THE UNIVERSITY OF HULL

Preparation of Porous Polymeric Scaffolds via High Internal Phase Emulsion Templating

being a Thesis submitted for the Degree of MSc

in the University of Hull

by

Lamyaa Alsuhaymi

April 2014

Supervisor: Dr Tommy Horozov

Acknowledgment

Lamyaa Alsuhaymi gratefully acknowledges the financial support of the government of the Kingdom of Saudi Arabia.

The two diblock copolymers have been synthesised in the presence of Dr Theoni Georgiou. The techniques for their characterisation, such as NMR and gel permeation chromatography (GPC), have been done with help from other specialists in the Chemistry Department of Hull University.

Abstract

The aims of this project are to synthesise polymeric surfactants, investigate their ability to stabilise high internal phase emulsions (HIPEs) and the structure of porous polymeric materials obtained after polymerising the oil phase of water-in-oil HIPEs (poly-HIPEs). In the first part of the work, group transfer polymerisation (GTP) has been used to synthesise novel diblock copolymer of poly (ethylene glycol) methyl methacrylate-b-poly tetrahydropyranyl methacrylate. ^{1}H NMR and GPC have been used to characterise their composition and molecular weights, respectively. Two diblock copolymers were successfully synthesised, although their composition and molecular weights were significantly different from those anticipated. The second part of the project has studied the stability against coalescence and sedimentation of W/O emulsions in the presence of the amphiphilic PEGMA-b-THPMA diblock copolymers synthesised by us or a commercial low molecular weight surfactant Span 80 (sorbitan monooleate). Finally, the porous polymeric foam with interconnected pore structure has been produced by polymerisation of the styrene containing continuous phase of HIPEs. A remarkably strong effect of the nitrogen gas treatment on the viscosity and stability of HIPEs and the morphology of the porous polymeric scaffolds was obtained from them after polymerisation.

List of Figures

List of Tables

List of Abbreviation

AIBN	Azbisiobutyronitrile
Al_2O_3	Aluminium Oxide
$CaCO_3$	Calcium Carbonate
CaH_2	Calcium hydrid
$CaCl_2.2H_2O$	Calcium chloride dihydrate
DHP	3, 4-dihydro-2H-pyran
DMDCS	Dichlorodimethylsilane
DP	Degree of Polymerisation
DPPH	2, 2-diphenyl-1-picrylhydrazyl hydrate
DVB	Divinyl benzene
GPC	Gel Permeation Chromatography
GTP	Group Transfer polymerisation
HDDA	1,6-Hexanediol-diacrylate
HIPE	High Internal Phase Emulsion
HLB	high hydrophilic– lyophobic balance
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
MMA	Methyl methacrylate
Mn	Number average of molecular weight
MTS	1-methoxy-1-trimethylsiloxy-2-methylpropene
MWD	Weight average of molecular weight
N_2	Nitrogen gas
$NaHCO_3$	Sodium hydrogen carbonate
Na_2SO_4	Sodium sulphate anhydrous
O/W	Oil-in-water
O/W/O	Oil-in-water-in-oil
PEGMA	Poly (ethylene glycol) methacrylate
Poly-HIPE	Polymerisation of High Internal Phase Emulsion
Span 80	sorbitan monooleate
SEM	Scanning electron microscopy
St	Styrene
TBABB	tetrabutyl ammonium bibenzoic acid
THPMA	2-therahydropyranyl methacrylate

THF	Tetrahydrofuran
W/O	Water-in-oil
W/O/W	Water-in-oil-in-water

Table of content

Chapter 1. Introduction

Porous polymeric materials have been prepared and subject to much study in recent years, due to their applications (both potential and actual) in numerous fields of advanced material science, such as low dielectric substrates for microelectronics, scaffolds for tissue engineering, catalyst supports, etc. One relatively simple and reliable method for their preparation is the use of emulsion templates. This chapter provides an overview of emulsions, polymers and porous polymeric materials. The synthesis of diblock copolymers and their use as emulsion stabilisers is also considered.

1.1 Emulsions

1.1.1 Basic definitions

Emulsion refers to a dispersion of two immiscible liquids.[1] It is usually made up of water, which is the aqueous phase, and oil, which is the organic phase. They can be classified as two types; oil-in-water (O/W) and water-in-oil (W/O) emulsions. O/W emulsion has water as the external (continuous) phase while oil forms the internal (dispersed) phase, whereas W/O emulsion includes water droplets dispersed within oil, which constitutes the continuous phase (Fig. 1.1).[2] More complex types of emulsion, such as O/W/O or W/O/W, called multiple emulsions are also known.[3] High internal phase emulsions (HIPEs) are those with a volume fraction of the internal (droplet) phase bigger than 75 vol. %.[4]

Emulsions are unstable systems and therefore surface active species (emulsifiers) are used to enhance their preparation and improve the stability.[5] Emulsifiers play an essential role in the formation and stabilisation of emulsions by reducing the interfacial energy of the dispersed droplets and suppressing their coalescence. Frequently used emulsifiers involve small molecular surfactants, amphiphilic block copolymers and solid particles.

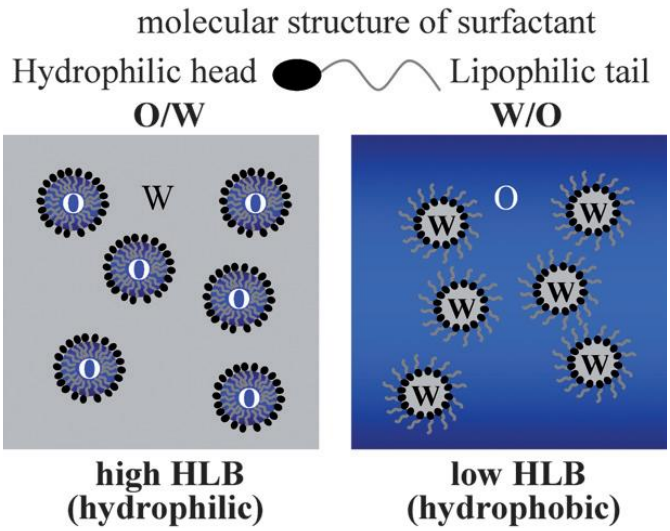


Figure 1.1. Emulsion types (O/W ,W/O) stabilised by surfactants with relevant hydrophile-lypophile balance (HLB).[2]

1.1.2 Surfactants

The unusual properties of aqueous surfactant solutions can be ascribed to the presence of a hydrophilic head group and a hydrophobic chain (or tail) in a molecule. The polar or ionic head group usually interacts strongly with an aqueous environment, in which case it is solvated via dipole-dipole or ion dipole interactions.[6] Depending on the properties of the hydrophilic head in water, surfactants are classified as anionic, cationic, non-ionic and zwitterionic (amphoteric).[6] A high hydrophilic–lyophobic balance (HLB) value of the surfactant indicates a strongly hydrophilic character, while a low value is an indication of a strong hydrophobic nature.[2] A widely used nonionic surfactant with a low HLB of 4.3 is Span 80 (sorbitan monooleate). It belongs to the group of the fatty acid esters of anhydro sorbitols which are good, oil-soluble, emulsifying agents.[7] Fig. 1.2 shows the molecular structure of the Span 80 surfactant,[8] where the hydrophilic sorbitan group acts as a ‘polar head’ and the hydrophobic fatty acid group acts as the ‘non-polar tail’.[9] In the food and cosmetic industries, Span 80 is generally known as sorbitan monooleate, although commercial Span 80 is a heterogeneous mixture of sorbitan mono-, di-, tri- and tetra esters. Span 80 is used extensively in cosmetics and topically applied pharmaceutical preparations and, to a lesser extent, as a food additive.

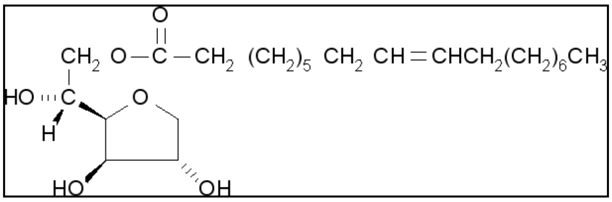


Figure 1.2. Chemical structure of sorbitan monooloeate (Span 80) surfactant.

1.1.3 Emulsion stability

The term "emulsion stability" refers to the ability of an emulsion to keep its properties unchanged over a certain period of time. However, as emulsions are thermodynamically unstable, changes of emulsion properties will occur; the more slowly the properties change, the more stable the emulsion is.[5] There are many phenomena that can alter emulsion properties: coalescence, flocculation, creaming, Ostwald ripening, etc.[10] Two or more of these instability phenomena may occur at the same time. Then, it is important to understand the cause(s) of instability, in order to select suitable components to form stable emulsions. Two factors affect the stability and formation of droplets: firstly, the migration of the surfactant at the droplets' interface (stabilising process); secondly, the droplets' coalescence (destabilisation process).[11] The main processes involved in the emulsion break down are illustrated in Fig.1.3.

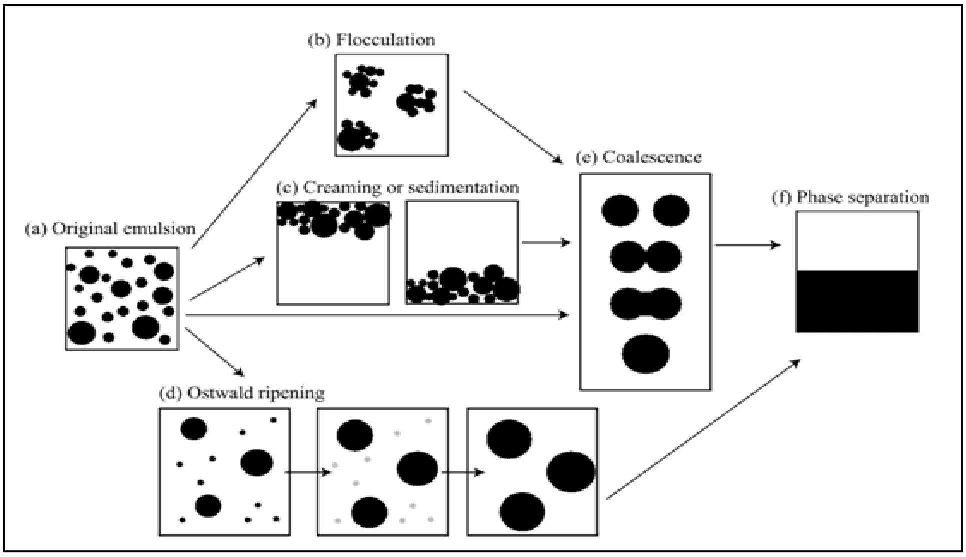


Figure 1.3. Break down processes of an unstable emulsion.[12]

1.1.3.1 Sedimentation or creaming

The creaming or sedimentation process occurring in an emulsion can be easily assessed by optical observations. Indeed, in most cases, creaming is characterised by a whitish/yellowish layer at the top of the emulsion, while a layer appears at the bottom of an emulsion if sedimentation occurs.[13] The creaming/sedimentation rate can be determined by measuring the volume of cream/sediment in the emulsion over time. This is done by placing the emulsion into a calibrated beaker or tube and measuring the height of the cream/sediment every second, then every minute. The droplet size is the same and also the number of droplets remaining does not change. The sedimentation and creaming are caused by gravity and depend on the density difference between both liquid phases.[13]

1.1.3.2 Flocculation and coalescence

Flocculation is the process in which emulsion drops aggregate without merging, in contrast to coalescence that refers to the process of merging of two or more drops into one bigger drop. Coalescence is frequently observed in everyday life. Whenever two miscible liquid drops or a liquid drop and its liquid bulk come into contact, they may coalesce. This has important consequences, e.g. the droplet size distribution in rain is (among other processes) determined by the coalescence probability. The coalescence reduces the total interface area and is driven by interfacial tension. [11, 14]

1.1.3.3 Ostwald ripening

Ostwald ripening occurs when there is a limitation of solubility of the dispersed phase in the continuous phase, so that large drops grow as smaller drops decrease in size, due to the transport of molecules from the small droplets to the large droplet through the continuous phase.[15]

1.2 Polymers and their main characteristics

A polymer is a large molecule built up from numerous smaller molecules. These large molecules may be linear, slightly branched or highly interconnected. In the latter case, the structure develops into a large three-dimensional network. The small molecules used as the basic blocks for these large molecules are known as monomers. The size of polymer may be defined either by its mass or by the number of repeat units in the molecule. This latter indicator of size is called the degree of polymerisation, or DP. The relative molar mass of the polymer is thus the product relative molar mass of the repeat unit and the DP.[16] Molecular weight is one of the most important characteristics that have an effect on polymer properties. The average number of repeat units in polymer chains is called the number average degree of polymerisation $(\overline{X}_n)$. , which is very much linked with $\overline{X}_n$ that is the number average of molecular weight$(\overline{M}_n)$.[17]

Polymer architecture describes the shape of a single polymer molecule, which often determines its physicochemical properties. Every natural, semi-natural and synthetic polymer falls into one of these categorised architectures; linear, graft, branched, cross-linked, block (see figure 1.4), star-shaped and with dendron/dendrimer topology. when two homopolymers or copolymers with a different chemical structure are linked together through a common conjunction point, it is called a diblock copolymer, which is the simplest polymer architecture.[18]

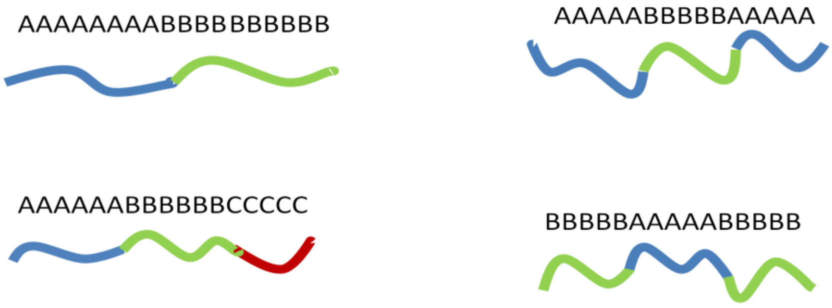


Figure 1.4. Schematic diagrams of block copolymers.

1.3 Diblock copolymer synthesis methods

1.3.1 General synthetic strategy

Group transfer polymerisation (GTP) is one of the methods of addition polymerisation. There are two major types of polymerisation method used to convert small molecules (monomers) into polymers. These major types were originally referred to as addition and condensation polymerisation. Depending on the additions, polymerisation is now called chain, chain-growth, or chain reaction polymerisation. The condensation reaction is now referred to as step-growth or step-reaction polymerisation.[17] Step-growth normally employs a difunctional monomer that is capable of undergoing a typical organic reaction.[17] In step-growth polymerisation, the polymer chains grow step-wise by reactions that occur between two molecular species. This type of polymerisation is concerned with the formation of polyamide and polyester. In this case, diacid can be allowed to react with diole in the presence of acid catalysed to obtainable polyester. Therefore, chain growths initiated by the reaction of different techniques are available to offer a high yield and a high molar mass. For chain reaction polymerisation, (Addition), the monomers usually employed in this type of polymerisation contain a carbon-carbon double bond that can participate in a chain reaction.

As a matter of fact, the majority of synthetic polymers used today are copolymer; the procedure that is referred to is copolymerisation and the resulting products are called copolymers. There are often two or more different monomers employed in a chain reaction polymerisation, to yield a polymer containing the corresponding repeat units. Hence, by varying the copolymerisation technique and the amounts of each monomer, one can use as few as two monomers to prepare a series of copolymers with considerably different properties. The amounts of different materials that can be prepared increases dramatically as the number of monomers employed increases.[17]

A diblock copolymer can be synthesised by using two developing methods: 1) a sequential addition to monomers and 2) a coupling of two appropriate end functionalised chains.[19, 20] The first method, sequential addition reaction, is the most widely used to provide copolymers of well-defined compositions. In sequential ionic polymerisation, the ionic initiators are where an active site is kept “alive” on the end of an initial block, which is then capable of initiating the chain growth of a second monomer on the end of the first chain. Furthermore, it usually involves three steps; initiation, propagation and termination. There are some important requirements to successfully implement the technique. The living chain from polymerisation must be able to efficiently initiate the polymerisation of the second monomer; more to the point, the conversion of the first monomer must be quantitative in order to achieve control over the molecular weights, in addition to the chemical and structural analogy.[20] Chain growth polymerisation can be subdivided into radical or ionic, depending on the mechanism.[21] Here, we will define most of the approaches which are used to synthesise diblock copolymers, such as synthesis of a diblock copolymer by conventional free radical polymerisation[21]

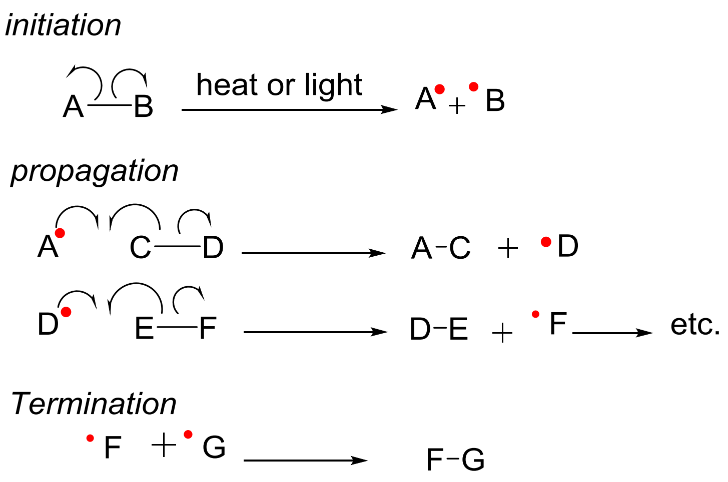


Figure 1.5. General mechanism of free radical polymerization.

