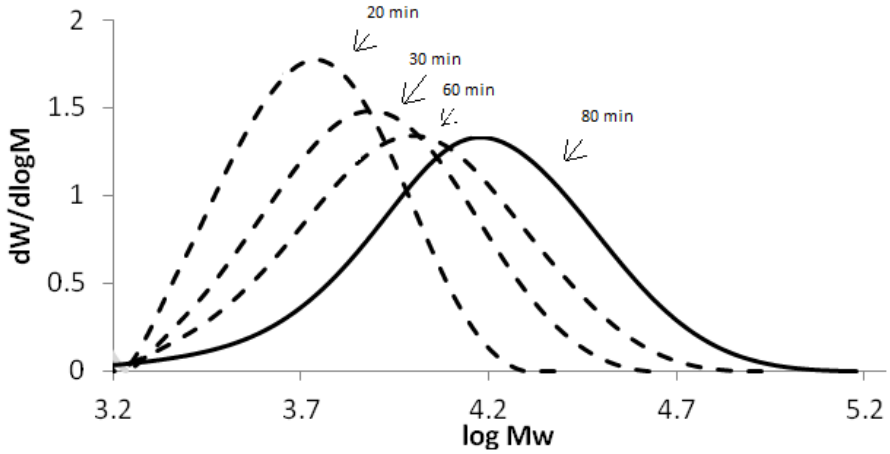


Figure 5-2: Effect of monomer conversion on molecular weight distribution for linear copolymer of AA and HEMA - (AA/HEMA):I:R, (20/80):1:1, entry 3 in Table 5-1 for aliquots taken at time 20, 30, 60 and 80 min respectively. The reaction was conducted in n-butanone.

Figure 5-3 (a, b, c) demonstrated that this reaction (entry 3 in Table 5-1, p.143) was progressing at a controlled manner. Figure 5-3 (a) of conversion against time provides further proof of controllability, with the graph having a relatively stable and linear progression. With longer reaction time, the GPC trace showed the conversion of monomers into polymer, with the significant change in *Mw* shifting peak to higher molecular weight. During the first stage of polymerisation in n-butanone, CTA did not control the reaction well, between 10 to 20 min reaction run as a FRP, an autoacceleration or similar effect occurs and then suddenly RAFT was employed and played role in controllability of the reaction, as seen on Figure 5-1. The relatively constant progression of those peaks shows that at this stage the reaction is more controlled than in entry 1 or entry 2 (Table 5-1).

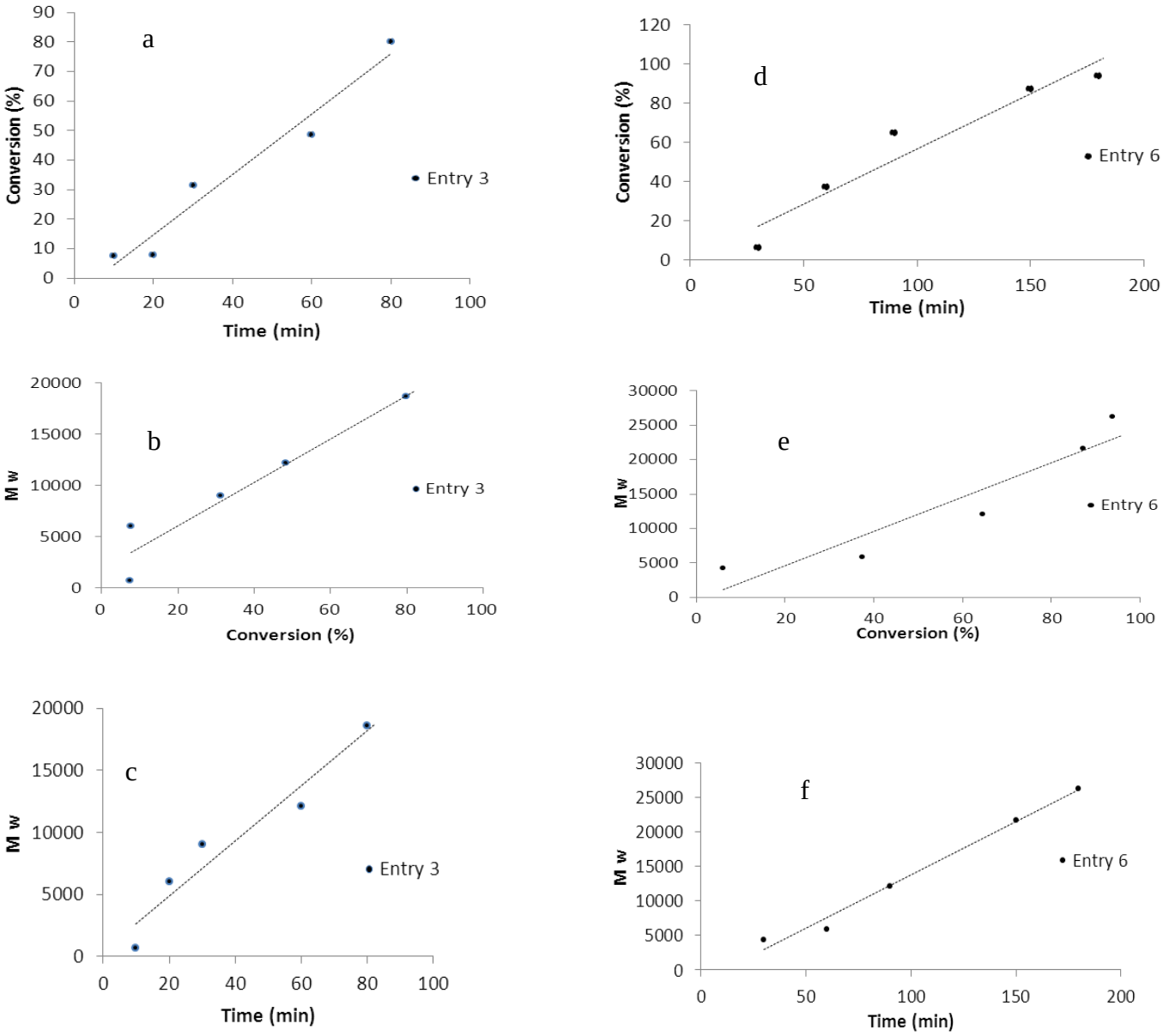


Figure 5-3: Graphs presenting the effect of the solvent on copolymerisation of AA and HEMA, related to (Table 5-1); a, b, c are for the reaction entry 3 in Table 5-1 conducted in n-butanone; d, e, f are for the reaction entry 5 in Table 5-1 conducted in DMF. (a) Conversion *via* Time (entry 3); (b) Weight-average molecular weight *via* Conversion (entry 3); (c) Weight-average molecular weight *via* Time (entry 3); (d) Conversion *via* Time (entry 5); (e) Weight-average molecular weight *via* Conversion (entry 5); (f) Weight-average molecular weight *via* Time (entry 5).

The effect of the ratio of RAFT agent and initiator was also studied (entry 4 in Table 5-1, p.143). In this case a reduced amount of initiator and RAFT was used; samples were taken at t = 0, 30, 60 and 90 minutes. As a result the gelation time increased slightly up to 90 minutes, but showed little effect on the *Mn*, *Mw* or PDI of the polymer. This led to the decision to keep the RAFT agent and initiator levels at 1% mol for subsequent reactions. N-Butanone is a reasonably polar solvent; further research was done in the aprotic more polar solvent DMF, because DMF is a better solvent when EGDMA is introduced to the polymerisation system and delay gelation by slowing the rate at which the initiator and RAFT agent can react with the monomers. To compare the effect of the solvent, the experiments were conducted (entries 5 and 6 in Table 5-1), where the monomer feed molar ratio, initiator and RAFT agent were same as in entries 3 and 4 in Table 5-1, and the only difference was using DMF instead of n-butanone as the solvent. The samples for kinetic study of this reactions were taken at t = 0, 30, 60, 90, 150 and 180 minutes for GPC analysis Figure 5-3 and Figure 5-4), as shown entry 5 (Table 5-1) is comparable to entry 3 (Table 5-1).

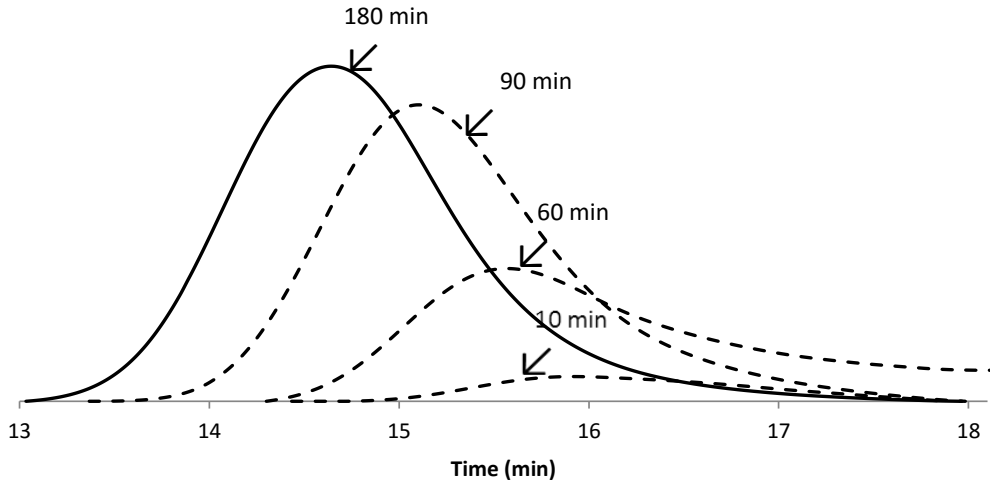


Figure 5-4: GPC traces from RI detector for copolymer of (AA/HEMA):I:R, (20/80):1:1, entry 5 in Table 5-1) with final molecular weigh 26.2 kDa, a polydispersity 1.7 after 180 min. The reaction was conducted in DMF.