1.3.2 Advantages of using GTP method

By the mid-1980s researchers at DuPont had developed the group transfer polymerisation (GTP) method.[22-24] This method is particularly useful for synthesis of acrylate and methacrylate polymer (see figure 1.6.).[23] Poly (2 (dimethyl amino) ethyl methacrylate) is an interesting polymer, which is soluble in water that has been synthesised by GTP.[25] GTP has become a valuable method for preparing block copolymers that stabilise both hydrophobic/hydrophilic interfaces and biological interfaces.[25] Owing to the unique structure, GTP has many helpful applications to prepare novel polymers.[26]The polymerisation of methacrylics and acrylics is allowed at ambient temperatures via GTP polymerisation, with great control of polydispersities and molar masses. Furthermore, it is a synthetic approach of preference to complex architecture, such as star-like or homogeneous gels based on polymethacrylates.[24]

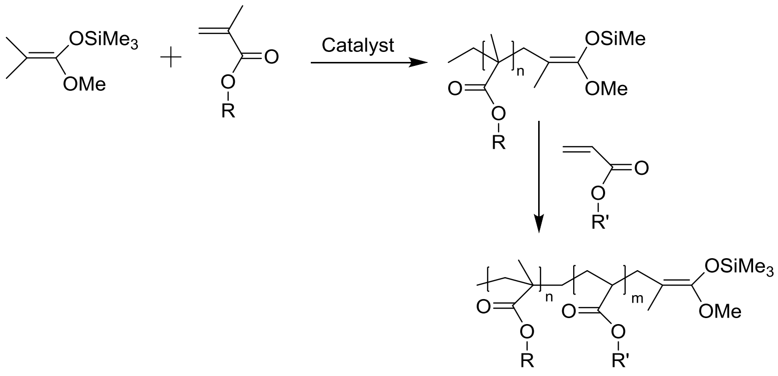


Figure 1.6. Example of using GTP for synthesis acrylate and methacylate**.**

1.4 Amphiphilic diblock copolymers and their importance

Block copolymers are a well-known and representative class of macromolecules that have been synthesised in the laboratory during the last 50 years.[27] Moreover, recent progress in the synthetic strategies that can be applied to vary the chemical composition and architecture of block copolymers opens new areas of development[27]. The main reason for the current interest in these systems is related to the wide variety of micro and nanostructures that can be obtained.[27] It can now be established, both from a theoretical and an experimental point of view, that linear AB diblock copolymers composed of immiscible blocks undergo micro phase separation.[27] They can form micellar aggregates when dissolved in a selective solvent for one of the blocks and phased separately into a variety of organised structures in the bulk state of the corresponding morphologies, (shape and size), depending on the total degree of polymerisation N which is proportional to the molecular weight, and on the chemical $\mathrm{A}(f_B = 1 - f_A)$.[25, 27] The thermodynamic interaction between the two blocks is given by the dimension-less Flory Huggins interaction parameter χ_{AB} (or χ). Over the past few years, increasing attention has been given to the supramolecular organisation of water-soluble block copolymer surfactants and their potential use in applications, such as coating and drug delivery systems, nanoparticles or nanoreactors.[25, 27] An important issue in making these self-assembled systems useful for specific applications is their capability to respond to external stimuli, such as temperature and/or pH.[27] Finally, another important factor in the design of a self-assembled material is its stability. In fact, most aggregates are only stable within a certain range of concentration, temperature or pH. The copolymer consists of hydrophobic and hydrophilic monomers concatenated randomly with equal density.[25, 27] The emulsion consists of large blocks of oil in water arranged in a percolation type of distribution.[27]

1.5 Porous polymeric scaffolds

Porous polymer has attracted much study in recent years, due to its application (both potential and actual) in numerous fields of advanced material science; for instance, low dielectric substrates for use in the microelectronic industry, scaffolds for tissue engineering and 3D cell culture, support for catalysts, reagents and other species used in synthesis chemistry, membranes for separation processes and as templates for the production of porous inorganic materials.[28] There are many formats that can be involved to shape these porous polymers into particular particles (usually known as beads), membranes, monolith and films. Generally, two main approaches, templating and non-templating, have been used for preparing nonparticulate formats.[28]

The templating methods are based on solidifying a two-phase mixture of porogen, the substance yielding the porous nature of particles called porogen, (the templating) and polymer precursor, followed by removing the porogen to leave behind pores.[28] They are called 'endo-templating approaches’ that include, for instance, emulsion templating, microemulsion, salt crystal and other particulate leaching.[28] In contrast, a non-templating method begins with only a one phase liquid system that undergoes phase separation at some point, yielding a fluidic porogen and a solid matrix, then removing porogen to leave behind a porous material.[28] Specific examples of this non-templating approach include thermally induced phase separation (TIPS), gas blowing, phase separation during crosslinking and directional freezing.[28]

1.5.1 Preparation of porous polymeric scaffolds via emulsion templating

Herein, the present work will focus on the emulsion templating method, a novel, porous, polymer process and a key technique route to highly porous and permeable polymeric materials with well-defined porosity. The process has been recognised since the 1960s, along with extensive development in the 1980s by workers at Unilever.[28-30] Over the past two decades, there has been significant growing interest from both industry and academia.[28, 29] A high internal phase emulsion (HIPE) system consists of the internal (aqueous) or droplet phase which is usually greater than 70% of the volume of the emulsion.[28, 31, 32]In fact, above this volume, it is expected in the real system that droplets become poly-dispersed or adopt a polyhedral geometry, so droplets are still spherical and packed in the most efficient performance at 0.75 of the internal phase volume ratio, Φ, and the organic phase less than 26% of the total volume, then a thin film of the continuous phase separates these concentrated droplets.[32, 33]The external (non-droplet) phase becomes solid polymer; moreover, most of the time, a highly interconnected network of micron-sized pores of a quite well-defined diameter are yielded after removing the emulsion droplets.[28] The resulting product of monomer polymerized by radical initiator is often termed a polyHIPE, or porous polymer.[28] Indeed, many previous studies have already reported the synthesis of water-in-oil (w/o) HIPE where the aqueous phase includes a water soluble radical initiator solution and the oil phase includes a monomer of styrene and is cross-linked with divenylbenzene.[32, 33] As described previously,[28, 32] the three types of pores have been defined as follows. Firstly, the term “void” refers to the spherical cavities formed due to the loss of water. Secondly, the interconnecting holes formed inside three-dimensional voids are called “windows”. To avoid confusion when cell materials are used as parts of works which involve cell culture, Cameron and his co-workers have suggested that it is preferable to use the term “void” rather than “cell”, which belongs to the class of open-cell (solid) foam. Finally, the small pores that appear on the walls of polyHIPE are called “pores”. [32]

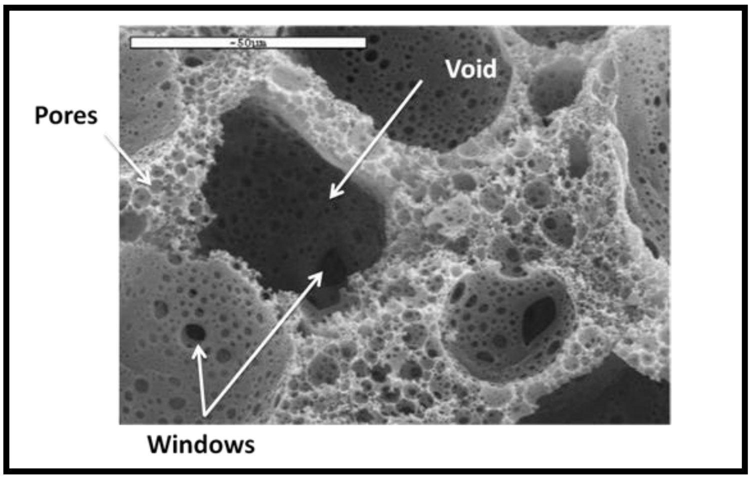


Figure 1.7. SEM image of a PolyHIPE with three types of pores. Scale bar = 50 μm.[32]

The procedure of PolyHIPEs preparation is typically simple.[32] In addition to monomer(s), often a crosslinker and an appropriate surfactant are mixed together along with a gradual addition of the liquid phase droplets.[32] Mixing is continued throughout addition to break up large droplets.[32] Once the addition of the internal liquid phase is completed, the emulsion product is treated with some techniques, then a Soxhlet extraction is used to wash the resulting porous material; after that, it is dried using a vacuum oven.[32, 33] The aqueous phase, which is one of the liquid phases involved in the emulsion system, could be the non-droplet or the droplet phase, although the two immiscible organic liquids produce non- aqueous HIPEs. [32]

It was found that the capability of preparation emulsions is influenced significantly by the polymeric nature of the surfactant phase, separated immediately in HIPEs as a result of using low molar mass non-ionic surfactants; for example, the mixture of

Span 80 and Tween 80. Obviously, only one successful HIPE has been synthesised by the use of low molar mass surfactant.[32]

HIPE preparation conditions were identical to those used to prepare polystyrene PolyHIPEs. Polystyrene is the most commonly used material investigated by PolyHIPE. To generate polystyrene PolyHIPEs, the water-in-oil (w/o) m HIPE has been used as the styrene is a water-immiscible liquid. Structural stability has been enhanced by varying the concentrations of hydrophobic crosslinkers, such as divenylbenzene. Other monomers, such as butyl acrylt (BA), 2-ethylhexyl methacrylate (HEMA), acrylate (EHA) and isobornyl acrylate (IBA) are examples of hydrophobic monomers that have been used to generate PolyHIPEs from w/o emulsion.[32]

According Cameron, w/o HIPEs can form the homogenous PolyHIPEs only if the organic continuous phase is sufficiently hydrophobic. In the same way, o/w can form the hydrophilic PolyHIPEs materials. Additionally, homogeneous PolyHIPE is formed by stable HIPE (the start of gelation). Thus, a relative hydrophilicity of the organic liquids is required to form w/o emulsion. As a consequence, using a monomer of intermediate hydrophobicity, in particular methyl methacrylate (MMA), has proved to be more difficult, because MMA is unstable and the phase separates quickly, owing to the separating of the organic matter between the two phases.[32]

1.5.2 Modifying the polyHIPE morphology

PolyHIPE morphology is controlled by the various parameters involved in the emulsion process.[33] The first and most important parameter is the choice of surfactant, its chemical nature and concentration.[32] For a non-ionic surfactant, the HLB (hydrophilic-lipophilic balance) is an important factor in deciding whether it is suitable for a particular type of emulsion.[28] Surfactants with low HLB values (ideally 2- 6) are more oil-soluble and so suitable for w/o systems.[32, 33] The most common surfactants used for w/o emulsions are non-ionic fatty acid esters, such as sorbitan monooleate (Span 80).[32, 33] However, ionic surfactants such as cetyltrimethyl ammonium bromide (CTAB), dodecylbenzenesulphonic acid (DDBS) or a combination of surfactants have also been used.[31]

Complex morphologies are the most common features for PolyHIPE materials, which include voids (spherical cavities), windows (interconnecting holes), the small pores within the walls and variation in the dimensions of PolyHIPE materials, from thin membranes to very large, monolithic articles.[28, 32] Furthermore, many different parameters have been considered to control morphology and their properties.[32] One of the most important parameters that affect the cellular nature of PolyHIPEs, which can be varied from closed and open cells, is the choice of surfactant. [32, 33]In fact, the nature of the surfactant used in the preparation affects the PolyHIPE morphology.[32] [33]Different types of surfactants would give various results for PolyHIPE properties. PolyHIPE with Span80 as a surfactant with low HLB balance (HLB 4.3) is, according to Williams, the most suitable surfactant to generate a stable emulsion for a Poly (S/DVB) PolyHIPE system, to create a stable emulsion and uniform cell size.[32, 33] Moreover, Span 20, a surfactant with a high HLB balance (HLB 8.6), was also capable of producing an emulsion with uniform cell size distribution and small cell size.[33] Earlier studies point out that there is an increase in the surface area and a reduction in the cell size, due to use of a mixture of anionic, cationic and non-ionic surfactants.[32]Thus, a mixture of surfactants helps every emulsion droplet to be surrounded by a strong interfacial film, in order to enhance emulsion stability and avoid the destabilisation mechanism of the system, in particular, the Ostwald ripening process.[33]

In addition to the effectiveness of the surfactant's chemical nature, the result of a PolyHIPE monolith has also been influenced by the surfactant's concentration. A thinning of the monomer films separating adjacent emulsion droplets was observed because of increasing surfactant concentration.[32] Actually, the windows between adjacent droplets could be formed at a certain critical thickness of film.[32] In other words, the volume concentration on conversion of monomer to polymer, well recognised features in vinyl polymerisation, leads to the production of the windows.[32]

Later studies have confirmed this hypothesis; it was found that the gelation point of the polymerisation corresponded with the point at which windows first appeared; therefore, the volume constriction on the polymerisation generates the windows.[32] This appearance of shrinking creation is owing to the fact that the thinnest position of the monomer films separating emulsion droplets is at the nearest contact between adjacent droplets.[32] The fact of the matter is, that there is no bulk shrinkage on treating of HIPEs because the shrinkage takes place between adjacent emulsion droplets (internally).[32]

Variations of the average void diameter have been also established. Workers at Unilever, and other researchers, have successfully provided first examples of the parameters that control the void diameter.[32] It was observed that a small but important reduction in the average void diameter from 15 to 5 m_m was due to increasing the DVB: styrene ratio in a styrene/ DVB HIPE, from 0 up to 100 DVB.[32] DVB is more hydrophobic than styrene, so it is assumed that the reduction in the HIPE droplet diameter, which results in an increase in emulsion stability (as a part of the high level of DVB), leads to the decrease in void diameter.[32] This is because increasing the emulsion stability to produce a smaller average size of droplet, owing to the (supposed) lower interfacial tension, allows for a larger interfacial area.[32] Furthermore, a decrease in average void diameter was caused by an increase in surfactant concentration, a gain due to increased emulsion stability, even though weak, unconnected, porous materials were noticed, due to an increase in surfactant concentration above 50% (w/w), relative to the monomer content.[32]

Preparing materials with much larger void diameters, for example, up to 200 $m_{m.,}$ is useful for many applications, particularly tissue engineering.[32] Biological cells have diameters between microns and tens of microns.[32] For this reason, cells are cultured in biodegradable and biocompatible porous support materials, which need average void and window diameters that allow cells to migrate through the material.[32] Undoubtedly, the 'controlled coalescence' technique can be used to form PolyHIPE materials with such large void diameters. Indeed, results from the ligatures found that, while the internal phase played a role, there are other ways to improve the surface properties of PolyHIPE materials.[32] Types of inert diluents and a concentration of crosslinking comonomer strongly affected the cell size and surface area of PolyHIPE materials.[32, 34]

As a final point, it has been demonstrated that the average void diameter is influenced dramatically by the electrolyte content in the aqueous phase.[32] The void diameter decreases up to 10-fold (from about 50-5m_m) due to the fact of increasing the concentration of aqueous solution of K_2SO_4 from 10_{k6} to 10 g/100 ml, with the initiator (AIBN).[32]This was associated with emulsion stability; increasing the concentration of the electrolyte resulted in the reduction of the Ostwald ripening, a method where the growth of large droplets at the rate of smaller ones, owing to the migration of droplets phase through the continuous phase.[32] The progressive coarsening of the emulsion that resulted from Ostwald ripening is followed by coalescence, and lastly, emulsion breakdown. Thus, limiting or preventing Ostwald ripening is required to reach a stable emulsion with a smaller average droplet diameter.[32]

1.6 Aims and organisation of the thesis

The project aims are to synthesise polymeric surfactants, investigate their ability to stabilise high internal phase emulsions (HIPEs) and the structure of porous polymeric materials obtained after polymerising the oil phase of water-in-oil HIPEs (poly-HIPEs). The thesis is organised as follows. The present introductory chapter is followed by the experiment chapter which constitutes **Chapter 2**. It describes all the experiments and methods used within the project. **Chapter3** starts with information about the importance of synthesis diblock copolymer, which could be an alternative to the low molecular surfactants in making stable w/o HIPE templates. This section describes the synthesis and characterisation of THPMA monomer and two novel diblock copolymers (PEGMA-b-THPMA). **Chapter 4** investigates the effect on w/o emulsion stability of these two novel diblock copolymers (synthesised by us) and other commercial surfactants, such as Span 80, Oleic acid and $CaCO_3$, in order to estimate their ability to stabilise HIPEs. **Chapter 5** describes the preparation of HIPEs, as the polymerisation of these emulsions will produce the polymeric scaffolds. The HIPEs are also treated with N_2 gas flow to remove dissolved oxygen, to enhance the polymerisation of HIPEs templating and, for the first time, the strong effect of the N_2 flow on the emulsion stability is investigated. Finally, **Chapter 6** summarises the achievements and contribution of this thesis to emulsions and polymer science.

Chapter 2 :Experimental

2.1 Materials

Materials used in the synthesis of diblock copolymers were: Tetrahydrofuran, (THF, polymerization solvent, 99.8%, Fisher Scientific), 3, 4-dihydro-2H-pyran (DHP, 97%, Aldrich), 2, 2-diphenyl-1-picrylhydrazyl 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH, free radical inhibitor, 99%, Aldrich), Dichlorodimethylsilane (DMDCS, 99.5%, Aldrich), sodium sulphate anhydrous (Na_2SO_4, Fisher Scientific) sodium hydrogen carbonate ($NaHCO_3$, Analytical regent, Fisher Scientific), activated alumina (Al_2O_3, Hull University store, basic), calcium hydride (CaH_2, 90-95%, Fisher Scientific), poly(ethylene glycol) methyl methacrylate (PEGMA, monomer, MW = 300 g/mol, Aldrich), 1-methoxy-1-trimethylsiloxy-2-methylpropene (MTS, initiator, 99%, Aldrich) and tetrabutyl ammonium bibenzoic acid (TBABB, GTP catalyst, Aldrich).

Materials used in the emulsion experiments were: styrene monomer (St, 99%, Aldrich), methyl methacrylate (MMA, $\geq$ 98.5%, Aldrich), 1,6-Hexanediol-diacrylate (HDDA ,90%, Aldrich) as cross linked, Sorbitan monooleate (Span 80, Aldrich), 2,2 Azobisisobutyronitrile (AIBAN), methanol (99.8 %, Fisher Scientific), isopropanol (>99.5, Fisher Scientific) activated alumina (Al_2O_3, Hull University store, basic) and Calcium chloride dihydrate ($CaCl_2 .2H_2O$, 99%, Fisher Scientific).

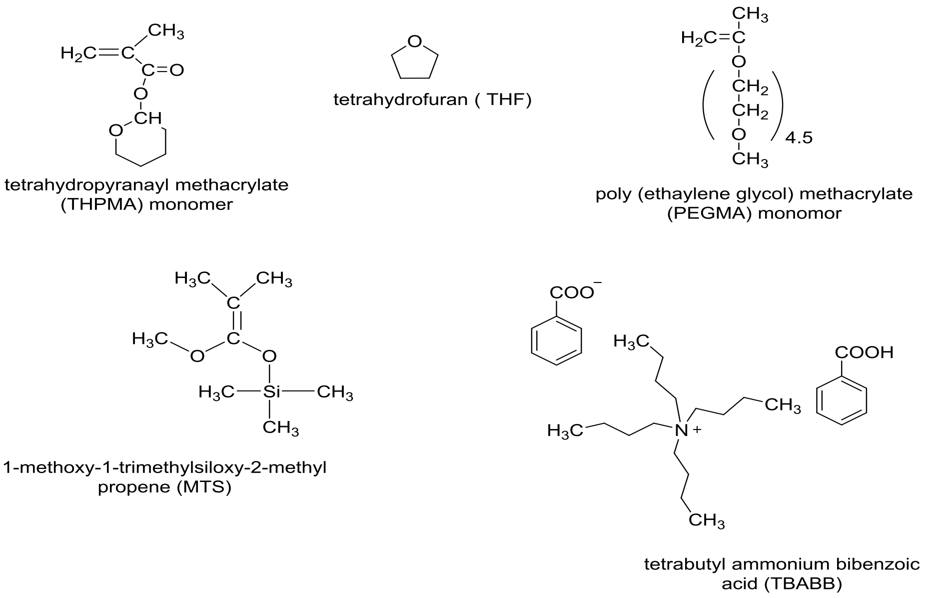


Figure 2.1. Chemical structures of monomers, initiator, GTP catalyst and solvent used for diblock copolymers synthesis.

2.2 Experimental procedures and methods

2.2.1 Cleaning of glassware

All glassware used for synthesis or distillation was left overnight in a 2M NaOH in an ethanol bath, and for at least one hour in a 2M HCl bath. Then it was washed with acetone and dried in the oven overnight at 140°C before use.

2.2.2 Synthesis of THPMA monomer

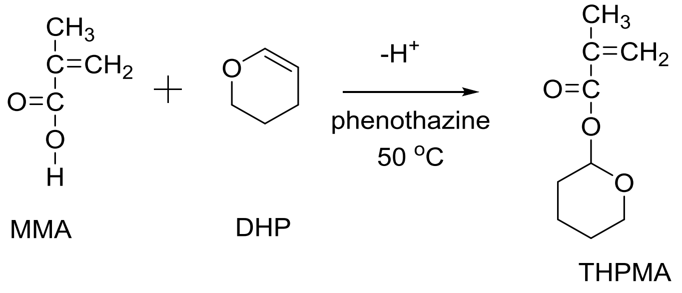


Figure 2.2. Synthesis of THPMA monomer

The synthesis of THPMA monomer was done via the acid-catalytic etherification of methacrylic acid (MAA) with 100% excess of 3, 4-dihydro-2Hpyran (DHP) at 50^oC, using a modification of the method reported by Hertler.[35] To a 1 L one-necked round-bottomed flask containing a magnetic stirring bar through the use of an addition funnel, were added 375 ml DHP (345.75 g, 4.11 mol), 174 ml MAA (164 g, 1.36 mol) and 1 g of phenothiazine in the presence of DPPH to stop polymerisation. Once the addition was complete (the reaction was warmed to 60^oC), the temperature of the reaction mixture rose to 60^oC for an hour and was maintained at 50^oC for three days. After cooling to room temperature the mixture was passed three times through basic alumina mixed with calcium CaH_2 to remove all inhibitors and impurities. Subsequently, it was stirred over CaH_2 for 5 hours and then purified by vacuum distillation to give pellucid liquid. ^1H NMR (300MHz, $CDCl_3$) δ ppm 1.53-1.7(m, CH_2),1.83 (s, 7H, CH_3), 3.57 and 3.76 (m, 2H, CH_2O), 5.47 (s,1H,=CH), 5.9 (s, 1H,OCHO) and 6.03 (s,1H,=CH).

2.2.3 Purification of THPMA monomer before polymerisation

The THPMA monomer was distilled from a 500ml one neck round-bottom flask under heated stirred conditions. The flask was connected to a vacuum line via distillation arms which were also connected with another 100 ml round-bottom flask to collect the distilled products. The distillation arm was cooled using liquid nitrogen. During the distillation process, the temperature was increased gradually from 25ºC to 80 ºC. The collected product, distilled at 30-40 ºC, was DHP as confirmed by ^1H NMR. The temperature was then increased from 40^oC to 80^oC and the distilled product was collected in a new round-bottom flask. It was confirmed by NMR that the entire product collected was THPMA monomer.