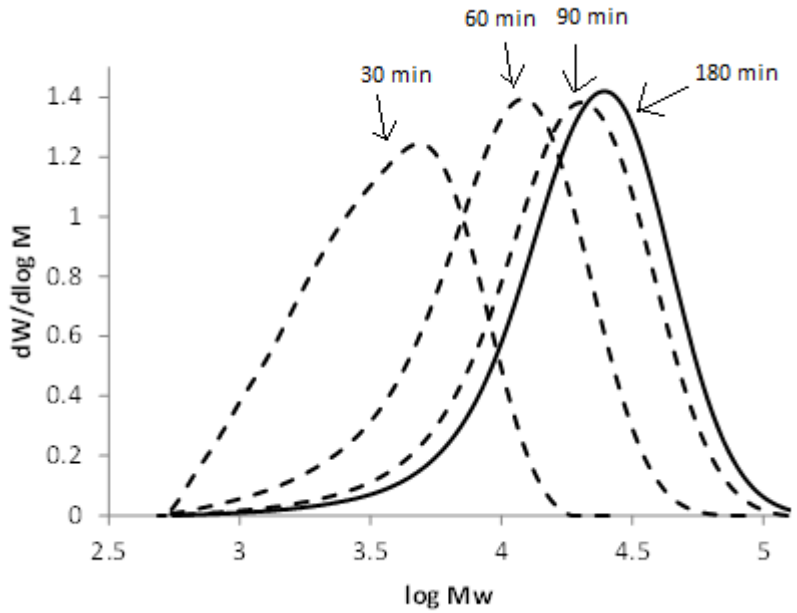


Figure 5-5: Effect of monomer conversion on molecular weight distribution for linear copolymer of (AA/HEMA):I:R - (20/80):0.25:0.75, entry 6 in Table 5-1) for aliquots taken at time 30, 60, 90 and 180 min respectively. The reaction was conducted in DMF.

It was observed that the reaction progressed steadily and controllably (Figure 5-4, Figure 5-5 and Figure 5-3 d, Figure 5-3 e, Figure 5-3 f). The use of DMF instead of n-butanone had a significant effect on the polymerisation rate, and demonstrating a delayed gelation, but had less impact on the molecular weight and PDI of the polymers. Entry 6 (Table 5-1) was conducted by varying the amount of initiator and RAFT agent, in comparison with entry 4 (Table 5-1). The reduced amount of RAFT agent (0.75% mol) and AIBN (0.25% mol) led to a further increase in the gelation time, resulting polymer with higher *Mw* (31 KDa) and a low PDI of 1.59. Samples from t = 0, 60, 150, 180, 240 and 300 minutes are analysed using GPC (Figure 5-6 and Figure 5-7). This reaction produced the polymer with the *Mw* of 31.7 KDa with a high monomer conversion (93%) and a low PDI (1.59). However, the conversion against time presented in Figure 5-6 (c) does not show a good linearity (R^2 = 0.805) comparing Figure 5-3 Figure 5-3 (d) (p.146, thus the molar ratio of RAFT and initiator of 1:1 was used in further studies.

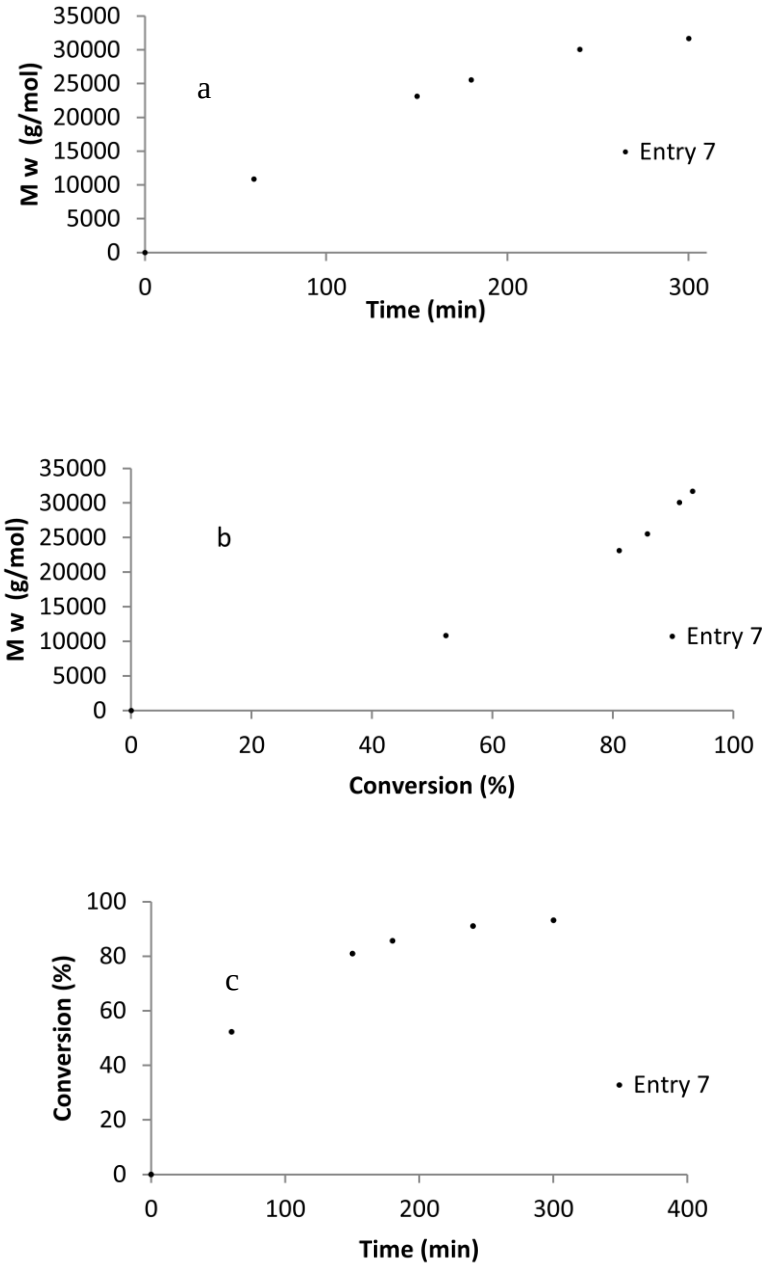


Figure 5-6: Graphs related to Table 5-1, shows (a) Weight-average molecular weight *via* Time (Entry 6, AA/HEMA):I:R - (20/80):0.25:0.75); (b) Weight-average molecular weight *via* Conversion (Entry 6); (c) Conversion *via* Time (Entry 6).

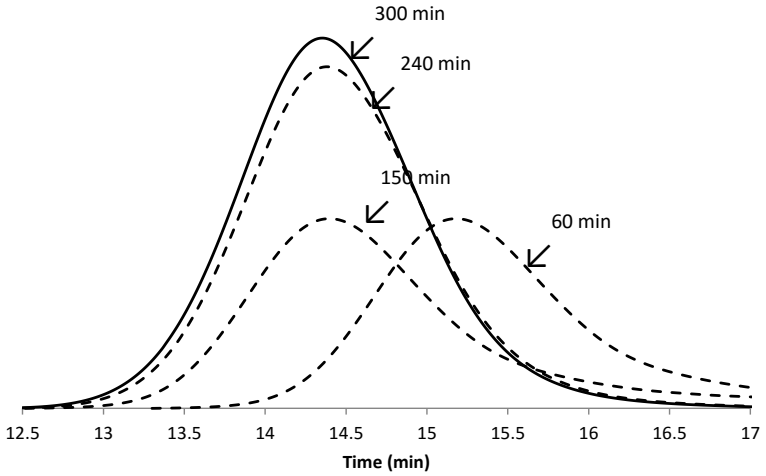


Figure 5-7: GPC traces from RI detector for (AA/HEMA):I:R - (20/80):0.25:0.75, entry 6 in Table 5-1 with final molecular weight 31.7 kDa, a polydispersity 1.6 after 300 min.

Branching agent

The synthesis of hyperbranched polymers using EGDMA as the branching agent was conducted according to Table 5-2 (p.143) by solution RAFT polymerisation. In entries 1-6, DMF was used as the solvent. Solution and bulk free radical polymerisations of AA, HEMA and EGDMA were also conducted as a comparison (entries 7 and 8 in Table 5-2). Entries 1 and 2 of hyperbranched structures can be compared to similar linear structures represented by entries 5 and 6 in Table 5-1, p143. Entry 1 in Table 5-2 had a shorter reaction time than entry 5 in Table 5-1, which indicates the addition of the branching agent (EGDMA) leading to an increase in the polymerisation rate. The *Mw* of the copolymer achieved in entry 1 (Table 5-2) is lower than those for others. When the monomer conversion is taken into account, it is clear that the reaction gelled (crosslinked) at a low monomer conversion (37.7%) where many unreacted monomers were trapped inside the gel network. Product could not be purified by a precipitation method, as proved impossible to dissolve. After drying it in vacuum oven yellow gel like copolymer was achieved.

The reaction listed in entry 2 (Table 5-2) was conducted using the lower amounts of RAFT (0.75%) and initiator (0.25%), as per entries 4 and 6 (Table 5-1). The results of this

reaction when compared to the other related reactions: the *Mw* is higher than in related entries from Table 5-1 but conversion is lower and the PDI much higher. To increase the monomer conversion while obtaining soluble hyperbranched polymers, the amount (DMF) in the reaction was increased from the ratio of 1:1 to 5:1, to delay gelation. Entry 3 (Table 5-2, p.143) is comparable to entry 5 (Table 5-1) but with the ratio of solvent to reactants as 5:1, rather than 1:1. The gelation time was increased dramatically up to 270 minutes from 180 minutes (Figure 5-8). Comparing entry 1 and entry 3 in Table 5-2, it shows that the dilution of the polymer solution suppressed the crosslinking reaction significantly, leading to a soluble hyperbanched polymer with *Mw* of 155 KDa at 88% monomer conversion but a relatively high PDI of 9.21. For the last copolymer sample from this reaction the GPC analysis was difficult to perform due to problems with solubility and pre filtration of polymer before loading on the columns. For that reason the response on GPC trace is much lower in final stage. The high PDI is not uncommon for hyperbranched structures made by RAFT controlled polymerisation. As in the end stage, reaction reached gel point and created copolymer was unable to dissolve in common solvents we conducted another set of two reactions entry 4 and 5 (Table 5-2), in an attempt to reduce PDI of the final polymer by stopping the reaction earlier at time t = 160 and t = 140 respectively. At this stage polymer was still soluble in DMF and it was possible to perform GPC analysis on it.

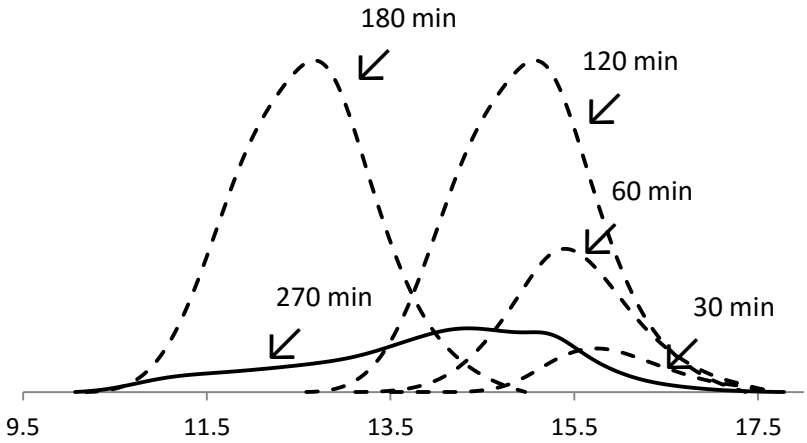


Figure 5-8: GPC traces from RI detector for (AA/HEMA/EGDMA):I:R - (10/80/10):1:1, entry 3 (Table 5-2) with final molecular weigh 155.2 kDa, a polydispersity 9.21 after 270 min.

We aimed to achieve a soluble hyperbranched polymer. From entry 3 (Table 5-2, p.143) it was clear that PDI increased significantly in the later stage of the reaction (see Figure 5-3 a, b, c), and control over reaction was lost, as more likely additional crosslinking occurred. The PDI was below 4 with the conversion 81% at t = 180 minutes. For that reason we repeated synthesis in the same set up but terminated entry 4 and 5 (Table 5-2) before that time. Entry 4 (Table 5-2) had a lower PDI of 3.29, but the *Mw* and conversion were both lower than entry 3 (Table 5-2). This suggested that there might be air in the system which delayed the initiation of radicals, especially when compared to entry 5 (Table 5-2). Entry 5 (Table 5-2) was stopped at t = 140 minutes, where the polymer was still in solution, in comparison to entries 4 and 3 this produced the highest conversion percentage and a reasonably high *Mw* of above 67 kDa. Entry 6 (Table 5-2) was conducted using a different monomer ratio to that of the previous five experiments that involved EGDMA, where the ratios of AA:HEMA:EGDMA was 10:80:10 in each entry. Entry 6 had the ratios changed to 15:80:5. The change in monomer ratios was a further attempt to prolong the reactions times before gel point was reached. Unfortunately, this condition did not produce expected results. The reaction reached gel point within 85 minutes, with twice higher *Mw* of 124 kDa, lower conversion rate and higher PDI than the comparable entry 6 (Table 5-2, p.143), where the only difference between the reactions was the monomer feed ratio. It shows that crosslinking occurred very quickly. Reactions in entry 7 and 8 (Table 5-2) were conducted as a FRP of AA-HEMA-EGDMA, in solvent and in bulk, with no addition of RAFT agent. In both cases gel point was reached very quickly.

Synthesis results

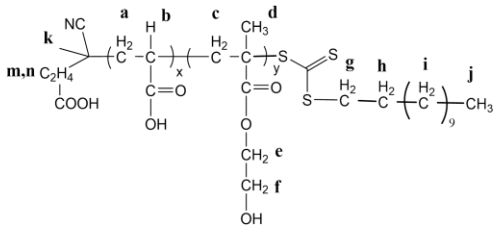
As the result of this work, it is deducted that the more C=C bonds have been converted into C–C bonds following a period of polymerisation time, involving the adjustments in the amount of monomers, RAFT agent, solvent and initiator ratio, the more cross-linking effects were initiated and occurred during the synthesis. The addition of 4[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid) noticeably had an impact on controllability of the linear and hyperbranched structures when comparing them to FRP.

The difference in appearance of final copolymers was also observed. Copolymers made through FRP were white crystalline/white hard solids, where polymers made through RAFT polymerisation were yellow powders, solids or gels. Yellow colour comes from incorporating chain transfer agent into the structure (Scheme 2-12a and Scheme 2-12b, p.58).

RAFT agent segments can be used for chain extension or be modified by aminolysis to introduce thiol functional groups. Linear polymers were soluble in methanol or DMF, but intended dendritic polymers were unable to dissolve in any common organic solvents. It was possible to purify resultants from entry 4 and 5 (Table 5-2) but after drying them in vacuum oven in room temperature, they lost their solubility in solvents. Probably some additional crosslinking occurred in the polymers while handling them. Moreover, linear structures were soluble in pH 7.44 and higher but not soluble in pH 2 and pH 4.

In this work, copolymers of AA and HEMA by RAFT polymerisation were successfully prepared in the absence and presence of EGDMA. They showed a clear shift from the longer retention time to the shorter retention time, indicating the increase of molecular weight with the monomer conversion.

The linear structures of the copolymers were analysed by ^{1}H NMR. The spectra showed the characteristic peaks at chemical shifts of 6.1 and 5.6 ppm which are attributed to the unreacted C=C groups in the copolymer, and some unreacted monomers. The other peaks are assigned as indicated in the Figure 5-9. The chemical shift bellow 1.5 ppm in ^{1}H NMR spectrum confirmed the existence of RAFT agent within the polymer structure.



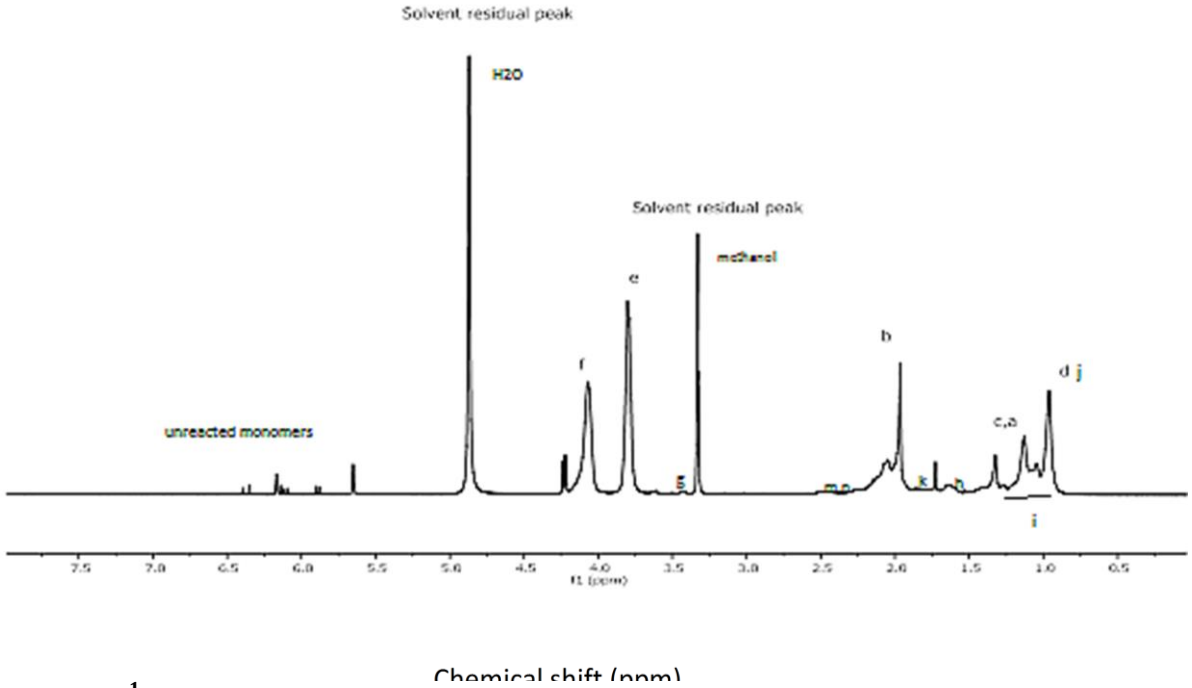


Figure 5-9: ^{1}H NMR of linear structures of acrylic acid and 2-hydroxyethyl methacrylate copolymers (in methanol).