To ensure there was no acid inside the collected product, solid sodium hydrogen carbonate (2g) and anhydrous sodium sulphate (8g) were added to the flask. The THPMA was twice passed through basic alumina to remove inhibitors and protic impurities, and stirred over CaH_2 for 3 hours in the presence of 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH). The monomer was kept refrigerated before its distillation through the use of a vacuum line. It was filtered thoroughly in the polymerisation reaction.

2.2.4 Purification of PEGMA monomer and MTS initiator

Polyethylene glycol containing monomer PEGMA (300 mL) was twice passed through basic alumina columns in a 50% v/v solution in THF and stirred overnight over CaH_2 (5g). It was kept refrigerated until use and was filtered directly into the reaction flask. The polymerisation initiator 1-methoxy-1-trimethylsiloxy-2-methyl propen (MTS) was distilled just before use.

2.2.5 Purification of the polymerisation solvent

Tetrahydrofuran (THF), used as a polymerisation solvent, was dried using a refluxing condenser over a potassium amalgam for several days under argon gas conditions. In addition, it was freshly distilled prior to use.

2.2.6 Synthesis of PEGMA-THPMA diblock copolymers

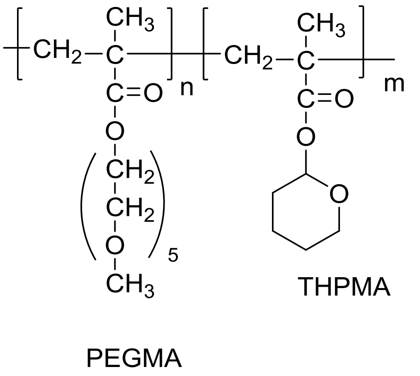


Figure 2.3. Diblock copolymer of PEGMA and THPMA

The two diblock copolymers were synthesised via Group Transfer Polymerisation (GTP) of the two monomers. The polymerisation was carried out at room temperature. The AB type diblock copolymer ($PEGMA_{7.2}$-b-$THPMA_{29.6}$) with a theoretical molar composition of 30 % PEGMA and 70 % THPMA was synthesised as follows. A small amount of the polymerisation catalyst (TBABB) was added under argon to a 250 mL round-bottom flask containing a magnetic stirrer. The flask was then sealed using a rubber septum. 57.59 mL of THF and 0.50 mL of MTS (0.34 g, 0.002461 mol) were added consecutively, followed by the slow addition of the first monomer, freshly distilled PEGMA (10 ml, 4.43 g, 0.01772 mol). The polymerisation exotherms were measured using a digital thermometer which recorded an increase in temperature from 25.6ºC to 30.6ºC. Once the exotherm had abated after couple of minutes, which was evidence of the end of polymerisation of the monomer, two 1 ml samples were extracted via syringe for GPC and NMR analysis. Afterwards, freshly distilled THPMA (12.40 ml, 12.40 g, 0.072965) was added dropwise into a reaction flask that contained the living PEGMA solution and the reaction exothem was observed. The temperature increased from 27.7 ºC to 28.4 ºC. Once the reaction had cooled down, two samples of the diblock copolymers solution were extracted for GPC and NMR analysis. The diblock copolymer solution was concentrated by removing the polymerisation solvent (THF) using a rotary evaporator. The same procedure was used to synthesise the second diblock copolymer $\mathrm{PEGMA_{6.0}}$-b-$\mathrm{THPMA_{31.8}}$ with a theoretical molar composition of 25 % PEGMA and 77 % THPMA (see Table 2.1).

Table 2.1 Reactants used and temperature changes in the synthesis of diblock copolymers in THF solvent using TBABB as a catalyst and 0.50 ml of MST as an initiator.

Polymer	**THF / ml**	**Monomer**	**Temperature / ^{o}C**	
			$T_{initial}$	**T_{max}**
$\mathrm{PEGMA_{7.2}}$-*b*-$\mathrm{THPMA_{29.6}}$	57.59	(i)10 ml PEGMA	25.6	30.6
		(ii)12.40 ml THPMA	27.7	28.4
$\mathrm{PEGMA_{6.0}}$-*b*-$\mathrm{THPMA_{31.8}}$	60.00	(i) 8.4 ml PEGMA	27.3	29.6
		(ii)13.5ml THPMA	24.7	29.3

Both copolymers were isolated by precipitation in ethanol. This was done by drop wise adding 50 ml polymer solution in THF to 500 ml of ethanol during stirring. After that, the ethanol was discarded as waste and the precipitated polymer was dried in a vacuum oven at room temperature for three days.

2.2.7 Gel permeation chromatography

The molecular weights (MWs) and the molecular weight distributions (MWD) of the diblock copolymer samples were determined by gel permeation chromatography (GPC) using PL-Mixed 'E' polymer laboratories column. The column temperature was maintained at 30^{o}C. The solvent (THF) was filtered and then used at a flow rate of 1 mL min^{-1} using Water’s 515 isocratic pump. The reactive index (RI) signal was measured by a VE-3580 detector. A series of PMMA standards were used for calibration.

2.2.8 Proton nuclear magnetic resonance spectroscopy

The proton nuclear magnetic resonance (^1H NMR) spectra were recorded using a JEOL 300 MHz device to identify the composition of the polymers and their precursors using $CDCl_3$ as a solvent.

2.2.9 Emulsions

The oil phase in all emulsions was a mixture of a monomer, methylmethacrylate (MMA) or Styrene (St), and a cross-linker, 1,6-Hexandiol-diacrylate (HDDA), in a volume ratio of 90:10. It also contained a thermo initiator (AIBN, 1 mol% with respect to the double bonds) and an emulsifier – either a diblock copolymer synthesised by us at concentrations 1 – 5 wt%, or a low molecular weight surfactant Span 80 at concentrations in the range 10 – 20 wt%. In some experiments, oleic acid and calcium carbonate particles were used as emulsifiers. The aqueous phase in all emulsions contained 0.27 M $CaCl_2$ and was prepared by dissolving 10 g of $CaCl_2.2H_2O$ in 250 ml of deionised water.

2.2.9.1 Preparation of emulsions by hand shaking or by using an Ultra Turrax homogeniser

In all cases, an equal ratio of oil phase and water phase were combined in a glass vial (7.5 cm height, 2.5 cm diameter), total volume 10 ml .The emulsification was made by either hand shaking (strongly 12 times for 30 seconds), or using a homogeniser Ultra-Turrax from IKA, T25 digital (model T25 Ds2) with an 18 mm head at a speed of 12,600 rpm for 1 min at room temperature.

2.2.9.2 Preparation of HIPEs by using an overhead stirrer

HIPEs with Span 80 were emulsified by using an overhead stirrer at 300 rpm. The stirrer paddle was made out of plastic which perfectly matched the inner bottom of a 250ml 3 neck round-bottom flask that already contained 5ml of the oil phase. The aqueous solution was injected by using a syringe pump (NE-1000, New Era) with a relatively slow rate (20 ml per hour). Some emulsions were treated with N_2 (7 min), and some without. The resulting HIPEs were divided into two samples; one to check their stability and the other one was polymerised overnight at 60 ^{o}C in the oven.

2.2.9.3 Determining the emulsion type and stability

The drop test method was used to identify the emulsion type (W/O or O/W). Using a pipette, a drop of emulsion was added to water or oil. The W/O type was identified where the drop of emulsion shows a spherical appearance and precipitate in the water; whereas, in the oil the W/O emulsion droplet dispersed and mixed with oil. The O/W type behaved in an opposite manner.

The emulsion stability was investigated by monitoring the changes in their appearance and phase separation with time, t. Slide images of the emulsion vessels were taken at different times and the height of oil, water and emulsion layers were measured from the images. The fractions of released oil, water and remaining emulsion ($\Phi_{OR,}\Phi_{WR}$ and Φ_{E}, respectively) were then calculated by the following equations.

$$\Phi_{\boldsymbol{OR}}(\boldsymbol{t}) = \frac{\boldsymbol{H_{OR}(t)}}{\Phi_{\boldsymbol{O}} \times \boldsymbol{H_{total}}} \tag{2.1}$$

$$\Phi_{\boldsymbol{WR}}(\boldsymbol{t}) = \frac{\boldsymbol{H_{WR}(t)}}{\Phi_{\boldsymbol{W}} \times \boldsymbol{H_{total}}} \tag{2.2}$$

$$\Phi_{\boldsymbol{E}}(\boldsymbol{t}) = \frac{\boldsymbol{H_{E}(t)}}{\boldsymbol{H_{total}}} \tag{2.3}$$

where H_{OR} and H_{WR} are the height of oil and water released, respectively, Φ_{O} and Φ_{W} are the volume fraction of oil and water in the system and H_{total} is the total height of the system.

In some experiments, microscope images of the emulsion were taken using a Nikon Labophot microscope supplied with a 12-bit digital camera (QICAM FAST 1394, Q Imaging) and Image-Pro Plus 5.1 software (Media Cyberntics) in order to obtain information about the droplet size.

2.2.10 Measuring the viscosity of the styrene containing oil phase

The viscosity of the oil phase composed of styrene (a monomer), HDDA (a cross-linker), 1 mol % AIBN (an initiator) and Span 80 (an emulsifier) was measured by using an Ostwald viscometer. To prevent the polymerisation of the oil inside the viscometer, 0.1 ml solution of phenothaizene in styrene with concentration 1.5 wt/v% was added to the samples (20 ml) before the measurements. The temperature was kept constant by immersing the viscometer in a water bath set at 25 ^{o}C by using a Grant thermostat.

2.2.11 Preparation of poly-HIPEs

Samples of HIPEs were placed in plastic conical centrifuge tubes and polymerised in an oven at 60 oC overnight. The polymers obtained were taken out of the tubes and purified twice by using Soxhlet extraction for 6 hours. The first purification was done with a mixture of 180 ml ethanol and 20 ml water to remove the aqueous phase of the template. The second purification was done with pure isopropanol to dissolve the surfactant trapped in the polymer. The purified porous polymeric blocks were dried in a vacuum oven at room temperature for 3 days. Scanning electron microscopy (SEM) images of the porous polymeric samples were taken by a Hitachi S2400 electron microscope operating at 25 kV in order to obtain information for the morphology of the samples.

Chapter 3: Synthesis and characterisation of amphiphilic diblock copolymers

3.1 Introduction

High internal phase emulsions (HIPEs) are widely used for the preparation of porous polymeric scaffolds. Usually water-in-oil (w/o) HIPEs are used as templates and the porous scaffolds are obtained after the polymerisation of the continuous oil phase at an elevated temperature (50-80 ^{o}C) using thermo-initiators (e.g. AIBN), thus forming polyHIPEs. Therefore, the HIPEs used as templates must be very stable. This is usually achieved by using low molecular weight surfactants at high concentrations (typically 10-25 wt %). For many applications, the excess surfactant must be removed from the polyHIPEs, thus increasing the efforts and costs of scaffold preparation. It is known that polymeric surfactants can make very stable emulsions at much lower concentrations (a few wt %). The aim of this study is to synthesise polymeric surfactants (amphiphilic diblock copolymers), which could be used as an alternative to the low molecular surfactants in making stable w/o HIPE templates.

In this investigation, two amphiphilic diblock copolymers of polyethylene glycol methacrylate (PEGMA) and tetrahydropyranyl methacrylate (THPMA) were synthesised via group transfer polymerisation, using tetrahydrofuran as a solvent. The resulting block copolymers were purified and characterised by nuclear magnetic resonance spectroscopy (^{1}H NMR) and gel permeation chromatography (GPC) to determine their composition and molecular weight respectively.

3.2 Synthesis and purification of THPMA monomer

THPMA (2-tetrahydropyranyl methacrylate) monomer was not commercially available, so it was synthesised by reacting methacrylic acid with 3, 4 hydro-2H-pyran at 50 ºC, as shown in Figure 3.1.

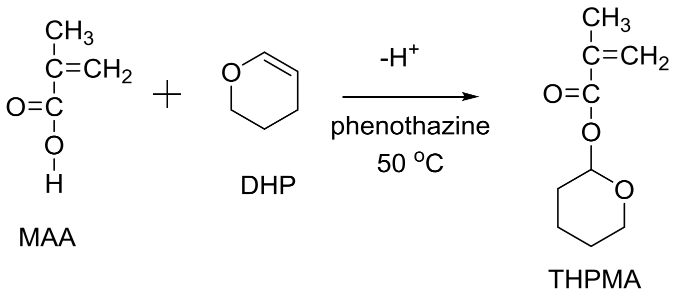


Figure 3.1 Synthesis of the THPMA monomer.

The mechanism indicates that the addition of the methoxy group to dihydropyran (DHP) was in carbon 1 rather than carbon 2, due to the stabilisation of the positive charge on carbon 1 by the (resonance) electron donating effects of the adjacent oxygen (Figure 3.2).

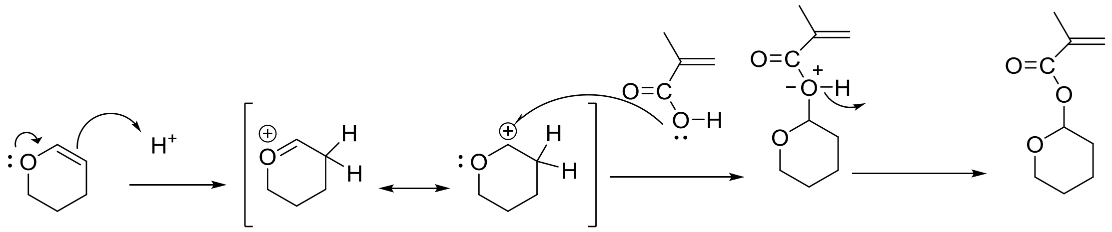


Figure 3.2. Mechanism of etherification to form THPMA monomer.

The experimental procedures and conditions used in the synthesis of THPMA monomer and its purification are described in Chapter 2, section 2.2.2. The ^{1}H NMR spectrum of the purified product with each peak assigned and peak integrals is shown in Fig. 3.3.

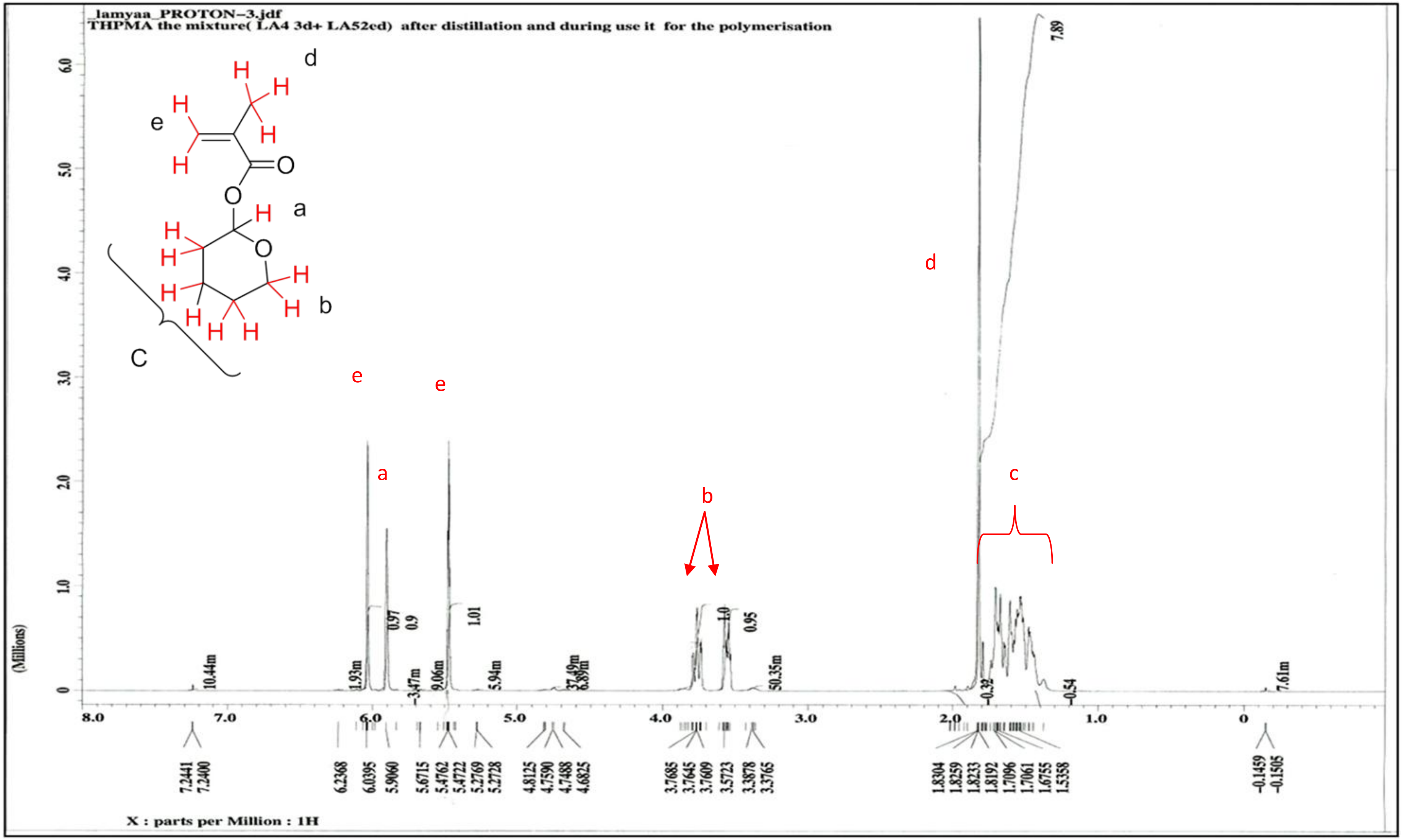


Figure 3.3. ^{1}H NMR spectrum of THPMA monomer in $CDCl_3$.

3.2 Synthesis of PEGMA-b-THPMA diblock copolymers

Group transfer polymerisation (GTP) was employed to synthesise two amphiphilic diblock copolymers with the first block of PEGMA (a hydrophilic block) and the second block of THPMA (a hydrophobic block). The reaction was carried out in THF solution at RT, using 1-methoxy-1-trimethylsiloxy-2-methyl-1-propene, MTS as an initiator, tetra-n-butyl ammonium bibenzoate, TBABB as a catalyst and the sequential addition of monomers. In this case of copolymers, the polymerisation of PEGMA is conducted first to give the correct defined product. Figure 3.4 shows a schematic representation of the synthesis procedure of PEGMA-b-THPMA, while the reaction scheme is shown in Figure3.5.

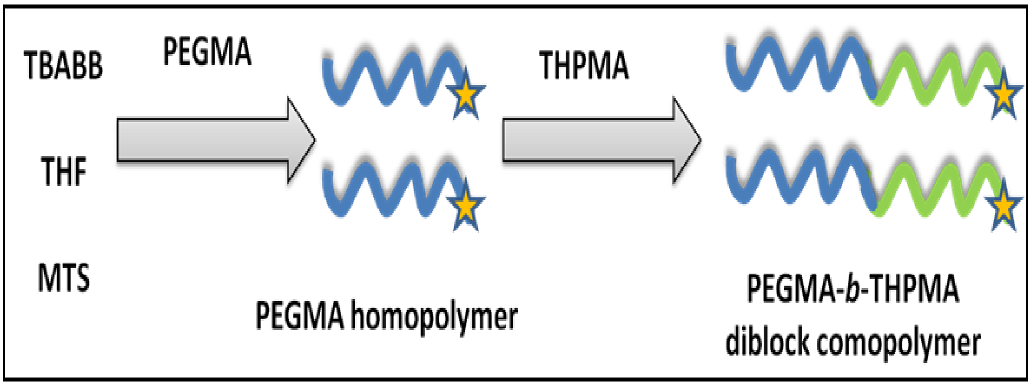


Figure 3.4. Schematic diagram showing the synthesis PPEGMA-*b*-THPMA diblock copolymers.