Attempts were also made to characterise the final dendritic/hyperbranched copolymers of AA – HEMA – EGDMA structurally by ^{1}H NMR but due to their insolubility in water and in any common organic solvents (including chloroform, tetrahydrofuran, dimethylformamide, acetone, acetonitrile, ethanol, cyclohexane) we could not perform this analysis; solid state NMR would be an option but unfortunately it was not available at the time of this study.

The FTIR spectra of the selected copolymers are shown in Figure 5-10 and Figure 5-11. We clearly see that functional groups from linear and branched structures are overlapping and for that reason FTIR was used mainly to detect additional groups introduced through RAFT and they are visible on spectres.

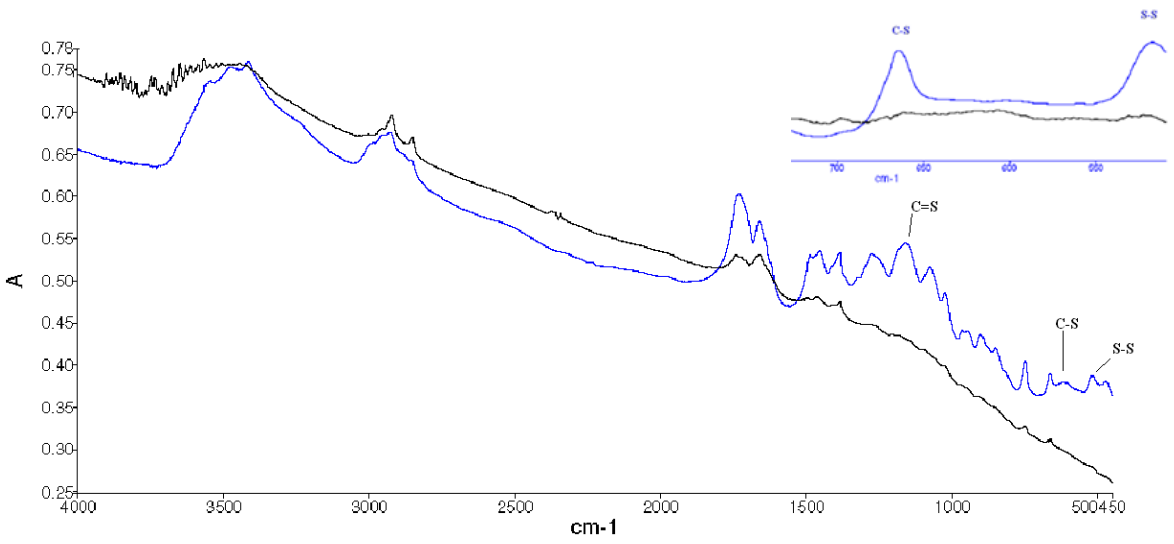


Figure 5-10: FTIR spectra of branched structures made by RAFT - blue (entry 5 (Table 5-2)) and FRP without the use of RAFT agent - black (entry 7 (Table 5-2)), respectively.

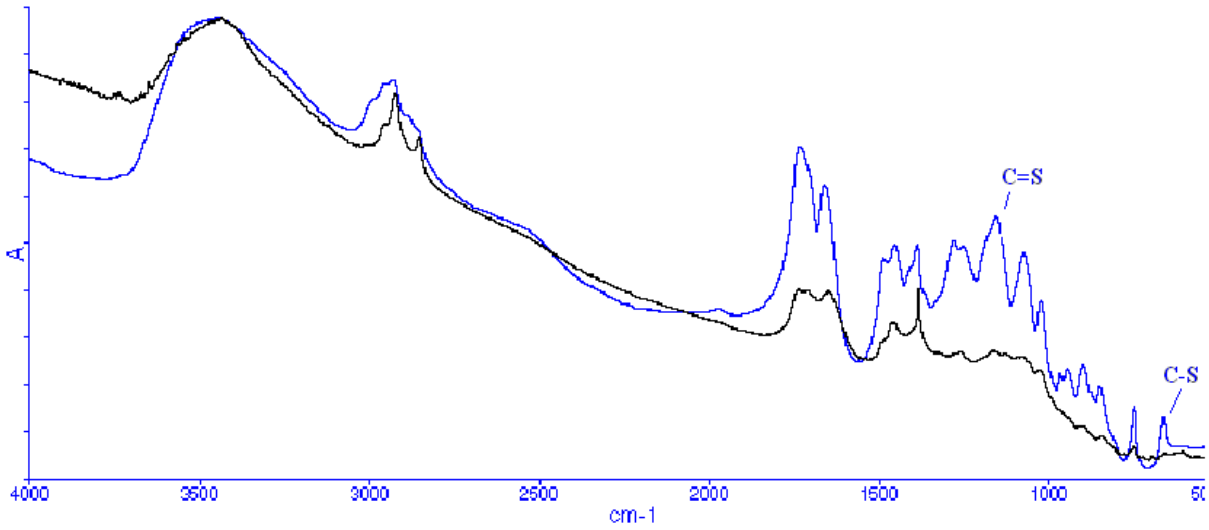


Figure 5-11: FTIR spectra of linear structures made by RAFT - blue (entry 6 in Table 5-1) and FRP - black (entry 1 in Table 5-1), respectively.

The broad peak at 3440 cm^{-1} was attributed to OH stretching, while OH bending was seen at region 1073 cm^{-1}. The band of aliphatic CH, CH_2 asymmetric and symmetric stretching peaks were observed at 2958 and 2864 cm^{-1} respectively. The strong band at 1453 cm^{-1} corresponded to CH, CH_2 bending. The characteristic C=O stretching was seen at 1730 cm^{-1} with small shoulder around 1652 from stretching C=C. The peaks between 1359 and 1078 cm^{-1} were assigned to C-C-O and O-C-C ester stretching vibrations. The functional absorption band for C-S group from RAFT agent was seen in 660 to 690 cm^{-1}, weak S-S groups in 500 to 540 cm^{-1} also stretching C=S seen in 1250 cm^{-1} region . The presence of chemical groups in structures of the resultant copolymers were confirmed by FTIR study and incorporation of RAFT in the structure was observed. Even though analysed copolymers presented a similar pattern of spectra, the peak intensities were strongly dependent on the reaction conditions and KBr pellets preparation.

5.3. Swelling and pH response

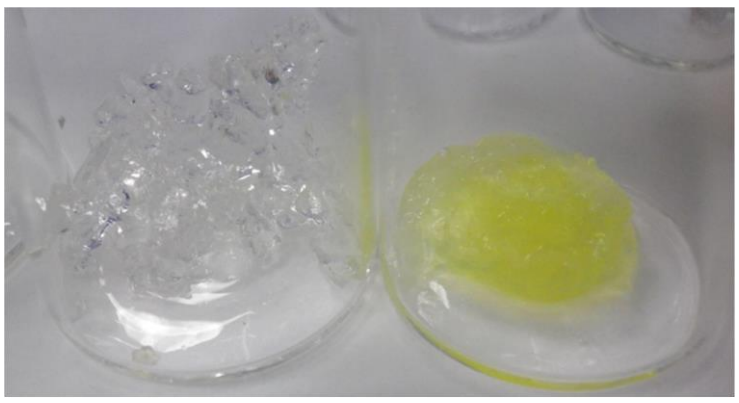


Figure 5-12: Picture of HEMA-AA-EGDMA hydrogel prepared from copolymers synthesised by the a) FRP and b) conventional RAFT process.

As HEMA-AA-EGDMA copolymers were not soluble in solvents, in order to assess swelling behaviour, selected samples were immersed in the required solvent solutions. Dry samples were weighted individually before being immersed into 3 mL of the solvent at

different pH for the time required (pH 4; pH 7 deionised water; pH 7.4 phosphate buffer). The excess solvent was removed and then the samples were weighted at regular time intervals. Measurements were performed in triplicates; the weights of the swollen samples were recorded on a digital balance at each time point.