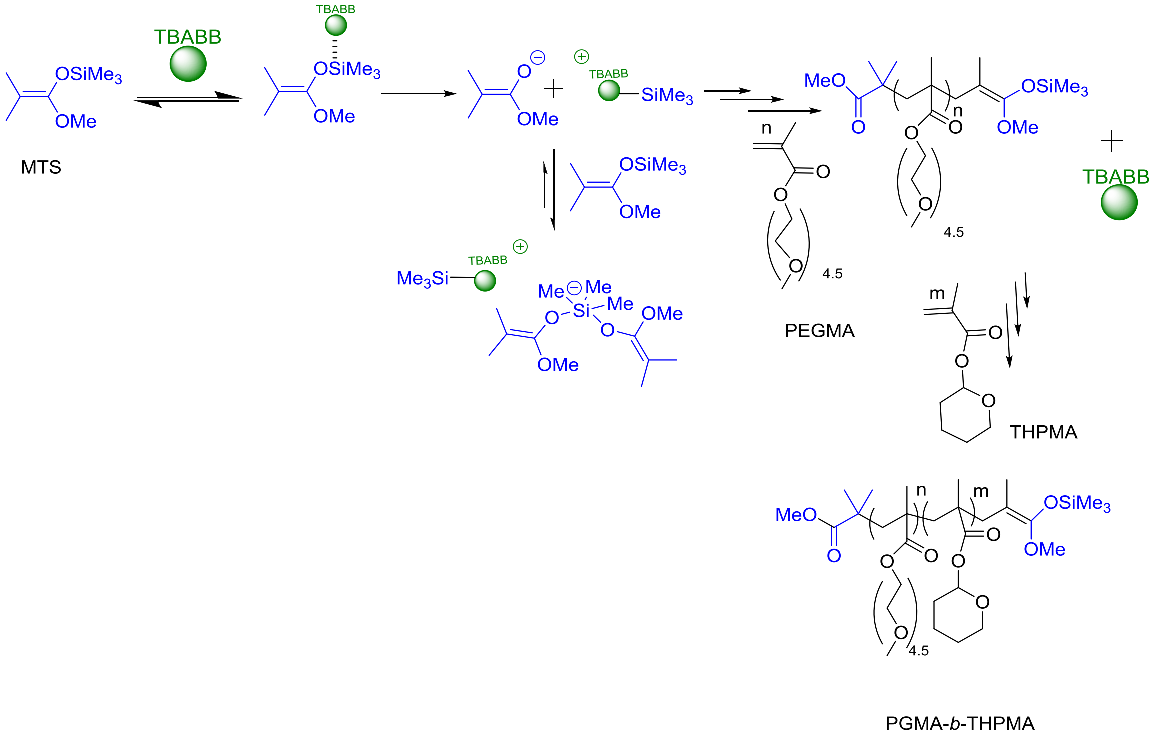
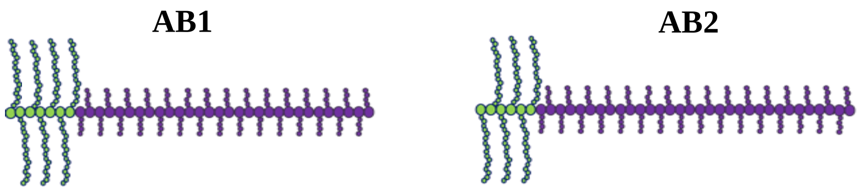


Figure 3.5. Reaction scheme for the synthesis of PEGMA-*b*-THPMA diblock copolymers.

Two diblock copolymers (AB1 and AB2) with slightly different compositions, but the same molecular weight were targeted in the synthesis (Fig. 3.6). The experimental procedure and conditions used for their synthesis are described in section 2.2.6 of chapter.



AB1

PEGMA$_7$-*b*-THPMA$_{30}$
70%-30%

AB2

PEGMA$_6$-*b*-THPMA$_{32}$
25%-75%

Figure 3.6. A schematic representation of the targeted block copolymers and their weight composition. The PEGMA units are coloured in green, whereas the THPMA are shown in purple.

3.3 Composition and molecular weights of the PEGMA-b-THPMA diblock copolymers synthesised

The composition of synthesised diblock copolymers were determined from their NMR spectra recorded in $CDCl_3$, whereas their molecular weights and polydispersities were obtained by gel permeation chromatography (GPC).

The NMR spectra of as synthesised polymers AB1 and AB2 without further purification are shown in Figures 3.7 and 3.8, respectively. The peaks “a” (THPMA block) and “f” (PEGMA block) were used to calculate polymer compositions by equations 3.1 and 3.2 below.

The following is an example of molar composition of AB1 before purification:

1- PEGMA

Peak (f): 3H at δ = 0.39

H= 0.13

2- THPMA

Peak (a):1H at δ = 0.15

Molar composition of PEGMA: $\frac{0.13}{0.13 + 0.15} \times 100 = 46\%$ (eq. 3.1)

%THPMA = 100 – 46 = 54% (eq. 3.2)

It was found that the actual composition of the polymers before purification was comparatively different from the theoretical composition (See table 3.1). Furthermore, the NMR spectrum of diblock of AB1 and AB2 shows that there were some traces of impurities from THPMA monomer. As evidenced from figures 3.13 and 3.14 these traces of impurities have been removed after purification.

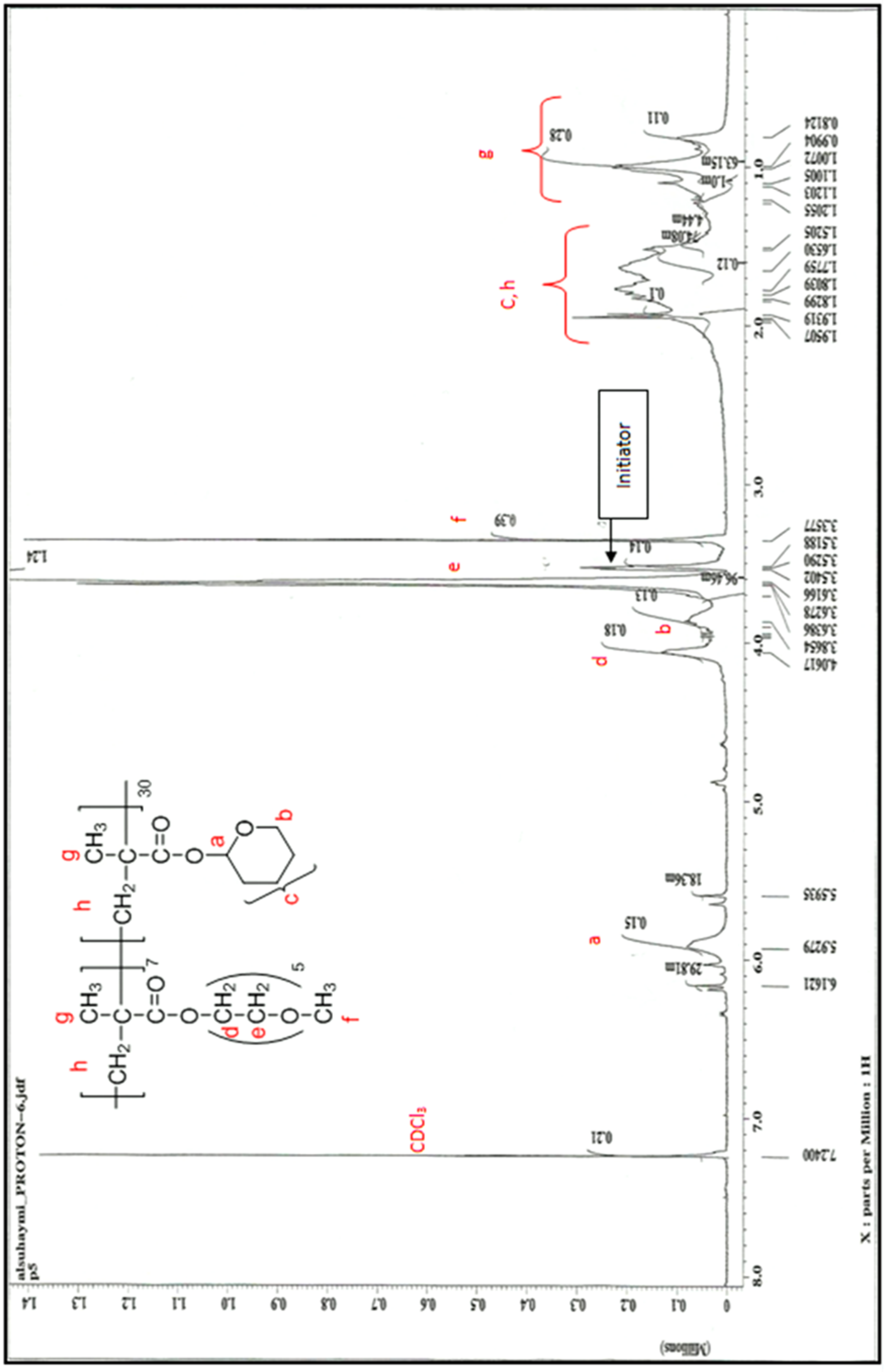


Figure 3.7. ^1H NMR spectrum of the as synthesised AB1 (PEGMA$_{7.2}$-THPMA$_{29.6}$) polymer recorded in $CDCl_3$.

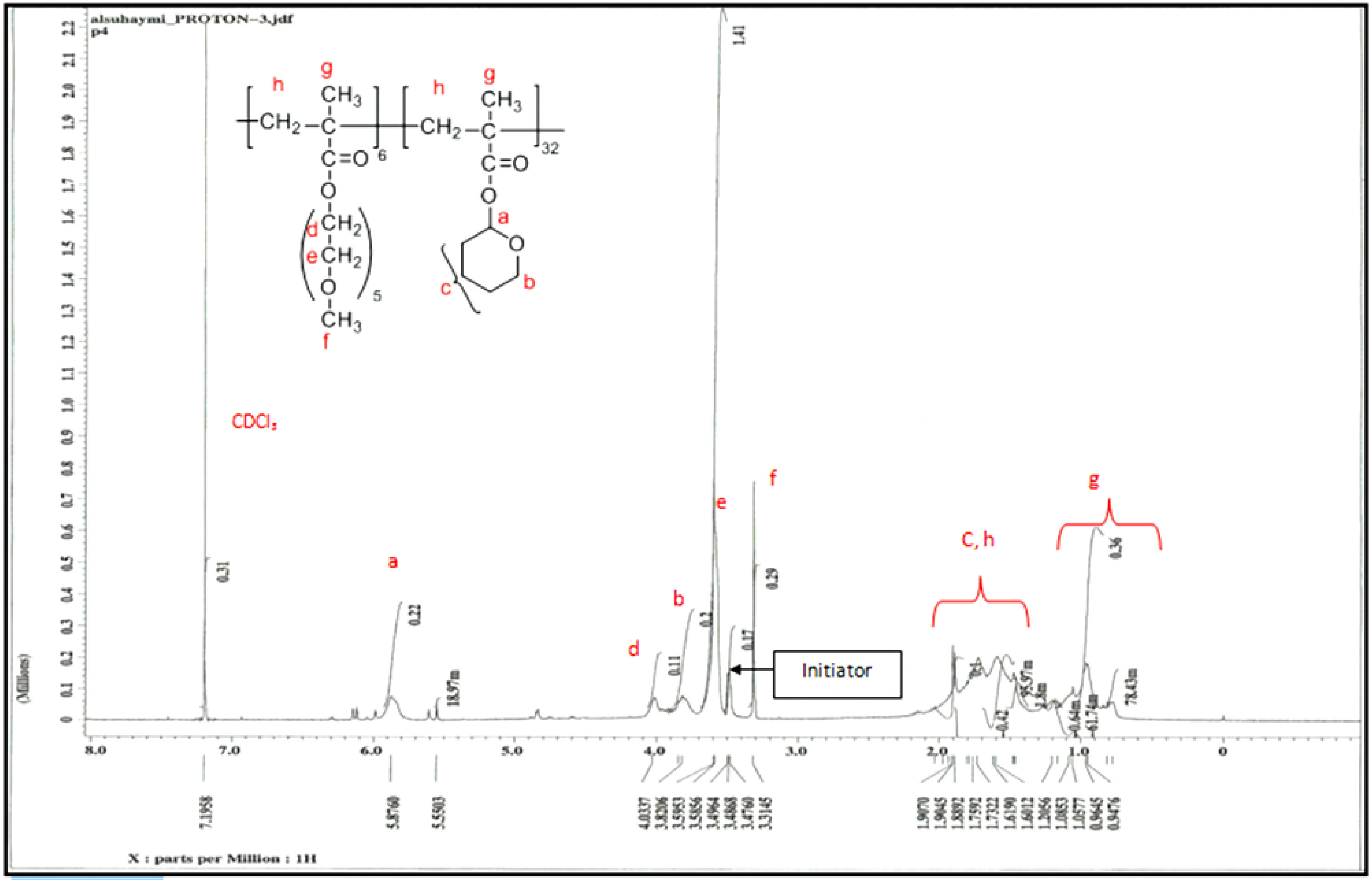


Figure 3.8. ^{1}H NMR spectrum of the as synthesised AB2 ($PEGMA_{6.0}$-$THPMA_{31.8}$) polymer recorded in $CDCl_3$.

Information about the purity, molecular weights and polydispersities of the polymers were obtained by GPC. GPC chromatograms of the as synthesised AB1 and AB2 polymers (i.e. without further purification) are shown in Figures 3.9 and 3.10.

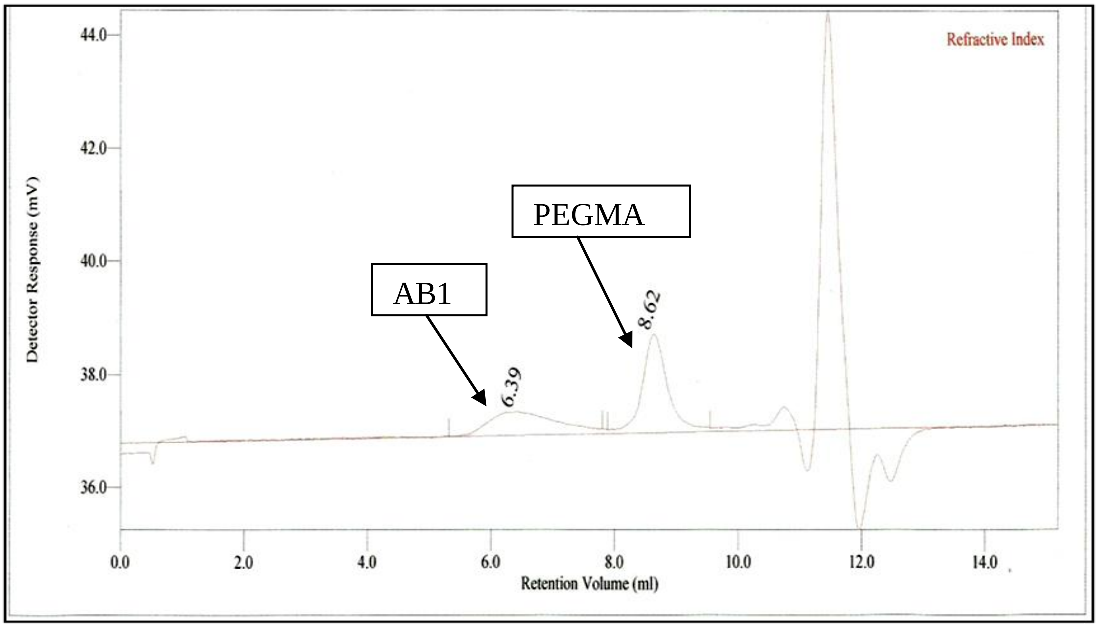


Figure 3.9. Gel permeation chromatogram of the $PEGMA_7$-b-$THPMA_{30}$ diblock copolymer (AB1) obtained just after its synthesis without further purification.

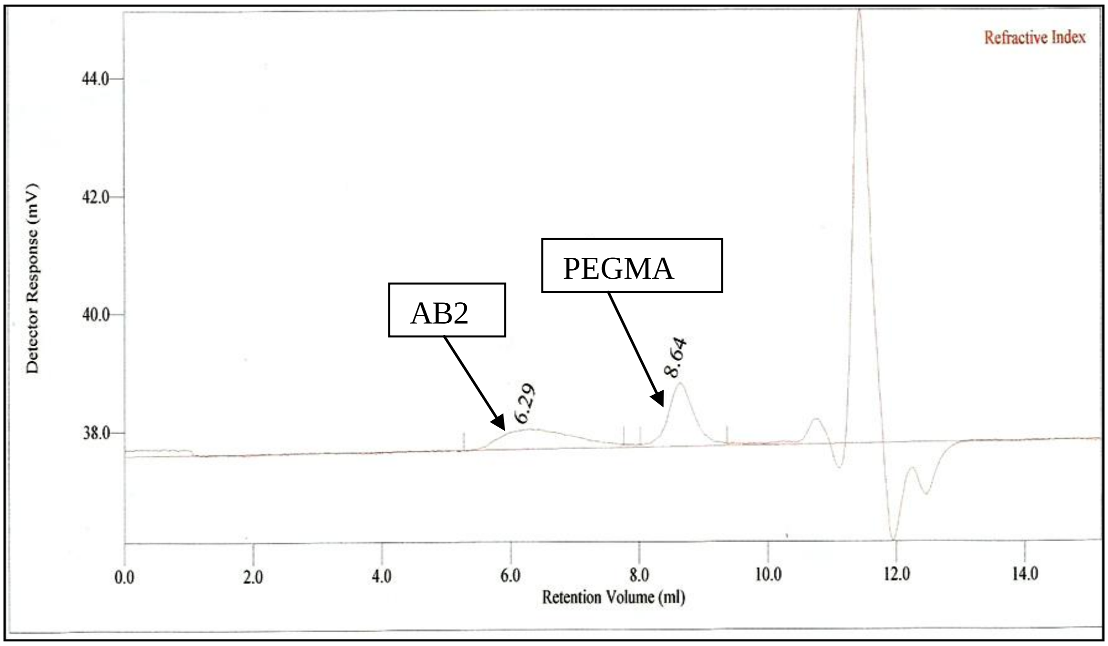


Figure 3.10. Gel permeation chromatogram of the $PEGMA_6$-b-$THPMA_{32}$ diblock copolymer (AB2) obtained just after its synthesis without further purification.

The data obtained for the number-average molecular weight (M_n) and polydispersities (Mw/Mn) of the two diblock copolymers are shown in brackets in Table 3.1.

Surprisingly, the experimentally determined molecular weights were much higher than expected. Furthermore, broad molecular weight distributions were observed evident from the large polydispersity indices (>2). Usually, the polydispersities of polymers synthesised via GTP are less than 1.2, which is considered to be a significant advantage over other polymerisation techniques e.g. the free radical polymerisation.[20] These results suggest that the control over the GTP was lost during the synthesis and perhaps the polymerisation also occurred via a free radical mechanism. This is supported by the fact that significant amounts of the PEGMA homopolymer were detected in the unpurified polymers (see Figs 3.9 and 3.10), while in previous studies on GTP, little or no homopolymer contaminations were evidenced by GPC analysis.[20, 36] It should be mentioned that both polymers were synthesised during the summer when the air humidity was high. It is known that the GTP is very sensitive to even traces of water that have a negative impact on the living polymerisation reactions. Therefore, the increased humidity is most probably the reason for the deviations from the expected molecular weights. Time constraints did not allow for repeating the synthesis in more favourable humidity conditions.

Table 3.1. Theoretical and observed compositions and polydispersities of the PEGMA-*b*-THPMA block copolymers with theoretical weight-average molecular weights of 7200 g mol^{-1}. The values in brackets are obtained before purification.

Polymer	Observed^{a} M_n / g mol^{-1}	Theor. molar composition	Observed molar composition^{b}	M_w/M_n^{a}
AB1	78800 (108000)	30:70	8:92 (46:54)	2.3 (2.4)
AB2	120000 (108000)	25:75	13:87 (30:70)	2.0 (2.6)

^{a} Determined by GPC; ^{b} determined by NMR spectroscopy.

The purification of polymers by precipitation significantly reduced the amount of free PEGMA homopolymer in the samples (see Figures 3.11 and 3.12). It also affected the data for the polymer composition determined from the NMR spectra shown in Figures 3.13 and 3.14. This is expected, because the NMR cannot distinguish between the PEGMA homopolymer and that in the copolymer. The data in Table 3.1.

shows that the purified diblock copolymers are much richer in THPMA than expected.

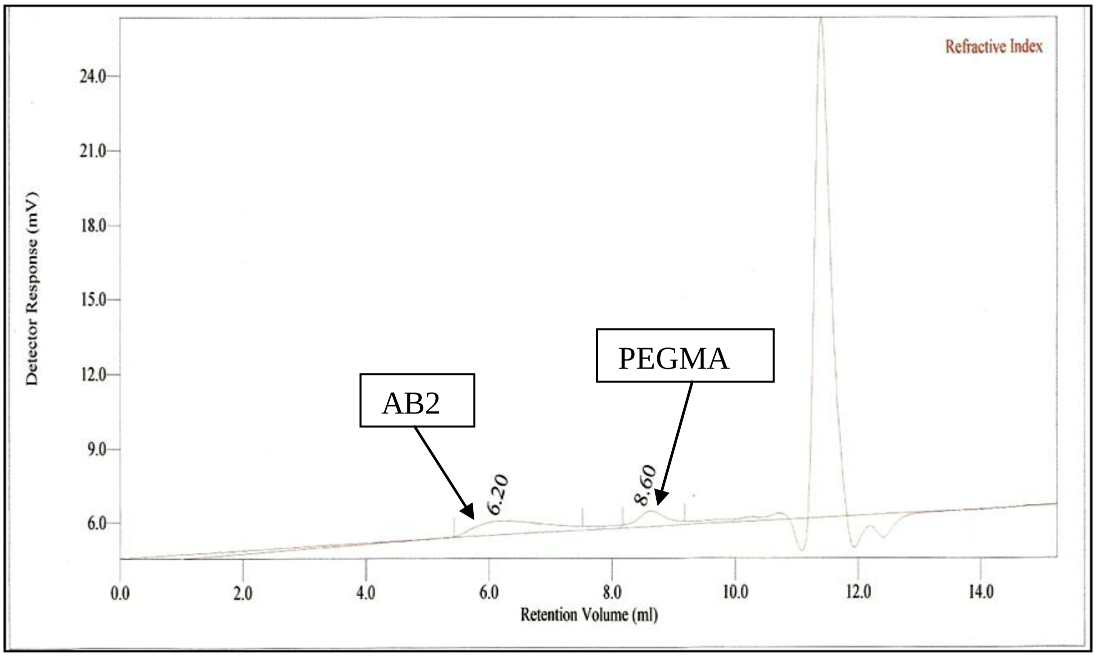


Figure 3.11. Gel permeation chromatogram of the $PEGMA_6$ -b-$THPMA_{32}$ diblock copolymer (AB2) obtained after its purification.

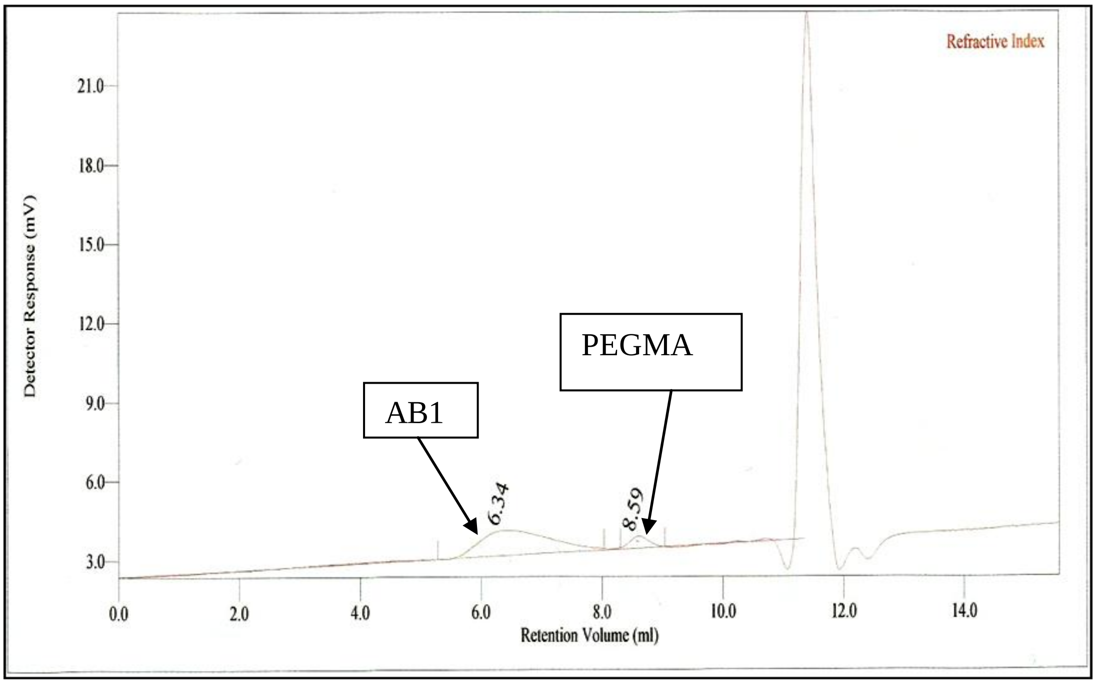


Figure 3.12. Gel permeation chromatogram of the $PEGMA_7$-b-$THPMA_{30}$ diblock copolymer (AB1) obtained after its purification.

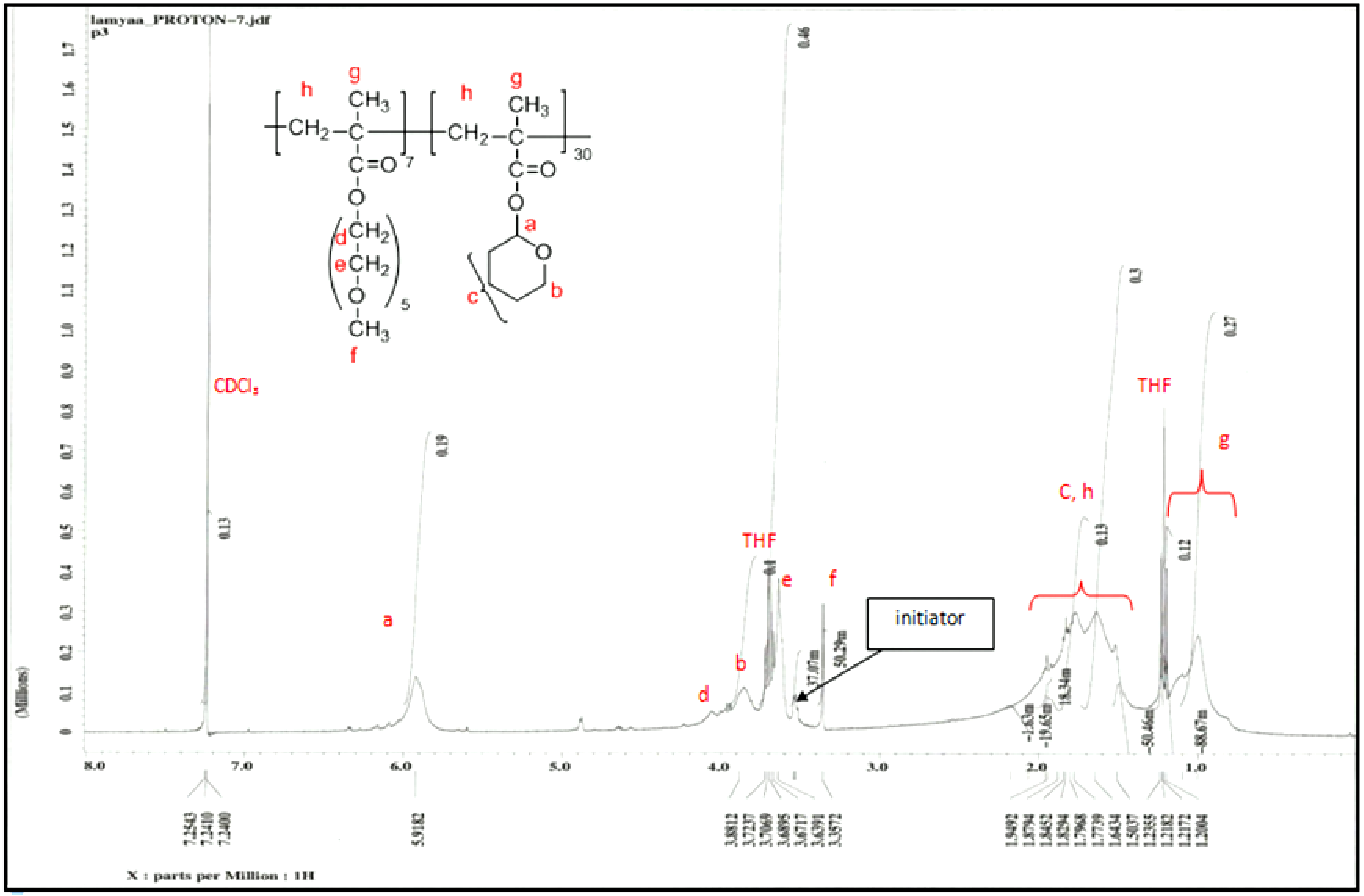


Figure 3.13 ^{1}H NMR spectrum of the AB1 (PEGMA$_{7.2}$-THPMA$_{29.6}$) polymer after purification recorded in CDCl$_3$.

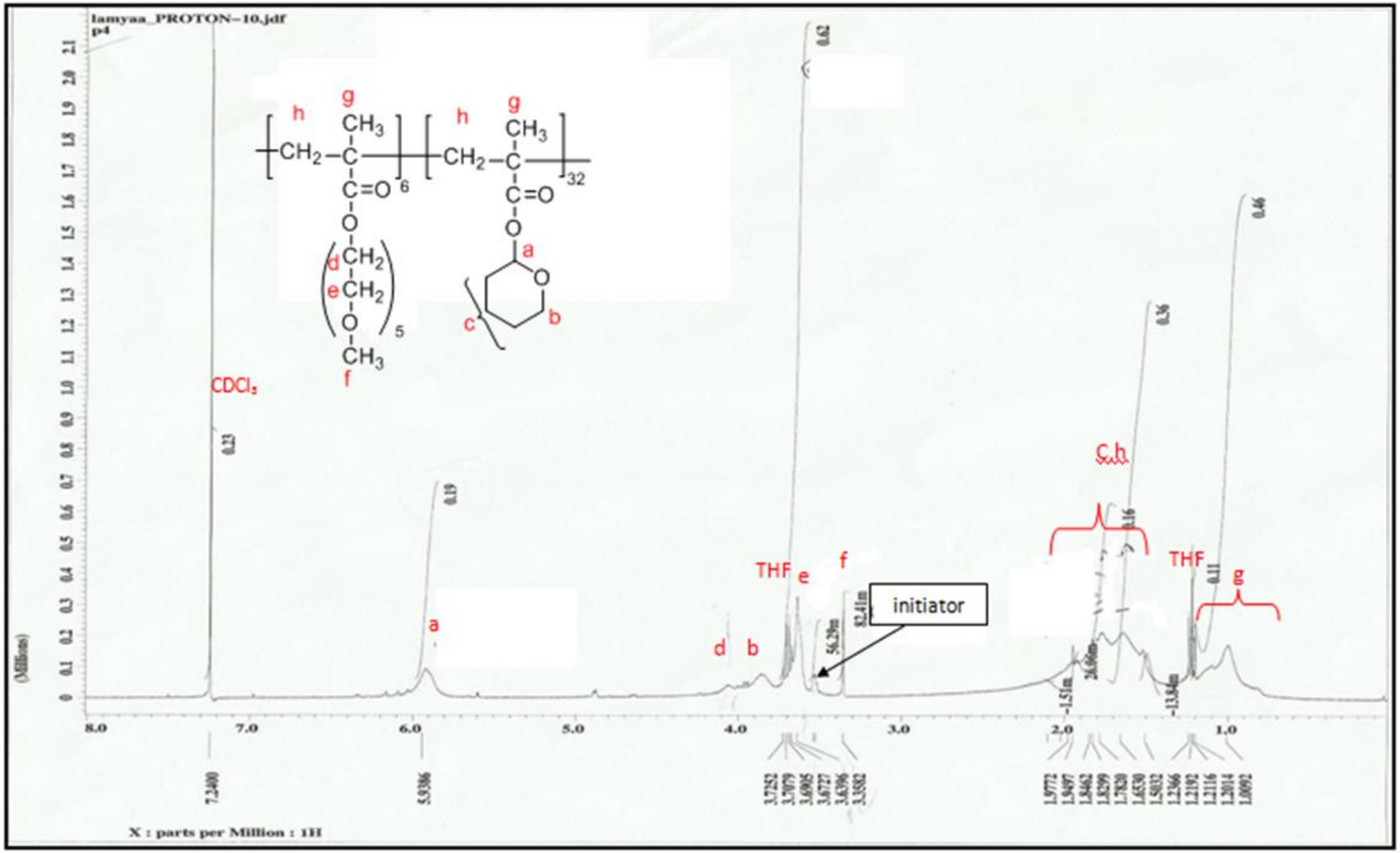


Figure 3.14 ^{1}H NMR spectrum of the AB2 (PEGMA$_{6.0}$-THPMA$_{31.8}$) polymer after purification recorded in $CDCl_3$.

3.4 Conclusions

The group transfer polymerisation (GTP) technique has been used to synthesise two novel amphiphilic diblock copolymers of poly ethylene glycol (PEGMA) and 2-tetrahydropyranyl methacrylate (THPMA). Gel permeation chromatography was used to determine block copolymer molecular weights and polydispersities, while proton NMR spectroscopy was used to assess their composition.

The THPMA monomer needed for the synthesis of the copolymers has been successfully synthesised and purified as demonstrated by ^{1}H NMR spectroscopy. Diblock copolymers were also produced, although their molecular weights and polydispersities were significantly higher than expected. This could be attributed to the poor control over the GTP due to significantly higher air humidity during their synthesis. The copolymer compositions were also affected, showing increased amounts of THPMA in the hydrophobic block and reduced amounts of PEGMA in the hydrophilic one. It is expected that this will affect the emulsifying ability of the amphiphilic diblock copolymers produced.

.

Chapter 4: Emulsifying ability of polymeric and common surfactants in systems with polymerisable oil phase

4.1 Introduction

The aim of this study is to obtain information about the ability of the two diblock copolymers, synthesised by us as described in chapter 3 and other commercially available surfactants (Span 80 or oleic acid and $CaCO_3$ solid particles), to stabilise W/O emulsions of polymerisable oils (MMA and St monomers) and water. This is an important first step in evaluating their potential application as stabilisers of high internal phase emulsion (HIPE) templates for fabricating porous polymeric scaffolds after polymerisation of the continuous oil phase.

4.2 Experimental

The oil phase used in all experiments was a mixture of a MMA or St monomer and a cross-linker, 1,6-Hexandiol-diacrylate (HDDA), in a volume ratio of 90:10. It also contained a thermo-initiator (AIBN, 1 mol% with respect to the double bonds of 1,6 Hexandiol diacrylate) and an emulsifier – either a diblock copolymer synthesised by us at concentrations 1 – 5 wt%, or a low molecular weight surfactant Span 80 at concentrations in the range 10 – 20 wt%. In some experiments, oleic acid and calcium carbonate particles were used in the oil as emulsifiers. The aqueous phase in all emulsions contained 0.27 M $CaCl_2$ because the emulsion stability was dependent on the addition of electrolyte to the water phase. Seeing that, Increasing the electrolyte concentration increased the refractive index of the water phase, and thus decreased the refractive index difference between oil and water phases. This decreased the attractive force between water droplets, which resulted in reducing the coalescence of droplets and increasing the stability of emulsions.[37]

The emulsions in these tests were prepared by hand shaking or/and by using an Ultra Turrax homogeniser, as described in chapter 2. The emulsion type was determined by drop tests and their stability was evaluated from images of the vessels with the emulsion taken at different times after their preparation (see chapter 2).

4.3 Results and discussion

4.3.1 Emulsions prepared with PEGMA-b-THPMA diblock copolymers as an emulsifier

4.3.1.1 Emulsions containing MMA

Images of the MMA containing emulsions prepared by hand shaking or a homogeniser are shown in Tables 4.1 – 4.4. It is seen that these emulsions made from equal volumes of oil and water are very unstable against sedimentation and coalescence. Those made by handshaking quickly separated to oil and water soon after the emulsification. Although some improvement in the stability of homogenised emulsions is observed, it is clear that both diblock copolymers are not efficient emulsifiers of MMA containing emulsions.

Table 4.1 Images of vessels of MMA emulsions with volume fraction of water 0.5 made by hand shaking in the presence of copolymer AB1, PEGMA-b-THPMA (8% - 92%), in the oil phase. Other details for the copolymer can be found in Table 3.1 of chapter 3.

Polymer concentration	**Time**			
	0 min	**1min**	**10 min**	**1 day**
1wt/v%				
2 wt/v%				

Table 4.2 Images of vessels of MMA emulsions with volume fraction of water 0.5 made by hand shaking in the presence of copolymer AB2, PEGMA-b-THPMA (13% - 87%), in the oil phase. Other details for the copolymer can be found in Table 3.1 of chapter 3.

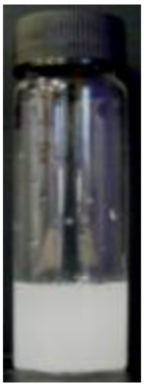
Polymer concentration	**Time**			
	0 min	**1min**	**30 min**	**1 day**
1wt/v%				
2 wt/v%				

Table 4.3. Images of vessels of MMA emulsions with volume fraction of water 0.5 made by a homogeniser at 12600 rpm for 1 min in the presence of copolymer AB1, PEGMA-b-THPMA (8% - 92%), in the oil phase. Other details for the copolymer can be found in Table 3.1 of chapter 3.

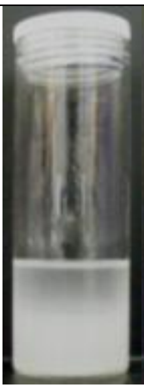
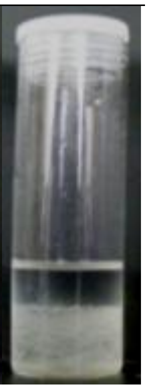
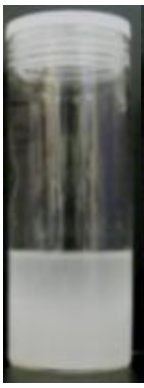
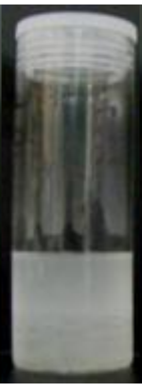
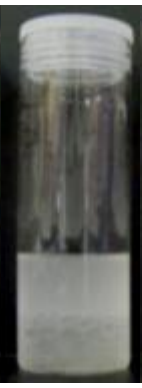
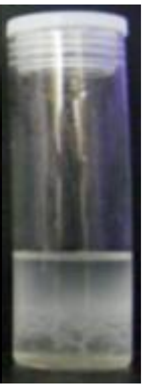
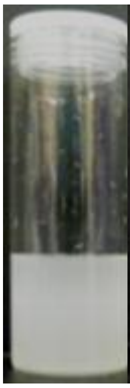
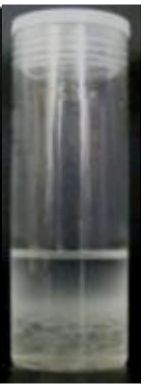
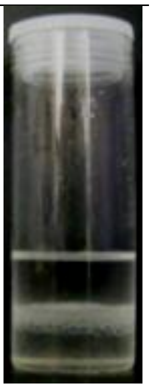
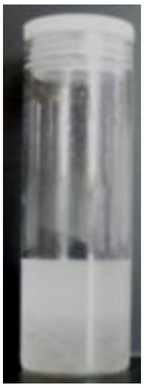
polymer concentration	**Time**				
	0 min	**1 min**	**10 min**	**30 min**	**1 day**
1wt/v%					
2 wt/v%					
5wt/v%					

Table 4.4. Images of vessels of MMA emulsions with volume fraction of water 0.5 made by a homogeniser at 12600 rpm for 1 min in the presence of copolymer AB2, PEGMA-b-THPMA (13% - 87%), in the oil phase. Other details for the copolymer can be found in Table 3.1 of chapter 3.

polymer concentration	**Time**				
	0 min	**1min**	**10 min**	**30 min**	**1 day**
1wt/v%					
2 wt/v%					
5wt/v%					

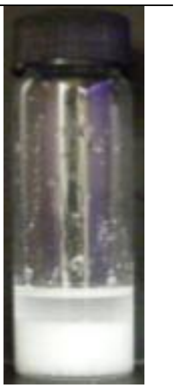
4.3.1.2 Emulsions containing Styrene

Styrene containing emulsions were significantly more stable than those containing MMA and improvement in the emulsifying ability of both copolymers was observed after purification. Tables 4.5 and 4.6 display images of vessels with the emulsion of styrene made by handshaking from an equal volume ratio of W/O, using 1w/v% and 2 w/v% purified copolymers. In all cases, the emulsions are unstable against sedimentation noticeable just 1 minute after emulsification. However, coalescence has not been detected in the emulsions for up to 1 hour after their preparation. Emulsions prepared with the same copolymers purified twice by using a homogeniser at 12600 rpm for 1 min were much less prone to sedimentation and completely stable against coalescence for 3 days (Table 4.7). The droplet diameters estimated from microscope images (Fig. 4.1) were in the range 1 – 20 µm.

Table 4.5. Images of vessels of Styrene containing emulsions with volume fraction of water 0.5 made by hand shaking (vigorously, 30 times) in the presence of copolymer AB1, PEGMA-b-THPMA (8% - 92%), in the oil phase. Other details for the copolymer can be found in Table 3.1 of chapter 3. All emulsions are w/o type as confirmed by drop tests.