Responsive polymers can be used to create hydrogels through a variety of interactions. Commonly hydrogel can be formed by self-assembly formation of polymers. In responsive hydrogels the ratio of pH sensitive and/or thermo sensitive polymers must be balanced correctly, to make sure that the polymer can respond in the true physiological settings.[22] Increase of temperature can cause decrease in hydrogen bonding with the surrounding environment of water and subsequently polymer can form physical crosslinking and self-assemble into hydrogel.

The swelling data of the studied HEMA copolymers were conducted at room temperature (read on the day of the study), are presented on Figure 5-13, Figure 5-14, Figure 5-15 and Figure 5-16. The SD are listed in Table 5-3, Table 5-4, Table 5-5 and were affected by the technique of solvent removal as well as by the nature of hydrogels.

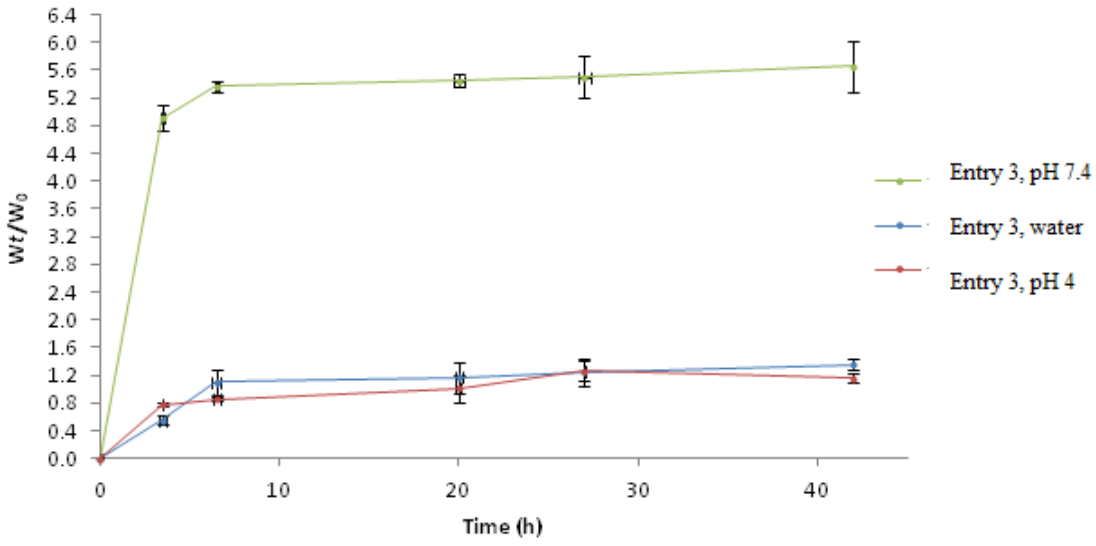


Figure 5-13: Swelling studies carried out on copolymer of AA:HEMA:EGDMA prepared *via* RAFT polymerisation (pH 4), neutral water and PBS buffer (1M, pH 7.4) at 20 ^{o}C. The number of test samples was 3 in each case. (AA/HEMA/EGDMA):I:R - (10/80/10):1:1, SR=5:1, entry 3 in Table 5-2.

Table 5-3: SD errors for swelling studies (in Figure 5-13) on copolymer of AA:HEMA:EGDMA prepared *via* RAFT polymerisation.

Time point (h)	SD (pH 4)	SD (pH water)	SD (pH 7.4)
3.5	0.18182	0.05634	0.03834
6.5	0.07348	0.17599	0.02649
20	0.10424	0.22311	0.20223
27	0.30771	0.19009	0.15746
42	0.36491	0.07221	0.07041

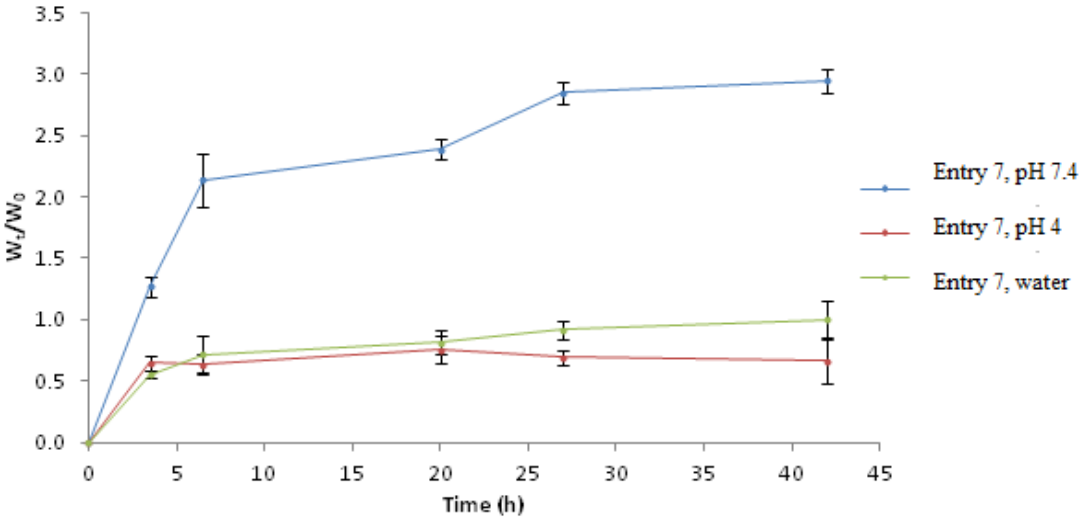


Figure 5-14: Swelling studies carried out on copolymer of AA:HEMA:EGDMA prepared *via* FRP polymerisation in pH 4 water, neutral water and PBS buffer(1M, pH 7.4) at 20 ^{o}C. The number of test samples was 3 in each case. (AA/HEMA/EGDMA):I:R - (10/80/10):1:0, SR=5:1, entry 7 in Table 5-2.

Table 5-4: SD errors for swelling studies (in Figure 5-14) on copolymer of AA:HEMA:EGDMA prepared *via* FRP polymerisation.

Time point (h)	SD (pH 4)	SD (pH water)	SD (pH 7.4)
3.5	0.05432	0.03495	0.08040
6.5	0.08161	0.14533	0.21354
20	0.11174	0.10217	0.08194
27	0.06064	0.07552	0.09262
42	0.17342	0.15053	0.09865

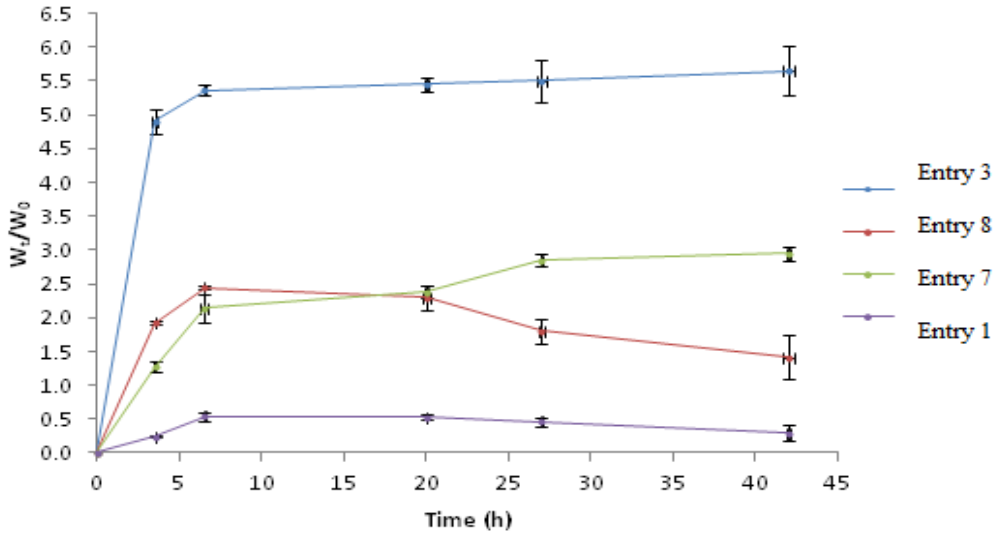


Figure 5-15: Swelling studies carried out on copolymers of AA:HEMA:EGDMA in PBS (1M, pH 7.4) at 20 ^{o}C. The number of test samples was 3 in each case. (AA/HEMA/EGDMA):I:R, entries: 1 - (10/80/10):1:1, SR=1:1; 3 - (10/80/10):1:1, SR=1:1; 7 - (10/80/10):1:0), SR=5:1 and 8 - ((10/80/10):1:0), SR=0 in Table 5-2.

Evident swelling was observed also in water comparing to other copolymers. Figure 5-15 shows some loss of integrity or stability of the copolymers represented by entry 8 and 1 (Table 5-2, p.143). This could be due to insufficient purification of the samples, thus when performing swelling the unreacted monomers were still present in the structure and were released or dissolved with time. When a dry polymer/hydrogel begins to absorb water molecules enters the matrix to hydrate hydrophilic groups (the most polar groups) leading to primary bound water. As the polar groups are hydrated network swells and exposes hydrophobic groups which hydrophobically (secondary) bound with water. Both primary and secondary bound water equals total bound water. The additional water that is absorbed beyond total bound water is called free (bulk) water. As the network swells, and network or crosslink chain are degradable, the gel can disintegrate and dissolve at the rate depending on its composition.