Polymer concentration	**Time**				
	0 min	**1 min**	**10 min**	**30 min**	**1 hour**
1wt/v%					
2 wt/v%					

Table 4.6. Images of vessels of Styrene containing emulsions with volume fraction of water 0.5 made by hand shaking (vigorously, 30 times) in the presence of copolymer AB2, PEGMA-b-THPMA (13% - 87%), in the oil phase. Other details for the copolymer can be found in Table 3.1 of chapter 3. All emulsions are w/o type as confirmed by drop tests.

Polymer concentration	**Time**				
	0 min	**1 min**	**10 min**	**30 min**	**1 hour**
1wt/v%					
2 wt/v%					

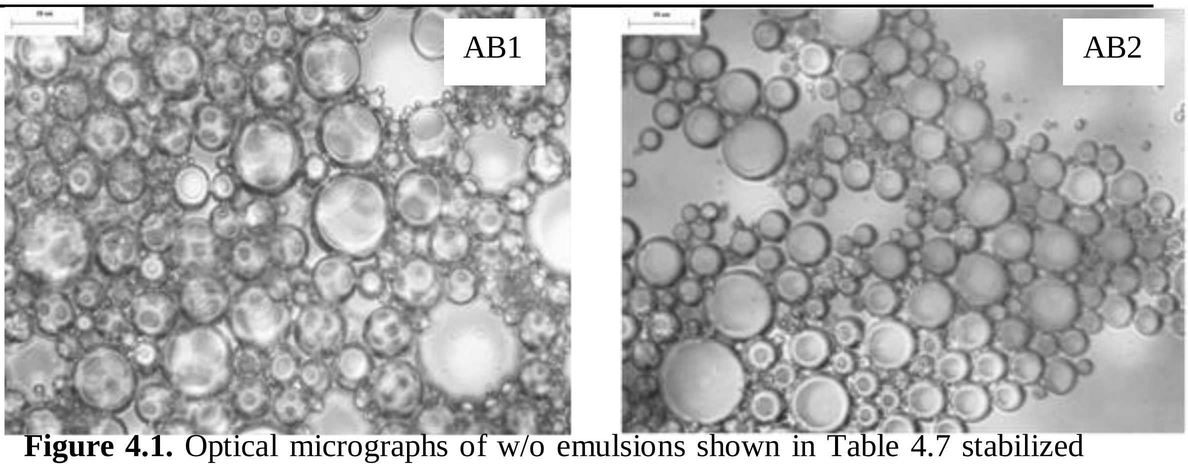


Figure 4.1. Optical micrographs of w/o emulsions shown in Table 4.7 stabilized by 1w/v% diblock copolymers purified twice by precipitation. Scale bars are equal to 20 µm.

Table 4.7. Images of vessels of Styrene containing emulsions with volume fraction of water 0.5 made by a homogeniser at 12600 rpm for 1 min in the presence of 1 wt/v% copolymer in the oil phase. Both copolymers have been purified twice by precipitation (see also Table 3.1 of chapter 3). All emulsions are w/o type as confirmed by drop tests.

Polymer	**Time**					
	0 min	**1 min**	**10 min**	**30 min**	**1 hour**	**3days**
AB1 PEGMA-b-THPMA (8% - 92%)						
AB2 PEGMA-b-THPMA (13% - 87%)						

4.3.2 Emulsions prepared with Span 80 as an emulsifier

The ability of Span 80 to stabilise emulsions containing MMA monomer was investigated and the effects of surfactant concentration and volume fraction of water were studied. All emulsions were prepared by putting the oil and water phases together and homogenising with an Ultra Turrax homogeniser at 12600 rpm for 1 minute. In order to check if the thermo-initiator AIBN present in the oil phase at 1 mol% has any influence on the emulsion properties, we carried out two series of experiments with and without AIBN present.

Images of the vessels with emulsions prepared with AIBN in the oil phase at water volume fraction of 0.5 are shown in Table 4.8. Some of the emulsions were not very stable, but they all were of w/o type as expected for a low HLB surfactant, such as the Span 80 used. Volume fractions of resolved water, oil and that of the remaining emulsion were monitored over time and are plotted in Fig. 4.2. The fraction of oil resolved is associated with the sedimentation, while that of resolved water – with the water drop coalescence. It was seen that there was a dramatic increase in the emulsion stability by increasing the concentration of Span 80. At 13 w/v% Span 80, the emulsions were unstable against coalescence and sedimentation. They separated quickly to the oil and water phases within 10 minutes after homogenisation. However, by increasing the concentration of Span 80 to 16.7w/v%, the emulsions were stable for an hour, while those at 20 w/v% Span 80 were completely stable to both sedimentation and coalescence for up to 4 hours.

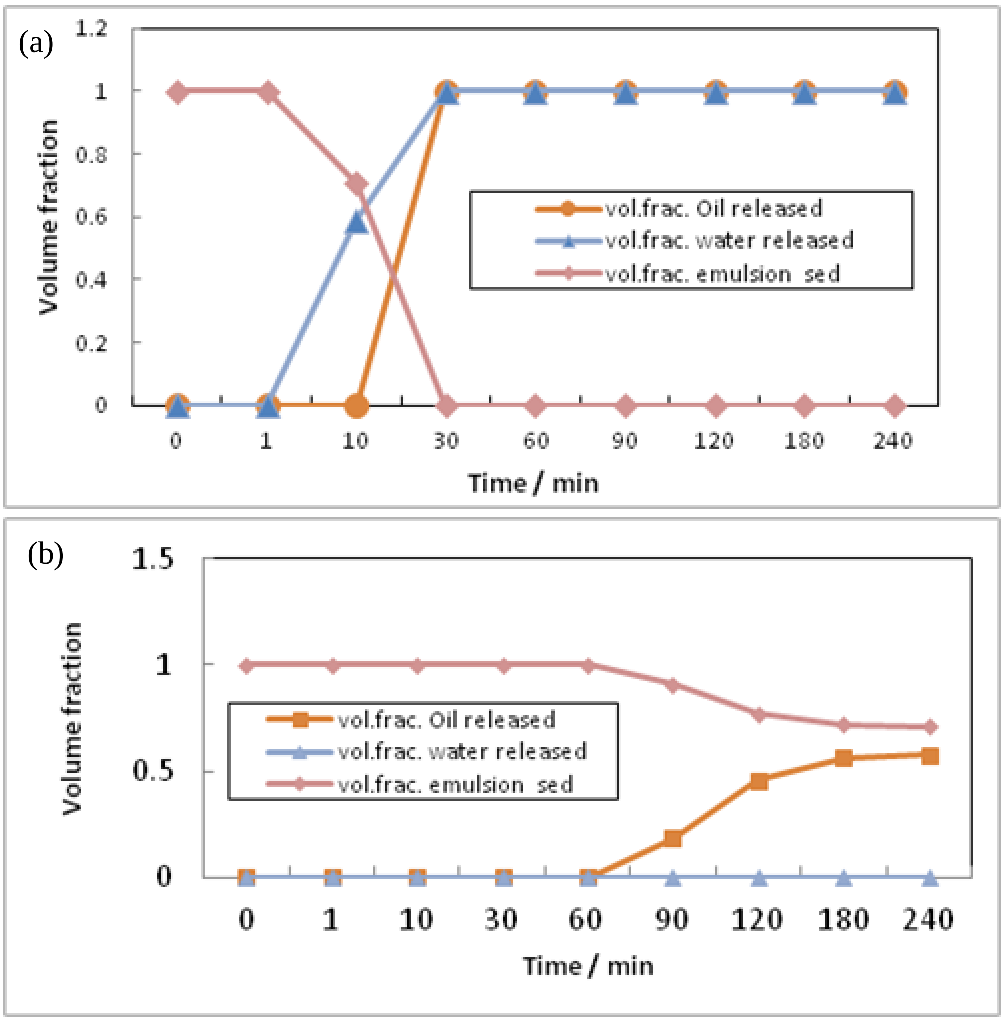


Figure 4.2. Volume fractions of released water, oil and remaining emulsion obtained from the images in Table 4.8 versus time after emulsification. (a) 13 wt/v% Span 80; (b) 16.7 wt/v% Span 80. The data for emulsions prepared at 20 wt/v% Span 80 are not shown because those emulsions were completely stable for 240 min (see the last row of Table 4.8).

Table 4.8. Images of vessels of MMA containing emulsions with volume fraction of water 0.5 made by a homogeniser at 12600 rpm for 1 min in the presence of Span 80 and AIBN initiator in the oil phase. All emulsions are w/o type as confirmed by drop tests

NO	**Span 80 concentration**	**Time**						
			0 min	**1 min**	**10 min**	**30 min**	**1 hour**	**4 hours**
1	**13 wt/v%**	W/O						
2	**16.7 wt/v%**	W/O						
3	**20 wt/v%**	W/O						

Table 4.9. Images of vessels of MMA containing emulsions with volume fraction of water 0.7 made by a homogeniser at 12600 rpm for 1 min in the presence of Span 80 and AIBN initiator in the oil phase. All emulsions are w/o type as confirmed by drop tests.

Span 80 concentration	**Time**					
	0 min	**1 min**	**10 min**	**30 min**	**1 hour**	**4 hours**
13 wt/v%						
16.7 wt/v%						
20 wt/v%						

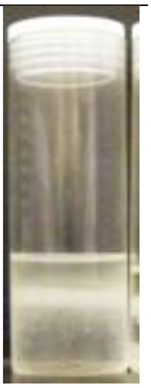
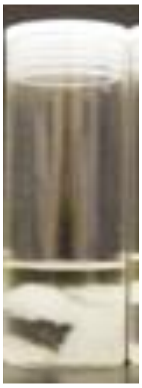
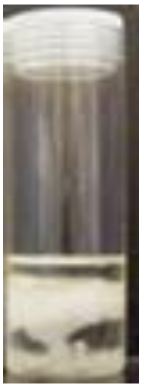
Images of the vessels with emulsions prepared with AIBN in the oil phase but at a higher water volume fraction of 0.7 are shown in Table 4.9. The emulsions were stable against sedimentation and coalescence for several hours, except those at the lowest Span 80 concentration. It seems that the increase of the internal water phase volume fraction improves the emulsion stability (compare with Table 4.8).

The results from the second series of emulsions prepared in the same conditions but without AIBN initiator added are shown in Tables 4.10 and 4.11 for water volume fractions of 0.5 and 0.7, respectively. The respective changes of the volume fractions of water, oil and remaining emulsion over time are shown in Figures 4.3 and 4.4. Surprisingly, all emulsions prepared without a thermo-initiator present in the oil phase are less stable than those containing an initiator. Since the AIBN initiator is not expected to be surface active, we can hypothesise that the AIBN has initiated the polymerisation of the MMA monomer and the hydrophobic tail of the surfactant (note that there is a double bond in the Span 80). Although the rate of polymerisation at room temperature should be slow, noticeable amounts of surface active oligomers capable of boosting the emulsion stability could be produced. This rather unexpected effect can be a subject of future investigation.

Table 4.10. Images of vessels of MMA containing emulsions with volume fraction of water 0.5 made by a homogeniser at 12600 rpm for 1 min without added AIBN initiator in the oil phase. All emulsions are w/o type as confirmed by drop tests.

Span 80 concentration	**Time**					
	0 min	**1 min**	**10 min**	**30 min**	**1 hour**	**4 hours**
13 wt/v%						
16.7 wt/v%						
20 wt/v%						

Table 4.11. Images of vessels of MMA containing emulsions with volume fraction of water 0.7 made by a homogeniser at 12600 rpm for 1 min without added AIBN initiator in the oil phase. All emulsions are w/o type as confirmed by drop tests.

Span 80 concentration	**Time**					
	0 min	**1 min**	**10 min**	**30 min**	**1 hour**	**4 hours**
13 wt/v%						
16.7 wt/v%						
20 wt/v%						

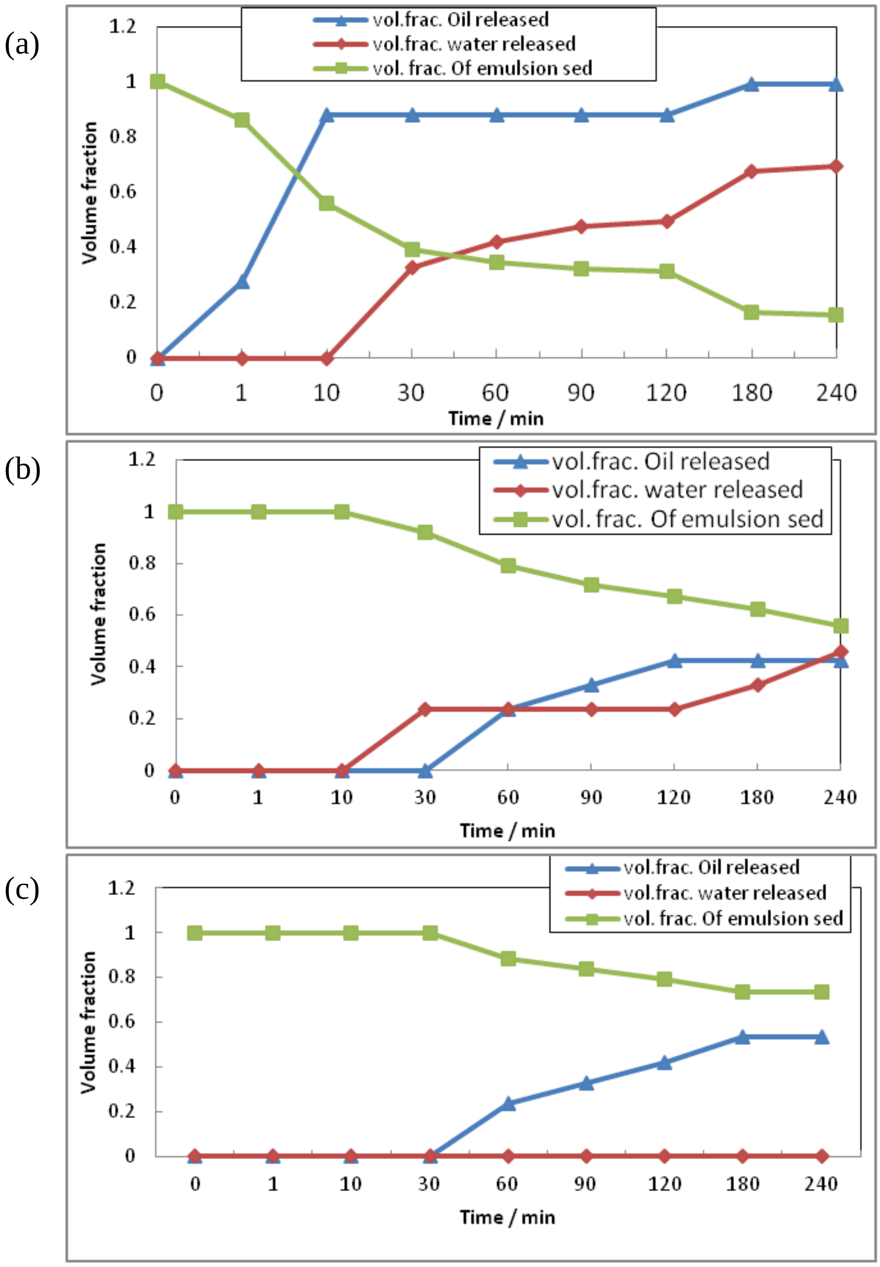


Figure 4.3. Volume fractions of released water, oil and remaining emulsion obtained from the images in Table 4.10 versus time after emulsification. Span concentrations are : (a) 13, (b) 16.7 and (c) 20 wt/v%. Emulsions have been prepared without added AIBN initiator to the oil phase. The volume fraction of water in emulsions is 0.5.

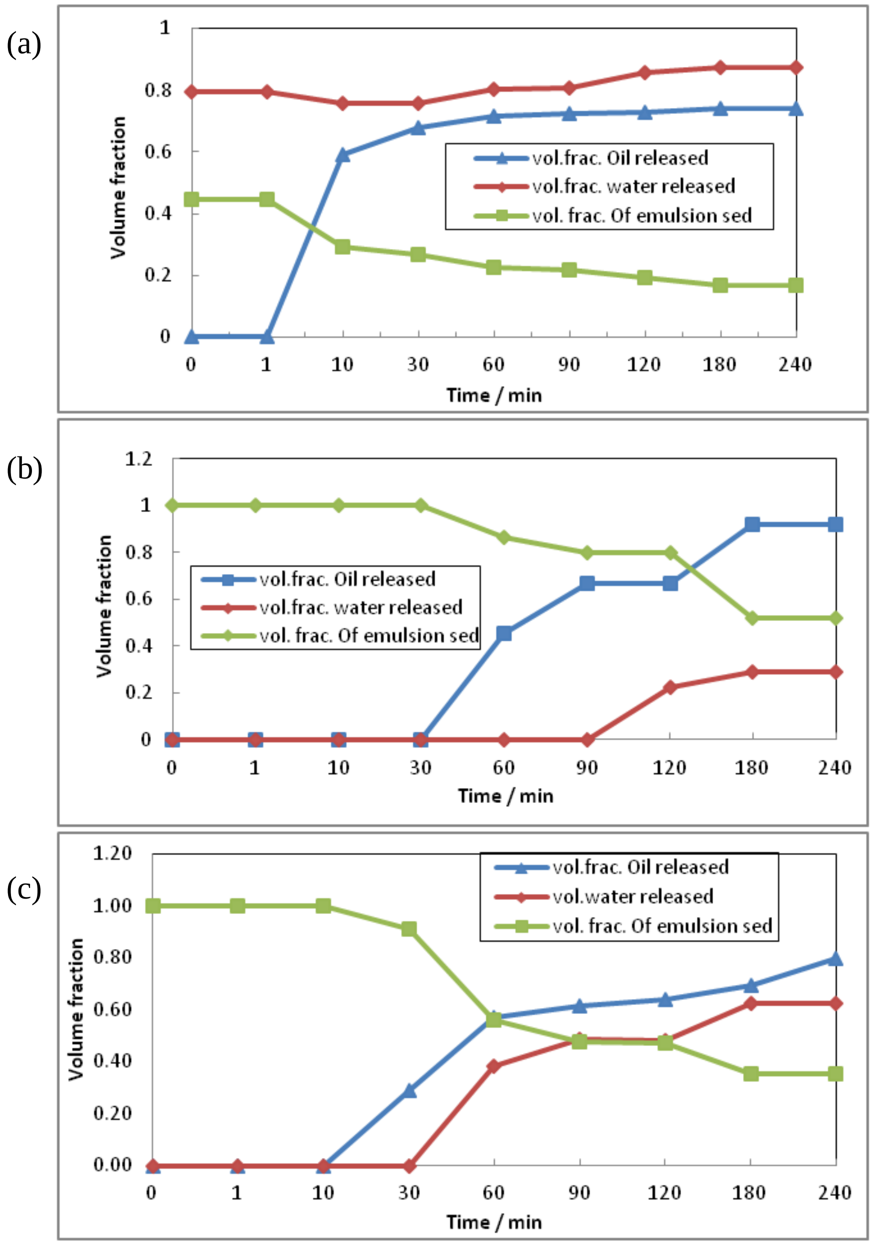
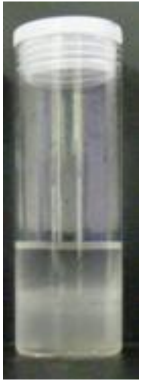


Figure 4.4. Volume fractions of released water, oil and remaining emulsion obtained from the images in Table 4.11 versus time after emulsification. Span 80 concentrations are: (a) 13, (b) 16.7 and (c) 20 wt/v%. Emulsions have been prepared without added AIBN initiator to the oil phase. The volume fraction of water in emulsions is 0.7.

4.3.3 Emulsions prepared with oleic acid and calcium carbonate particles as emulsifiers

The polymeric surfactants we synthesised were found to be poor emulsifiers of the MMA containing emulsion. Span 80 performed better, but only at concentrations larger than 15 wt/v%. This has motivated us to test the emulsifying ability of solid calcium carbonate particles in the current system. Since the particles are hydrophilic, it is expected that they will stabilise o/w emulsions. It is known that hydrophobic particles are needed to promote the formation of w/o emulsions. To make the calcium carbonate particles hydrophobic, we added oleic acid to the particle dispersion in the oil phase. The oil phase containing 5 wt/v% particles and 5 wt/v% oleic acid was mixed with an equal volume of aqueous phase (0.27 M $CaCl_2$ solution) and homogenised with an ultra Turrax homogeniser at 12600 rpm for 1 min. Images of the vessels with emulsions taken at different times after emulsification are shown in Table 4.12. Images of the emulsions prepared with particles or oleic acid alone are also shown for comparison. It appeared that the oleic acid alone was not able to form emulsion. Solid particles alone produced rapidly creaming o/w emulsion. The emulsion produced with the particle-surfactant mixture was of w/o type. It was not very stable to sedimentation, but showed significant stability against coalescence. These preliminary results suggest that such particle-surfactant mixtures could be used for stabilising MMA containing emulsions and probably also HIPEs. This seems an interesting direction for future research.

Table 4.12. Images of vessels of MMA containing emulsions with volume fraction of water 0.5 made by a homogeniser at 12600 rpm for 1 min in the presence of calcium carbonate particles or oleic acid or both in the oil phase.

Emulsifier	**Time**			
	0 min	**1 min**	**10 min**	**4 hours**
5 wt/v%CaCO$_3$				
5 wt/v% of CaCO$_3$ and 5wt/v %of Oleic acid				
5 wt/v% of Oleic acid				

4.4 Conclusions

The effect of the two amphiphilic diblock copolymers we synthesised on the emulsion stability was investigated and their ability to stabilise emulsion of MMA and styrene against coalescence and sedimentation was evaluated. It was found that both copolymers were not able to form stable emulsions of MMA. However, the water-in-styrene emulsions prepared with 1 wt/v% purified copolymers showed reasonable stability against both coalescence and sedimentation. These results suggest that the polymeric surfactants we synthesised could be able to stabilise styrene containing HIPEs. The experimental tests of this hypothesis are presented in the next chapter 5.

A commercial low molecular weight surfactant (Span 80) and mixtures of solid calcium carbonate particles with oleic acid were also tested as alternative emulsifiers in the systems studied. It was found that Span 80 was able to make stable water-in-MMA emulsions with water volume fractions of 0.5 and 0.7, but only at concentrations larger than 15 wt/v%. This concentration range is in agreement with previous studies on similar systems with Span 80 as emulsifier. A noticeable positive effect of the thermo-initiator AIBN on the emulsion stability was observed. Further studies are needed to reveal the role of the thermo-initiator in emulsion stabilisation at room temperature. Emulsions produced with particle-surfactant mixtures were of w/o type and had reasonable stability against coalescence. It could be interesting to study such systems in future investigations.

Chapter 5: Preparation of high internal phase emulsions and porous polymeric scaffolds

5.1 Introduction

The interest in the polymerisation of high internal phase emulsions (HIPEs) giving polyHIPEs has increased over the last decade, because it is a relatively simple technique for the fabrication of highly porous scaffolds with controlled morphology.[38] The oil phase of w/o HIPE templates is usually a mixture of monomers, such as styrene (St) or methyl methacrylate (MMA), a cross-linker and a thermo-initiator (e.g. AIBN). The polymerisation of the HIPE template is carried out at temperatures in the range 50-80 ^{o}C and occurs via a free radical mechanism that has been well investigated and understood (see Figures 5.1 and 5.2). It is known that the oxygen dissolved in the oil phase acts as a scavenger of free radicals, thus inhibiting the polymerisation reaction. Therefore, usually the HIPEs are prepared by the continuous addition of the internal water phase to the oil phase under a blanket of nitrogen gas to remove the dissolved oxygen and enhance the polymerisation of the HIPE template at the subsequent stage.

In this study, we have prepared w/o HIPE templates by the gradual addition of an aqueous phase (0.27 M $CaCl_2$) to the oil phase until the volume fraction of water (the internal phase) in the emulsion reached 0.8. The PEGMA-b-THPMA diblock copolymers synthesised by us (see chapter 3) and Span 80 (a low molecular weight surfactant) were used as emulsifiers. The aqueous phase in all experiments contained 0.27 M $CaCl_2$ to prevent Ostwald ripening. The effect of nitrogen gas treatment of the emulsions during or after their preparation on their properties was also investigated.

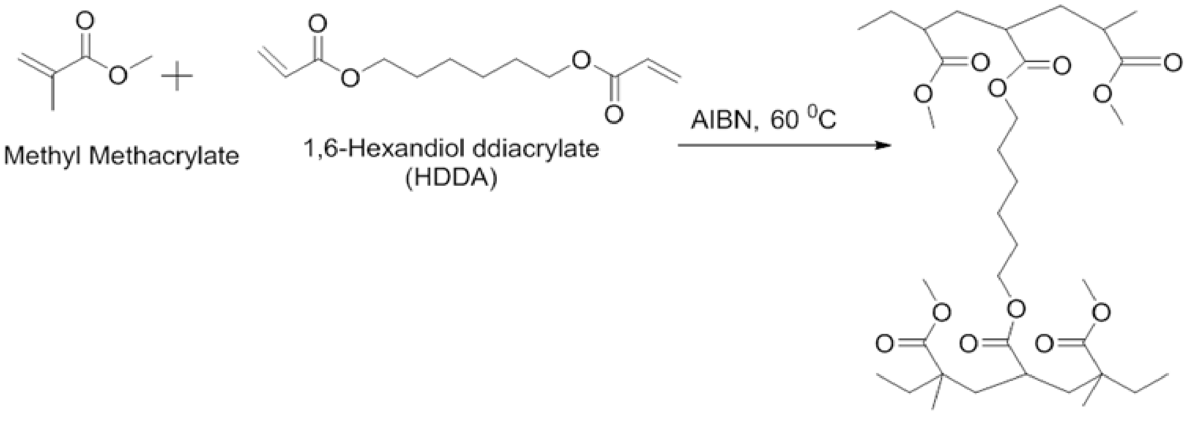


Figure 5.1. Synthesis of poly-MMA cross-linked with HDDA.

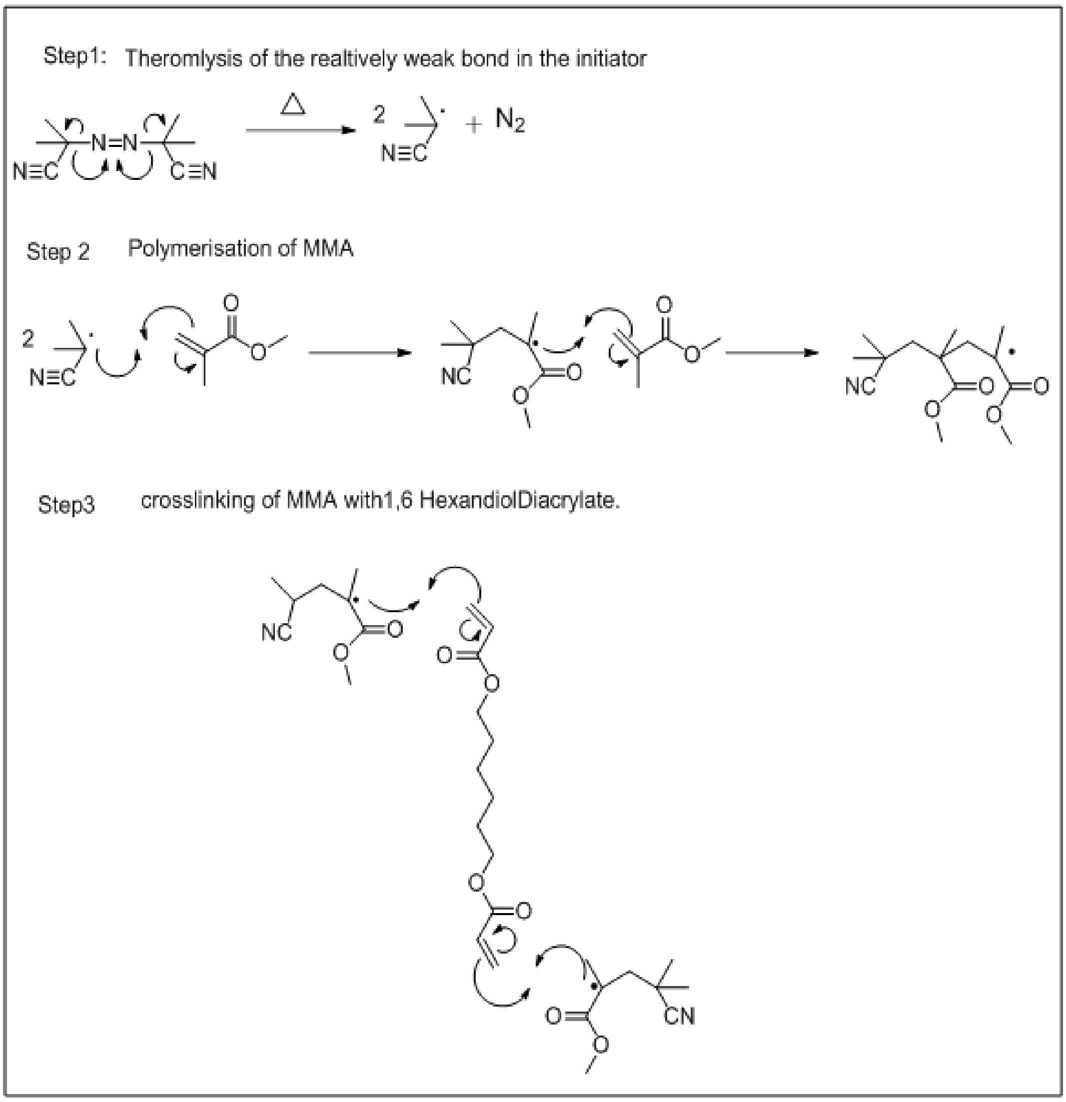


Figure 5.2. The mechanism of polymerisation of the continuous phase of MMA in the presence of HDDA cross-linker using AIBN as an initiator.

5.2 Experimental

The HIPEs were prepared as described in chapter 2. Briefly, a round-bottom three neck flask was loaded with the oil phase, containing a mixture of MMA or St and HDDA (a cross-linker) at a volume ration of 9:1, 1 mol% AIBN (a thermo-initiator) and emulsifier. The aqueous phase was added dropwise by a syringe pump under continuous stirring of the oil-water mixture at 300 rpm with an overhead stirrer supplied with a paddle stirring shaft. Some emulsions were treated with nitrogen gas for up to 15 minutes. The gas flow was supplied to the flask through one of its necks, thus filling the space above the emulsion. When the preparation of a HIPE was successful, it was polymerised in the oven at 60°C overnight, the polyHIPE obtained was purified by Soxhlet extraction and its structure revealed by using SEM imaging.

5.3 Results and discussion

5.3.1 Preparation of HIPEs using PEGMA-b-THPMA diblock copolymers as a emulsifier

Our previous results, described in chapter 4, have shown that both diblock copolymers synthesised by us were able to produce stable water-in-styrene emulsions with a volume fraction of water 0.5 at a polymer concentration of 2 wt%. Therefore, it was expected that they would also be able to stabilise HIPEs. It turned out that this was not the case. The emulsions were formed at low volume fractions of water, but they became unstable and phase separated during the preparation when the water vol. fraction exceeded ~ 0.65. Emulsions at higher polymer concentrations were not investigated due to the limited amounts of diblock copolymers available.

5.3.2 Preparation of HIPEs using Span 80 as an emulsifier

Our results, presented in chapter 4, revealed that Span 80 can stabilise water-in-MMA emulsions with water volume fractions up to 0.8 at high surfactant concentrations (13 – 20 wt%). Therefore, we have carried out the present study in the same range of Span 80 concentrations. Initially, the emulsions were formed in air, i.e. without flowing nitrogen gas through the flask containing the emulsion. Images of the vessels with emulsions obtained are shown in Tables 5.1 and 5.2. We found that the emulsions containing MMA were not very stable, in contrast to our previous findings when an Ultra Turrax homogeniser was used for their preparation (see chapter 4). This suggests that the method of emulsification plays a significant role in the preparation of HIPEs. The emulsions containing styrene, however, were much more stable (Table 5.2) and phase separation was not observed for several hours after their preparation.

To investigate if the removal of oxygen from the emulsion could affect their stability we have prepared HIPEs first and then passed nitrogen gas through the flask while still stirring for several minutes. To our surprise, the nitrogen gas flow had a dramatic effect on the emulsion viscosity. The emulsions treated with nitrogen gas for 7 minutes, in the case of styrene, or 15 minutes, in the case of MMA, were so viscous that we were able to take only small samples out of the flask. Images of the emulsion samples taken 3 hours after emulsification are shown in Table 5.3. In an attempt to understand the reasons behind the strong effect of nitrogen gas treatment observed on the emulsions, we have investigated in more detail as described in the next section.

Table 5.1. Images of vessels containing w/o MMA-HIPEs with volume fraction of water 0.8 made by using an overhead stirrer at 300 rpm without using nitrogen gas flow.

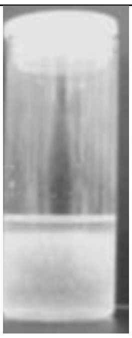
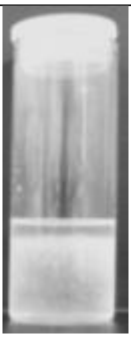
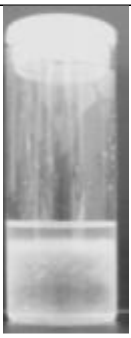
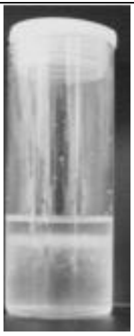
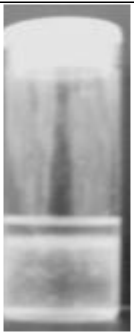
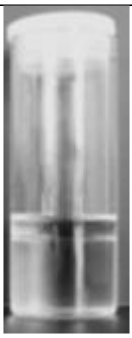
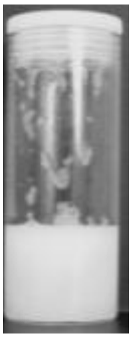
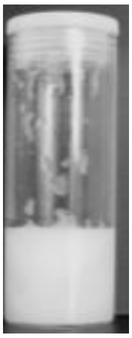
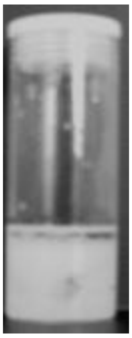
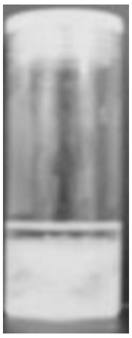
Span 80 concentration	Time					
	0 min	1 min	10 min	30 min	1 hour	1 day
13 wt/v%						
16.7 wt/v%						
20 wt/v%						

Table 5.2. Images of vessels containing w/o St-HIPEs with volume fraction of water 0.8 made by using an overhead stirrer at 300 rpm without using nitrogen gas flow.

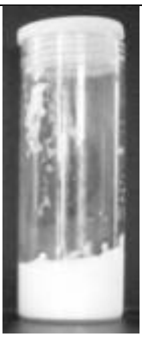
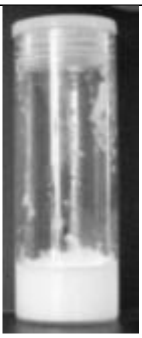
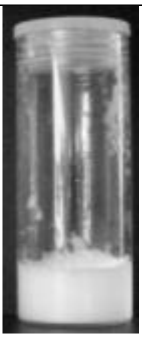
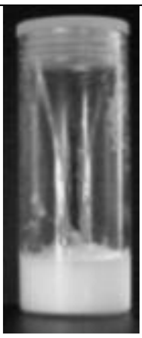
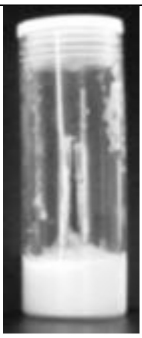
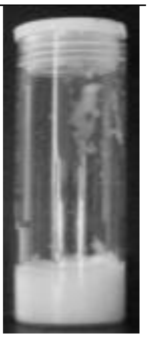
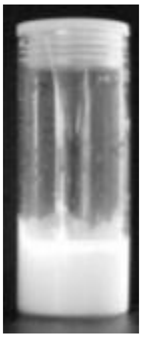
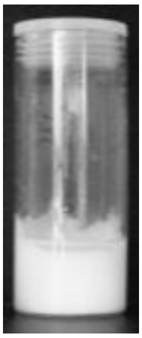
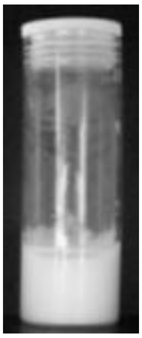
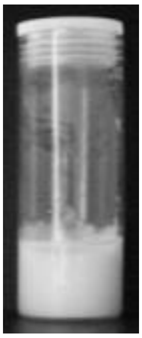
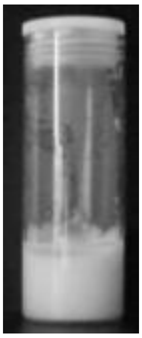
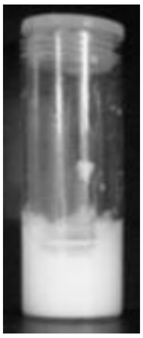
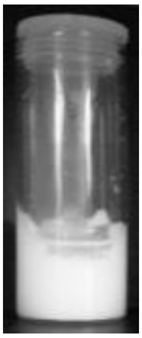
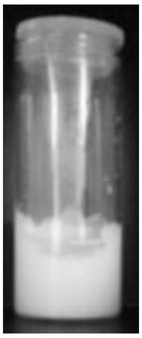
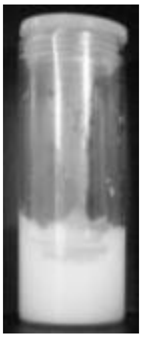
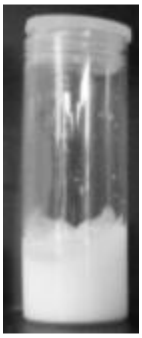
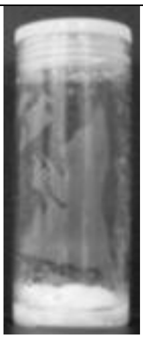
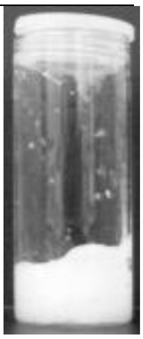
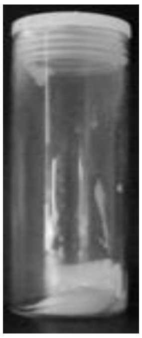
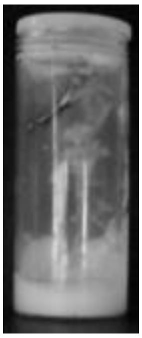
Span 80 concentration	Time					
	0 min	1 min	10 min	30 min	1 hour	3 hours
13 wt/v%						
16.7 wt/v%						
20 wt/v%						

Table 5.3. Images of vessels containing samples of w/o HIPEs with volume fraction of water 0.8 made by using an overhead stirrer at 300 rpm and subsequently treated with a nitrogen gas flow during stirring for 15 min (MMA) and 7 min (Styrene). The images are taken 3 hours after emulsification.

Monomer	**Span 80 concentration / wt/v%**		
	13	**16.7**	**20**
MMA (emulsion at 16.7wt/v of span 80treted with N_2 for 7 min.)			
Styrene			

5.3.3 Effect of nitrogen gas treatment on w/o emulsions stabilised with Span 80

In order to detect the effect of nitrogen gas treatment on the emulsion stability, we have made styrene containing emulsions with volume fraction of water 0.5. Two series of experiments were performed. In the first series, the oil phase was treated with nitrogen gas during stirring for different times. Then the gas flow was stopped and the aqueous phase added dropwise during stirring with an overhead stirrer at 300 rpm. The second series of experiments were performed by making the emulsion first, then passing nitrogen gas through the flask with the emulsion during stirring. The results from the first and second series of experiments are shown in Tables 5.4 and 5.5, respectively. A remarkable effect of nitrogen gas treatment was observed. Indeed, even after several minutes of treatment, the emulsions become viscous and very stable against sedimentation and coalescence. Since nitrogen is an inert gas and cannot react chemically with any of the species present, the observed effect should be related to the removal of oxygen (an inhibitor of polymerisation) from the system. The positive effect of oxygen removal by inert gas treatment of similar systems is well documented in the literature, but we are not aware that such a strong effect, as we observed, has been reported before.

Table 5.4. Images of vessels containing w/o emulsions with volume fraction of water 0.5 made by using an overhead stirrer at 300 rpm. The oil phase containing St/HDDA mixture (9/1 by vol.), 1 mol% AIBN and 20 wt/v% Span 80 has been treated with a nitrogen gas flow during stirring, before adding the aqueous phase to make the emulsions.