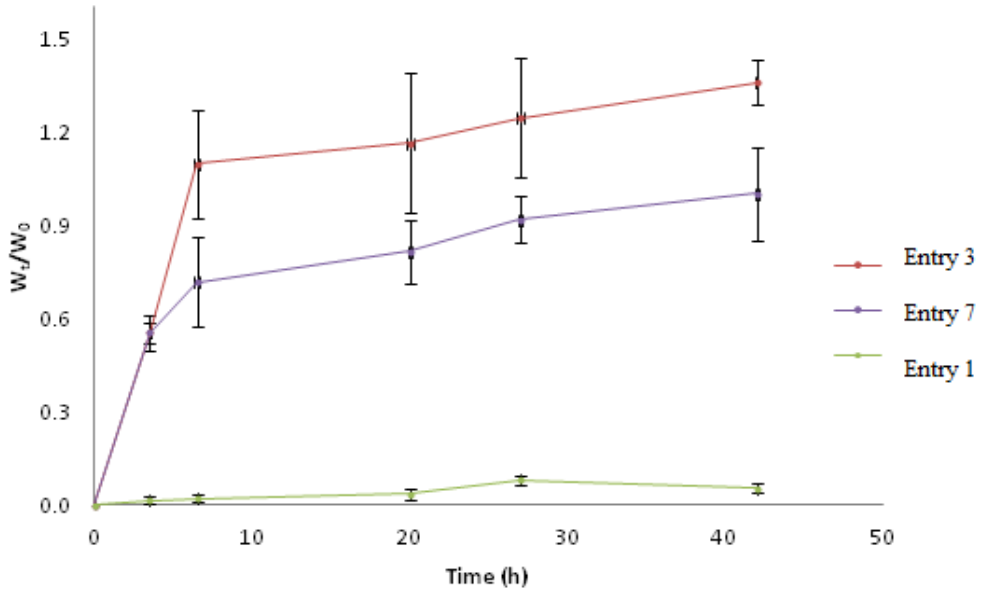


Figure 5-16: Swelling studies carried out on copolymers of AA:HEMA:EGDMA in neutral water at 20 °C. The number of test samples was 3 in each case. AA/HEMA/EGDMA):I:R, entries 3 - ((10/80/10):1:1, SR=1:1); 7 - ((10/80/10):1:0, SR=5:1); 1 - ((10/80/10):1:1, SR=1:1) in Table 5-2.

Table 5-5: SD errors for swelling studies (in Figure 5-15 and Figure 5-16) on copolymers of AA:HEMA:EGDMA.

	Entry 3	Entry 8	Entry 7	Entry 1	Entry 3	Entry 7	Entry 1
Time point (h)	SD (pH 7.4)	SD (pH 7.4)	SD (pH 7.4)	SD (pH 7.4)	SD (pH water)	SD (pH water)	SD (pH water)
3.5	0.03834	0.01542	0.08040	0.01647	0.05634	0.03495	0.01185
6.5	0.02649	0.02426	0.21354	0.06183	0.17599	0.14533	0.01194
20	0.20223	0.18656	0.08194	0.04063	0.22311	0.10217	0.01730
27	0.15746	0.18522	0.09262	0.05650	0.19009	0.07552	0.01684
42	0.07041	0.32499	0.09865	0.11786	0.07221	0.15053	0.01296

Significant swelling was observed in PBS buffer pH 7.4 for hyperbanched copolymer prepared according to entry 3 (Table 5-2) which is illustrated in Figure 5-15 and Figure 5-16. The difference in pH made large difference in the swelling ratio of this copolymer.

As it is shown in Figure 5-13 (p.157) the polymer did not swell appreciably in acidic pH 4 and swelling did not change significantly in natural water of pH 6.8, but changing pH to 7.4 increased swelling of this polymer almost five times at room temperature.

Swelling at 37 ^{o}C was also carried out for copolymers of AA:HEMA with EGDMA synthesized through FRP and RAFT solution polymerisations (ratio 5:1), and the results are presented in Figure 5-17.

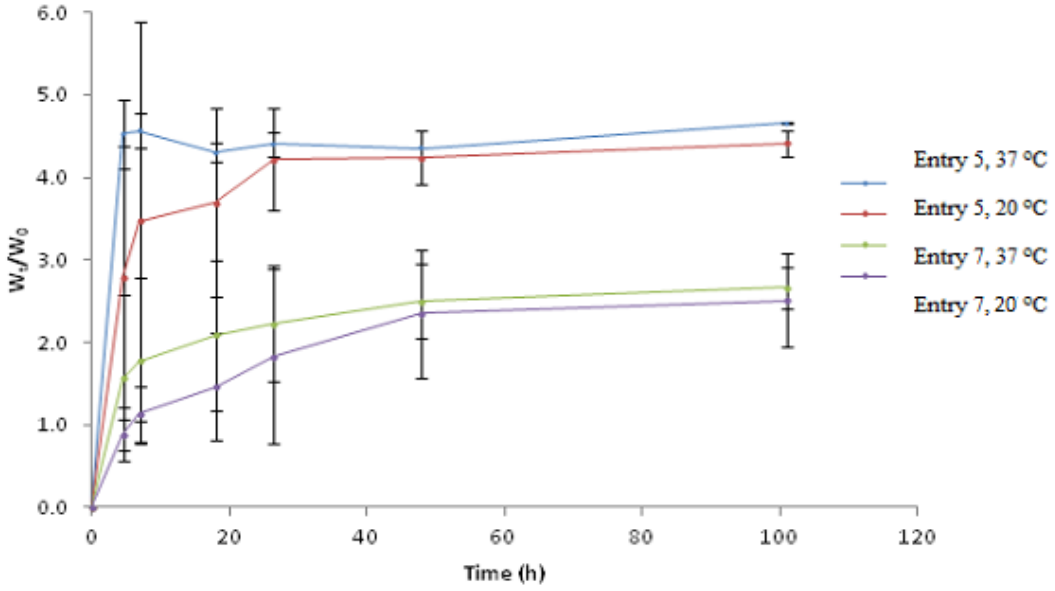


Figure 5-17: Swelling studies carried out on copolymers of AA:HEMA:EGDMA in PBS (1M, pH 7.4) at 37 ^{o}C. Comparing the hyperbranched copolymers prepared by RAFT (Entry 5) and FRP (Entry 7) polymerisation in solution ratio 5:1. The number of test samples was 3 in each case. AA/HEMA/EGDMA):I:R, entries 5 ((10/80/10):1:1, SR=5:1) and 7 (10/80/10):1:0, SR=5:1) in Table 5-2.

Copolymers synthesized by RAFT, showed enhanced swelling comparing to copolymers prepared by FRP, moreover swelling of those samples increased slightly at a higher temperature. A sharp increase in the swelling of the hydrogel was seen at the start, then in 3, 6, 9 and 12 hours swelling still increased but at the lower rate and after this point the gels reached an equilibrium state.

It has been demonstrated in this work that the swelling of the prepared polymers is linked to polarity of copolymer, its composition (hydrophobic and hydrophilic) and time. Acrylic acid is hydrophilic and can be referred to as a "polyelectrolyte" due to the carboxylic acid group within its structure.[274] This weak poly acid contributes to the pH sensitivity of the copolymer by accepting protons at a low pH but releasing them in neutral or high pH environments. Tests have confirmed that prepared copolymers of AA-HEMA-EGDMA have no swelling at low pH levels (pH 2 or pH 4), but there was some swelling of the polymer as the pH reached a neutral level at water (pH 6.8) and evident swelling in PBS buffer (pH 7.4).

These results are in agreement with literature, which says that the protons are lost at higher pH, where the carboxylic groups would become ionised and there would be electrostatic repulsion within the polymer, forcing it to increase in size.[22,21]

5.4. Thermal stability

Thermal decomposition of dried HEMA copolymers was investigated and stability data for selected AA-HEMA hydrogels prepared by RAFT and FRP polymerisation in the presence or absence of EGDMA were studied. Thermal analysis of the resultants was carried out to determine the degradation temperature and also the weight loss behaviour during continuous heating over a period of time. The temperature corresponding to 5% weight loss is defined as the initial degraded temperature of polymer (Td). The Td values for the curves presented in Figure 5-18 are listed in Table 5-6.

Table 5-6: Temperature (^{o}C) for 5% weight lost for AA-HEMA prepared *via* RAFT and FRP polymerisation.

Td (^{o}C)	Curve
73	Entry 5 (Table 5-1)
186	Entry 7 (Table 5-2)
208	Entry 3 (Table 5-2)
209	Entry 1 (Table 5-2)
288	Entry 5 (Table 5-2)

This values indicated that thermal stability of branched copolymers of AA-HEMA prepared by RAFT polymerisation in the same composition and the same solvent volume was much better than that of one prepared through FRP (compare entry 5 in Table 5-2 and entry 7 in Table 5-2). This clearly reflected the higher crosslinking in the copolymers.

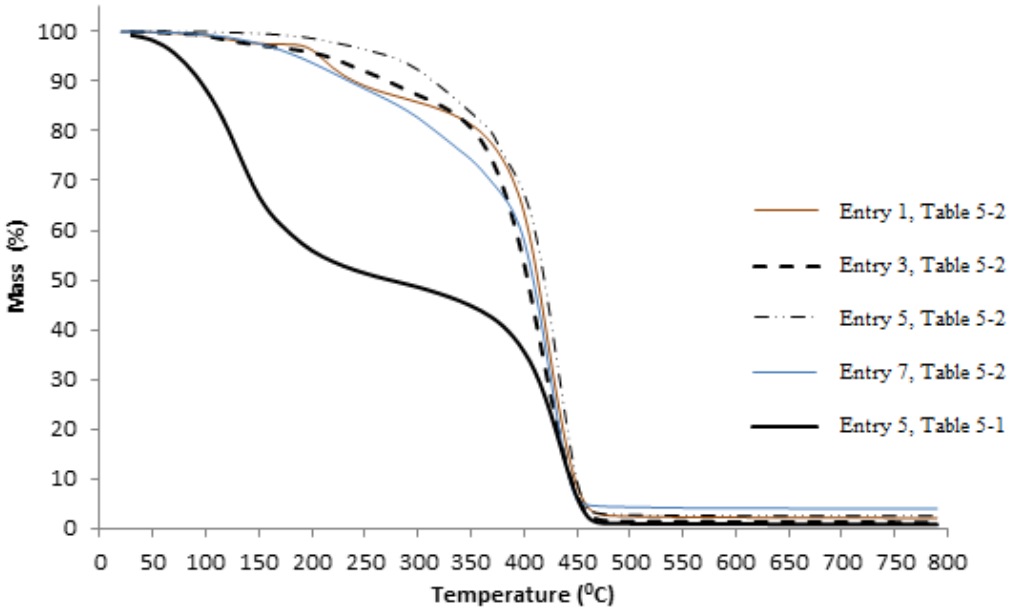


Figure 5-18: TGA curves of selected samples: branched copolymers entries 1, 3, 5, 7, respectively in Table 5-2; linear copolymer entry 5 in Table 5-1.

Selected Tg traces are shown in Figure 5-18, it is important to note that this set of data needs to be repeated. These analyses have to be done by cooling samples to -90 and heating up to maximum 100 ^{o}C. The reason for it is coming from the information given in above data, as it is seen copolymers start to lose mass and decompose above 100 ^{o}C. The weight loss was observed at different temperature regions and branched polymers were stable at temperatures below 100 ^{o}C, while we could observe less than 1% weight loss in analysed samples. The linear structures demonstrated lower stability below 100 ^{o}C and up to 12% weight loss was observed. This is associated with the evaporation of physically absorbed water in copolymers or compounds with low molecular mass.

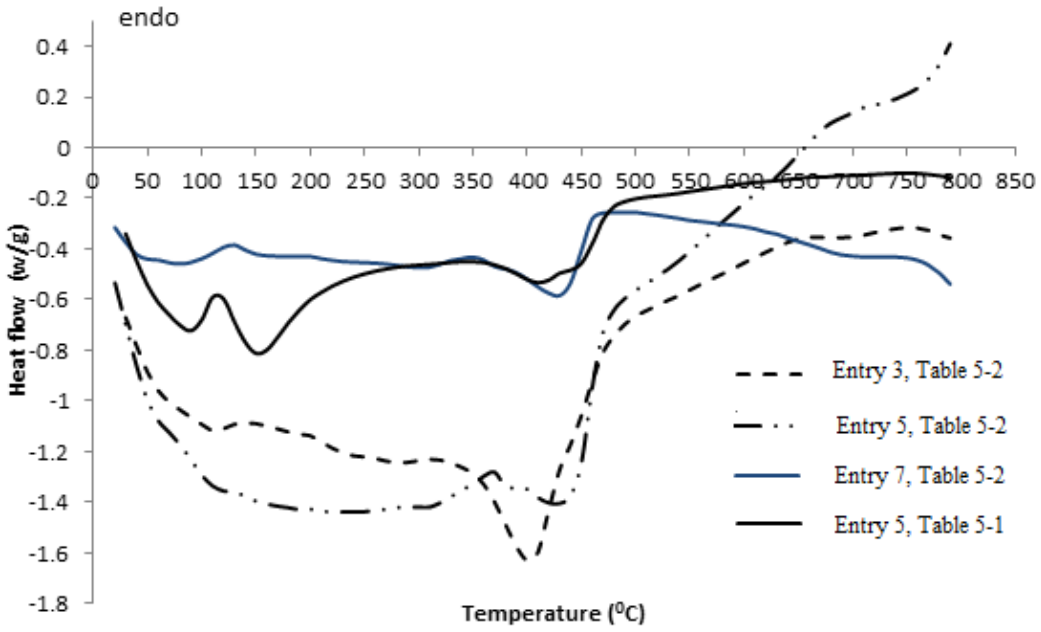


Figure 5-19: DSC thermograms for selected samples: branched copolymers entries 3, 5, 7, respectively in Table 5-2; linear copolymer entry 5 in Table 5-1.

Relatively slow weight loss (1 up to 17% loss) was seen in areas between 100 and 300 ^{o}C for branched structures and much higher weight loss for linear structures in this area (up to 51% loss). This could be associated to loss of water as it is possible that polymers did not dry completely, or it is also possible that purification of the polymers was not sufficient (which is also indicated on ^{1}H NMR spectrum), and in the first stage monomers with low molecular mass evaporated or decomposed. Rapid weight loss was undoubtedly seen in the temperature range above 300 up to 470 ^{o}C where up to 98% weight loss occurred and copolymers degraded. We can clearly see that the addition of EGDMA into the system increased thermal stability of the copolymers.

5.5. Solubility

This work aimed to prepare soluble copolymers of AA and HEMA with well controlled molecular weight (i.e. low PDI) at reasonable polymerisation rate (ideally to control the gelation time at about 5-24 hours).

Solubility of resultant copolymers of AA and HEMA was tested in water, methanol and in DMF. Data is presented in Table 5-1 and Table 5-2, p.143. Solubility is regarded as an important factor if the polymers/hydrogels are meant to be used in drug delivery applications.

Solubility of linear polymers prepared through RAFT polymerisation, was tested in water at the concentration of 100mg/ 1 mL and showed pH = 7.4 from 0 ^{o}C to 100 ^{o}C. The results showed the majority of the tested samples were not soluble under those conditions. Only one reaction produced linear polymer soluble in water which is entry 5 in Table 5-1, p.143. However, solubility was observable only at temperature above 75 ^{o}C (in pH=7.4 and higher but not soluble in pH 4 or lower). Dendritic polymers prepared by RAFT polymerisation of AA, HEMA and EGDMA (entry 1-6 in Table 5-2, p.143), were not soluble in water either. All linear polymers after purification were soluble in methanol and DMF at room temperature. Dendritic polymers were not soluble in methanol, and solubility of samples in DMF was lost when sample gelled. Importantly, final hyperbranched/dendritic structures were unable to dissolve in any common organic solvents. As mentioned in section 5.2, it was possible to purify resultants from entry 4 and 5 (Table 5-2) but after drying them in vacuum oven at room temperature, they lost their solubility. This could be due to crosslinking occurred during purification and drying procedures. Solubility of the prepared samples is a key aspect which should be further investigated.

Chapter 6 Conclusions and future work

6.1. Introduction

In this chapter, the summary of research results and general conclusions of work conducted for the preparation of this thesis are described. The main experimental findings are presented and discussed in chapters 3, 4 and 5. The work consists of four main parts: 1) development of an *in-situ* RAFT polymerisation approach; 2) synthesis, characterisation and property evaluations of new thermoresponsive hyperbranched polymer thru *in-situ* RAFT; 3) synthesis, characterisation and property evaluations of degradable PEGMEMA-PPGMA-DSDA copolymers prepared by RAFT polymerisation and 4) synthesis, characterisation and property evaluations of pH responsive dendritic hydrophilic polymers synthesized using RAFT copolymerisation.

6.2. Development of an *in-situ* Reversible-Addition Fragmentation Chain Transfer approach

The main objective is to establish a well-controlled, optimal reaction system where 2-cyanoprop-2-yl dithiobenzoate RAFT agent can be created *in-situ* and provide good control over polymerisation. Based on this objective, in section 3.1, the kinetics of one-pot and two-step *in-situ* RAFT polymerisations of MMA and St were studied in comparison to conventional RAFT approach. We focused on fine tuning of the *in-situ* synthesis. The impacts of variation of temperature, solvents, and ratio of initiator, on the control of the polymerisation and the properties of the final polymers obtained were assessed. An assessment of the results presented leads to the conclusions that 1) in the presence of disulphide, the FRP of vinyl monomers initiated by AIBN is inhibited; 2) RAFT agent was successfully formed *in-situ*; 3) reactions demonstrated similar controllability to

conventional method; 4) increasing the temperature significantly speed up the process, reducing time and cost of the synthesis; 5) PMMA and PSt prepared through *in-situ* approach successfully worked as a macro RAFT agent.

This *in-situ* route was successfully applied in the synthesis and produced polymers with relatively well controlled molecular weights.

6.3. Thermoresponsive hyperbranched polymer prepared *via in-situ* Reversible-Addition Fragmentation Chain Transfer polymerisation

The *in-situ* RAFT method has demonstrated good controllability over polymerisations of MMA and St as presented in section 3.1 and then in copolymerisation of PEGMEMA, PPGMA and EGDMA presented in section 3.2. The resultant copolymers of PEGMEMA-PPGMA-EGDMA from the *in-situ* RAFT were characterised by GPC and ^{1}H NMR analysis. The results confirmed that the copolymers exhibited multiple methacrylate groups and a hyperbranched structure, as well as RAFT functional residues. High levels of branching (up to 34%) and vinyl functionality (up to 27%) were achieved by utilizing high concentrations of multifunctional vinyl monomer EGDMA. The functional groups conferred useful properties to the polymers and can be used for further macromolecule design. These novel PEGMEMA, PPGMA and EGDMA water-soluble copolymers with tailored compositions demonstrated tuneable lower critical solution temperature (LCST) from 22 ^{o}C to 32 ^{o}C. In addition, the successful aminolysis of the copolymers has been achieved and we concluded that phase transition temperature can be altered by the introduction of thiol groups into polymer chains.

In-situ RAFT approach successfully used in the synthesis of novel hyperbranched PEGMEMA-PPGMA-EGDMA copolymers has many advantages over ATRP approach and conventional RAFT approach. We have provided the evidence that it simplifies the polymerisation and purification procedures by eliminating final purification and reaction steps, for example, the purification steps for removal of metal catalyst needed for ATRP approach and column chromatography needed for conventional RAFT approach. This *in-situ* RAFT polymerisation provided a facile and versatile strategy for the preparation of

thermoresponsive hyperbranched polymeric materials from commercially available vinyl monomers.

The synthetic pathway is very valuable but despite the successful study on dithiobenzoate-mediated polymerisation presented in chapter 3 (sections 3.1 and 3.2) the method cannot be generalized for all RAFT processes without further tests as results might differ and will depend on monomers, initiators used and the leaving group (-R) of created *in-situ* RAFT agent.

6.4. Degradable and thermoresponsive copolymers prepared *via* Reversible-Addition Fragmentation Chain Transfer polymerisation

New degradable and thermal responsive PEGMEMA-PPGMA-DSDA hyperbranched copolymers were successfully prepared using 2-cyanoprop-2-yl dithiobenzoate as the RAFT agent. The molar feed ratios of monomers were varied to tailor polymer properties and fine tune the LCST of the product between 17 and 56 ^{o}C. It has been reported that high monomer conversions (up to 95%) and a high degree of branching (up to 31 mol %) were both achieved. The polymerisation process was carefully studied by GPC and polymer compositions were calculated from peak integrations according to ^{1}H NMR analysis. No crosslinking or micro gelation was observed during the synthesis and purification processes. The copolymer solutions were found to form physical gels at the concentration about 20% w/v (and above) when the temperature was raised beyond their LCST. In addition, due to the presence of multifunctional vinyl monomer in polymer structures, Michael addition-type reaction was successfully employed to form chemical gelation.

Although a relatively high degree of branching (up to 31%) was achieved in the synthesized copolymers without gelation, a low level of free vinyl groups was obtained (about 1% molar ratio) which resulted in a very soft/mellow chemically crosslinked hydrogels with weak stability and integrity. For this reason, in the study described in chapter 4 (section 4.5), we were not able to observe the porous structure of chemically crosslinked gels due to amorphous state and nature of the samples. To overcome these limitations, other

approaches might be employed for the polymer synthesis and hydrogel preparation, some of which are discussed in following section as future recommendations.

6.5. pH responsive dendritic hydrophilic polymers synthesized *via* Reversible-Addition Fragmentation Chain Transfer copolymerisation

To our knowledge, a dendritic copolymer containing the three monomers, i.e. AA, HEMA and EGDMA, prepared by RAFT has not been reported to date. The objective of this part was to produce model, pH responsive, dendritic hydrophilic polymers through RAFT polymerisation where HEMA and AA copolymers were synthesized in the presence or absence of EGDMA, with tailored swelling and release profile. In chapter 5 we reported successfully prepared polymers/hydrogels. The addition of EGDMA into the system increased thermal stability of the copolymers. The hydrogels from the resultant linear and dendritic copolymers demonstrated responsive properties at different pH values and temperatures in swelling studies. The responsive behaviours of these hydrogels have also been compared to the hydrogels prepared directly from crosslinking of AA, HEMA and EDGMA monomers. Copolymers synthesized by RAFT, showed enhanced swelling comparing to copolymers prepared by FRP, moreover swelling of those samples increased slightly at a higher temperature. Tests have confirmed that prepared copolymers of AA-HEMA-EGDMA have no swelling at low pH levels (pH 2 or pH 4), but there was some swelling of the polymer as the pH reached a neutral point for water (pH 6.8) and evident swelling in PBS buffer (pH 7.4). It was also found that the hydrogels from copolymers of AA, HEMA and EGDMA demonstrated thermal and pH responsive properties, which were significantly affected by the chemical composition and topological structure of polymer chains.

In this project the solubility of prepared dendritic polymers and hydrogels presented a major challenge. Linear polymers were soluble in methanol or DMF, but intended dendritic polymers were unable to dissolve. Attempts were made to analyse the resultant dendritic samples by NMR but due to their insolubility in water and in any common organic solvents (including chloroform, tetrahydrofuran, dimethylformamide, acetone, acetonitrile, ethanol,

cyclohexane) we could not perform this analysis; solid state NMR would be an option in any future study. The difficulty caused by crosslinking mentioned previously, also affected purification process of the synthesized copolymers. We observed that e.g. Figure 5-15, p.159, shows some loss of integrity or stability of the copolymers as represented by entry 8 and 1 (Table 5-2, p.143). This could be due to insufficient purification of the samples, thus when performing swelling the unreacted monomers were still present in the structure and were released or dissolved with time. The lack of biodegrability in this system should be also considered.

6.6. Future work

Currently, to obtain hyperbranched polymers with well-defined and controlled molecular weights, and low PDIs in single step reaction is challenging. Hence, we strongly believe that the i*n-situ* RAFT system, as presented, opens up many possible doors, and polymers produced by this method might have a variety of functionalities including RAFT agent moieties which could supply new properties. Moreover, this route could be adapted by the polymer industry, with its aim of design and synthesis of polymers with structural complexity, conferring the advantage of reduction of the costs and time. The ease of the synthesis and multifunctionality of the resultant products will have a major impact on the preparation and application of functional hyperbranched materials. Such materials have great potential to be used as smart polymerisable precursors and building blocks for the design of functional materials with wide application, such as injectable scaffold and soft nanomaterials for tissue engineering, drug delivery, and diagnostics. We believe that this simple and convenient method is of great applicability and should be further tested using different monomers (such as poly(glycolic acid), poly(lactic acid), poly(lactide-co-glycolide)), initiators and different disulphide based RAFT agents.

Water-soluble thermoresponsive vinyl functional polymers PEGMEMA-PPGMA-EGDMA from *in-situ* RAFT have the potential for further modification. Individual hydrogels can be modified with synthetic short (RGD) adhesion peptides. Polymer bio-compatibility and cell adhesion can also be assessed. Cell culture experiments might be initially carried out using e.g. C2C12 myoblast cell lines or/and mouse 3T3 fibroblast to start with. Cell

adhesion can be assessed on the basis of analysis of cellular morphology. Cytotoxicity study for hydrogels can also be studied.

As a further study on of the reported PEGMEMA-PPGMA-DSDA copolymers, the amount of the free vinyl groups and branching degree could be tailored by changing the molar ratio of PPGMA, DSDA and PEGMEMA in the synthesis. Increase of free vinyl groups should significantly improve the reactivity during the Michael addition with consequent changes in the substantial form of the resulting hydrogel. Besides, further study on gelation conditions using the Michael addition-type reaction would be welcome. Crosslinking kinetics of these hyperbranched polymers and mechanical properties of the hydrogels can be studied using rheometry. Such gels may be optimised for tissue engineering applications that require different softness, pore sizes and porosity. Further investigation of the polymer behaviour after cleavage of disulphide bonds is required and studies involving particle size measurement of the samples would give the advantage of knowing what has happened in the polymer solutions after DTT treatment. Likewise, developed in chapter 3 and succesfuly used in copolymerisation of PEGMEMA-PPGMA-EGDMA i*n-situ* RAFT system could be adopted for the PEGMEMA-PPGMA-DSDA copolymer synthesis.

In the case of HEMA and AA copolymers synthesized in the presence or absence of EGDMA, solubility of the prepared samples is a key aspect requiring further investigation. Drug release study and further copolymer functionalization could follow up this research work.

In conclusion, this work I believe holds great potential. The polymers presented in this project synthesised through *in-situ* and conventional RAFT polymerisation could be functionalised further with short cell adhesion peptides to be used as drug delivery systems, cell carriers, and scaffolds for tissue engineering applications. In addition, as a result of thiol functionality, these copolymers may be capable of reacting well with metals (e.g. copper, silver or gold) through the formation of the metal-thiol bond. Consequently, these polymeric materials have the potential to be used in the preparation of systems such as RAFT polymer – nanoparticles.

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