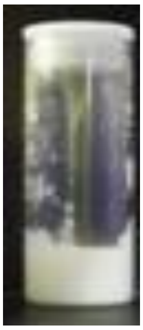
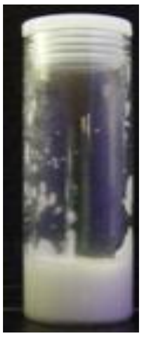
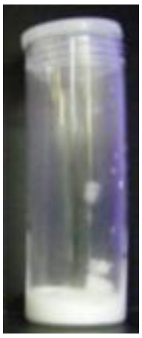
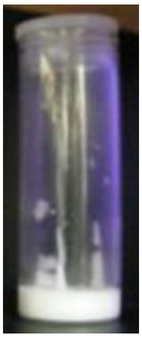
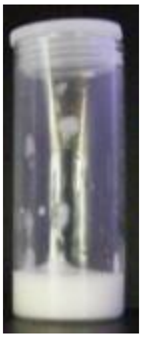
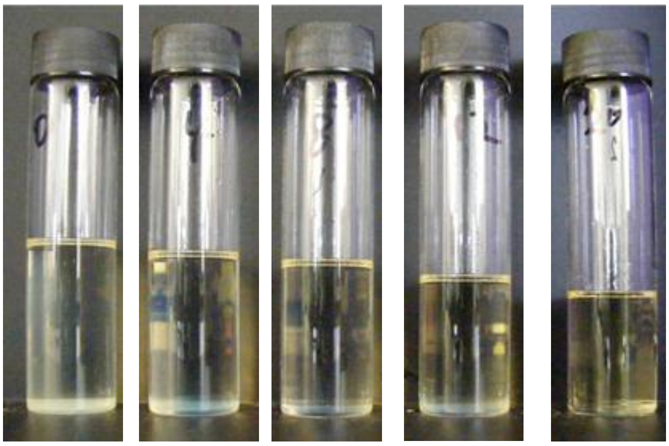
N_2 gas flow duration	**Time**					
	0 min	**1 min**	**10 min**	**30 min**	**1 hour**	**3 hours**
0 min						
2 min						
4 min						
8 min						

Table 5.5. Images of vessels containing w/o emulsions with volume fraction of water 0.5 made by using an overhead stirrer at 300 rpm. The oil phase contains St/HDDA mixture (9/1 by vol.), 1 mol% AIBN and 20 wt/v% Span 80. The emulsions have been treated with a nitrogen gas flow during stirring after their preparation.

N_2 gas flow duration	**Time**					
	0 min	**1 min**	**10 min**	**30 min**	**1 hour**	**3 hours**
0 min						
2 min						
4 min						
8 min						

One can hypothesise that the removal of oxygen (an inhibitor) from the oil phase could make it possible for the polymerisation of styrene to start even at room temperature, with a small but noticeable rate, thus increasing the continuous phase viscosity. It has been reported that increasing the viscosity of the polymerising phase increases emulsion stability, and affects the droplet size of the emulsion.[39] To test this hypothesis, we have treated the oil phase used in the emulsion preparation with a nitrogen gas during stirring, then measured its viscosity with an Ostwald viscometer, as described in chapter 2. The nitrogen gas treatment was carried out for different times in the same round-bottom flask used in the emulsion experiments. Images of the oil phase collected after the gas treatment are shown in Figure 5.3. A noticeable decrease in the oil volume is observed due to evaporation of styrene during the nitrogen gas flow treatment. The relative viscosity and volume changes measured are summarised in Table 5.6.



0 min 4 min 8 min 12 min 20 min

Figure 5.3. Images of vessels with an oil phase (a mixture of styrene and HDDA at a volume ratio of 9:1, 1 mol% AIBN and 20 wt/v% Span 80) collected after treatment with nitrogen gas for different times shown.

Table 5.6. Measured relative viscosity and volume of the oil phase, and estimated absolute and relative concentrations of Span 80 after treatment with a nitrogen gas flow for different durations**.**

Nitrogen flow duration / min	Relative viscosity	Volume / ml	Span 80 conc., C_t / wt/v%	C_0/C_t
0	1	20.0	20.0	1
4	1.10	18.9	21.2	1.06
8	1.14	17.9	22.3	1.12
12	1.33	15.9	25.1	1.26
20	1.67	14.0	28.5	1.43

The data in Table 5.6 shows that the viscosity of the oil phase progressively increases with the duration of nitrogen flow treatment. This could be attributed to the increase of the surfactant concentration due to a decrease of the oil volume caused by its evaporation. However, this data cannot explain the significant improvement of the emulsion stability observed after its treatment with nitrogen gas for just 2 min (see Table 5.4), because the changes of the viscosity and Span 80 concentration for such a short time are less than a few per cent. Further experiments are needed to reveal the reasons for the strong effect of nitrogen gas treatment on the emulsion stability we observed.

5.3.3 Preparation of polymerised HIPEs

The HIPEs prepared without and with a nitrogen gas treatment (see Tables 5-1, 5-2 and 5-3) were left to polymerise in an oven at 60 °C overnight. Those prepared with MMA without a nitrogen gas treatment were very unstable and phase separated completely just after emulsification or in the oven. The HIPEs prepared with nitrogen gas treatment were significantly more stable and survived the polymerisation stage The resulting material of polyHIPEs of MMA was of poor quality and very fragile whilst purified using a Soxhlet extractor.

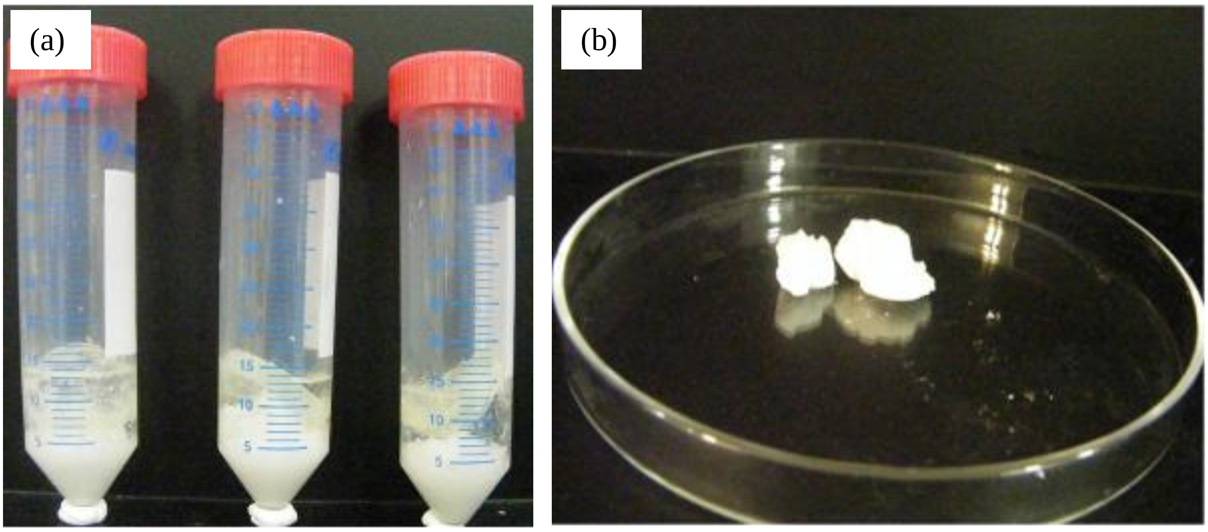


Figure 5.4. (a) Images of vessels with polymerised HIPEs of MMA at volume fraction of water 0.8 at a Span 80 concentration of (left to right) 13, 16.7 and 20 wt/v% prepared after treatment with nitrogen gas for 7 min. Note the polymerised layer of oil phase above the white material at the bottom of the vessels (b) An image of the white very fragile polymeric material obtained at the bottom of the vessels shown in (a).

The HIPEs of Styrene, however, were stable irrespective of the nitrogen gas treatment during their preparation. Images of the porous polymeric materials obtained after their polymerisation are shown in Fig. 5.5. The voids on the surface of the polymers obtained from nitrogen treated emulsions are due to air bubbles entrapped during the transfer of these rather viscous HIPEs into the tubes for polymerisation. These porous polymers showed lower mechanical strength in comparison to those obtained from HIPEs prepared without using nitrogen.

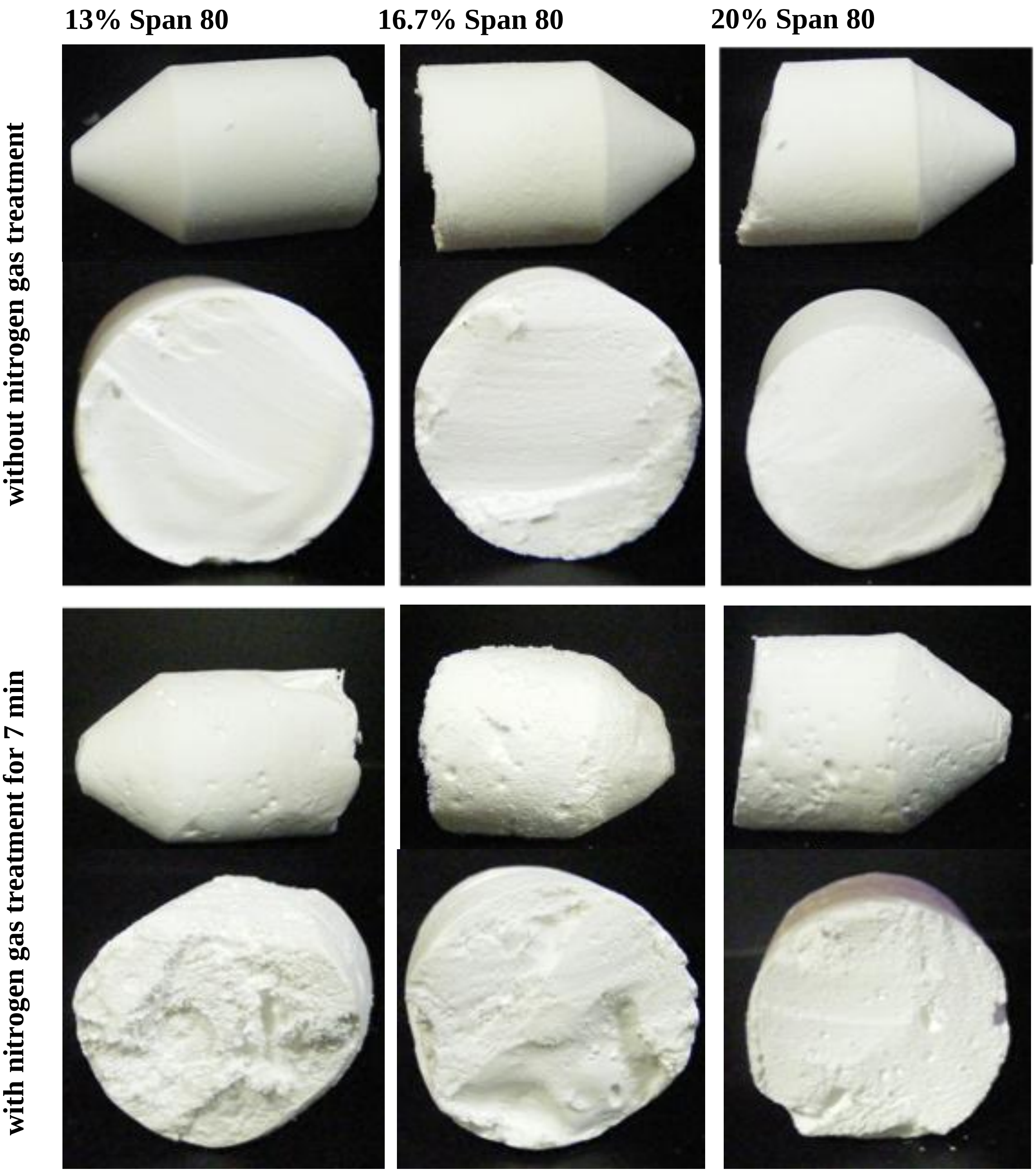


Figure 5.5. (a) Images of porous polymeric materials obtained after polymerisation of HIPEs of Styrene at volume fraction of water 0.8. All materials have been purified twice by Soxhlet extraction.

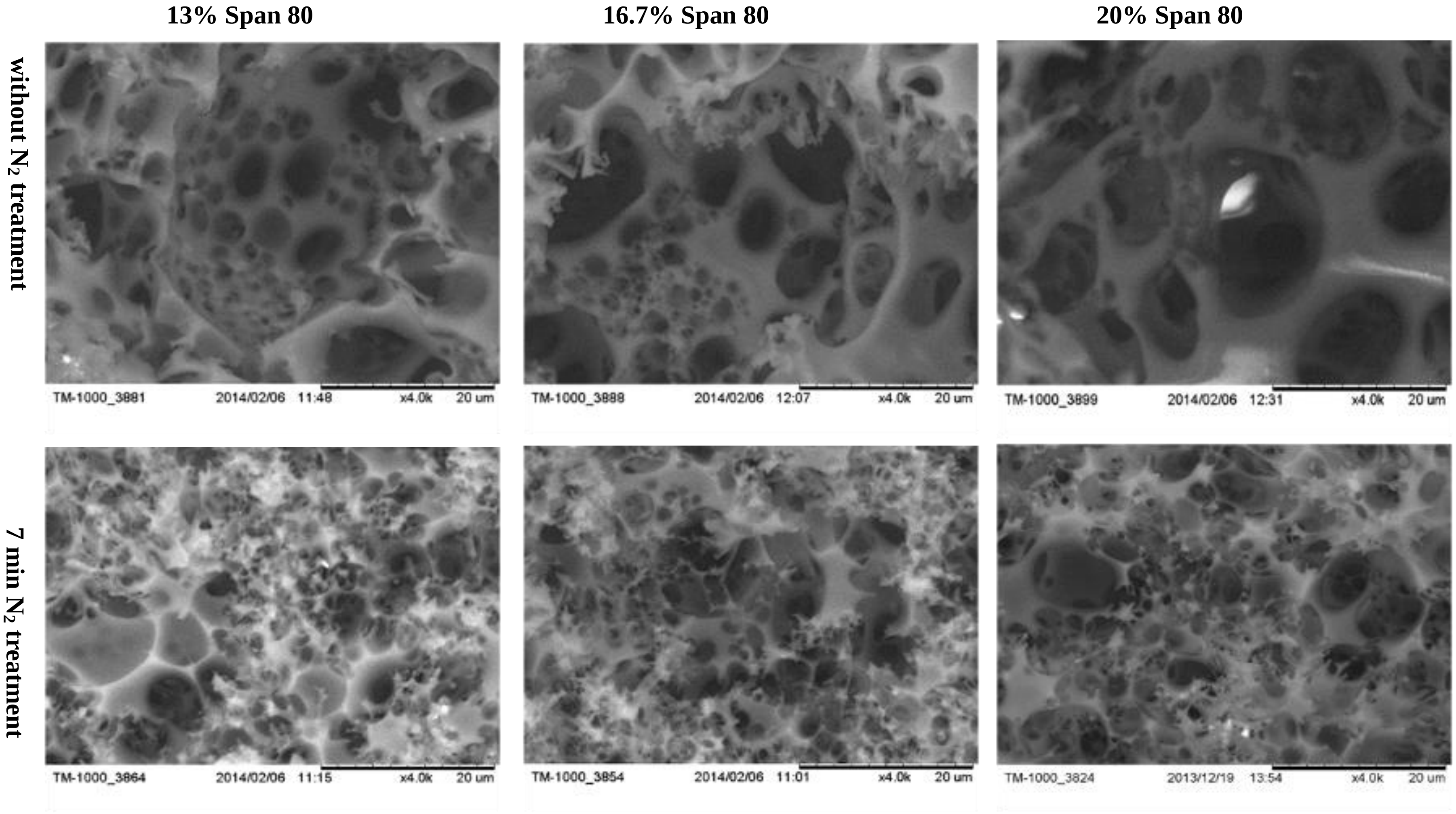


Figure 5.6. SEM images of porous polymeric materials obtained after polymerisation of HIPEs of Styrene at volume fraction of water 0.8. All materials have been purified twice by Soxhlet extraction. Scale bars are equal to 20 μm.

The SEM images of those materials (Fig. 5.6) revealed that they have interconnected pores. However, differences in their inner morphology were also noticed. The polymeric foams from HIPEs prepared with nitrogen treatment have smaller pore sizes separated by thinner walls. This could be attributed to the better stability against coalescence of nitrogen treated HIPEs and could explain the lower mechanical strength of the polymeric foams obtained from them. This is supported by reports in the literature that the pore size inversely correlates with emulsion stability.[40]

5.4 Conclusions

The stability of high internal phase emulsions (HIPEs) with MMA or St monomers as an oil phase has been investigated, with or without using nitrogen gas in their preparation, by homogenisation with an overhead stirrer at 300 rpm and gradual addition of the internal water phase to the oil. Porous polymeric foams have been produced from the most stable HIPEs after polymerisation at 60^{o}C and their structure revealed by SEM imaging.

It has been found that both amphiphilic PEGMA-b-THPMA diblock copolymers synthesised by us (chapter 3) were not capable of stabilising w/o HIPEs with volume fraction of water 0.8 at 2 wt/v% copolymer in the oil phase under studied conditions.

Stable w/o HIPEs with volume fraction of water 0.8 have been obtained when Span 80 was used as an emulsifier at concentrations in the range 13 – 20 wt/v%.

Porous polymeric foams with interconnected pore structures and of very good quality have been obtained from the styrene containing HIPEs after polymerisation.

A remarkably strong effect of the nitrogen gas treatment on the emulsion viscosity and stability has been observed. Even after several minutes of treatment, the emulsions became viscous and very stable against sedimentation and coalescence. This could be due to the removal of the dissolved oxygen from the system, thus diminishing its inhibiting action on the polymerisation of the oil phase. The positive effect of oxygen removal by inert gas treatment of similar systems is well documented in the literature, but such a strong effect has not been reported before. Further experiments are needed to reveal the reasons for the strong effect of nitrogen gas treatment on the emulsion stability we observed.

Influence of the nitrogen gas treatment on the morphology and mechanical properties of the polymerised foams has also been detected. The polymeric foams from HIPEs prepared with nitrogen treatment had smaller pore sizes separated by thinner walls.

These findings are of practical importance because they demonstrate that the stability of HIPEs templates and the morphology of porous polymeric scaffolds produced could be tuned by a careful optimisation of the nitrogen gas treatment.

Chapter 6 Conclusions and future work

The project described in this thesis focuses on the following goals:

- Synthesis of a THPMA monomer and amphiphilic PEGMA-THPMA a diblock copolymers through Group Transfer Polymerisation (chapter 3).
- Investigation of the emulsifying ability of PEGMA-b-THPMA diblock copolymers synthesised and selected low molecular weight surfactants or/and solid particles - Span 80, Oleic acid and $CaCO_3$ (chapter 4).
- Preparation and characterisation of porous polymeric foams of styrene and MMA from HIPE templates (chapter 5).

6.1 Summary of the main findings and conclusions

In Chapter 3, the group transfer polymerisation (GTP) technique has been used to synthesise two novel amphiphilic diblock copolymers of poly ethylene glycol (PEGMA) and 2-tetrahydropyranyl methacrylate (THPMA). Since the THPMA monomer was not commercially available, it was synthesised by reacting MAA with DHP. The THPMA monomer was then used to prepare block co-polymers with PEGMA. Gel permeation chromatography was used to determine block copolymer molecular weights and polydispersities, while proton NMR spectroscopy was used to assess their composition. It was found that the molecular weights of the diblock copolymers synthesised were unpredictably much higher than those targeted. Unpurified PEGMA-b-THPMA diblock copolymers showed significant amounts of PEGMA homopolymer impurities. This was attributed to the loss of control over the GTP process that could have been caused by the higher air humidity during their synthesis. This also affected the molecular weight and composition of the copolymers obtained.

In order to obtain information about the stability of W/O emulsions, the effect of PEGMA-b-THPMA diblock copolymers on the emulsion stability was investigated and described in Chapter 4. These emulsions were prepared by hand shaking homogenisation with an Ultra Turrax homogeniser at 12,600 rpm. We found that both copolymers were not able to form stable emulsions of MMA. However, the water-in-styrene emulsions prepared with 1 wt/v% purified copolymers showed reasonable stability against both coalescence and sedimentation. It was possible to stabilise emulsions of MMA with different volume ratios of the water to oil phase by using surfactant (Span 80) at concentrations larger than 15 w/v% in the oil phase. A noticeable positive effect of the thermo-initiator AIBN on the emulsion stability was observed, but further studies are needed to reveal the role of the thermo-initiator in emulsion stabilisation at room temperature. Using a mixture of oleic acid and $CaCO_3$ particles as an emulsifier provided some promising results.

In Chapter 5 we investigated HIPE templates using diblock copolymers or Span 80 as emulsifiers. HIPEs with styrene were successfully stabilised by Span 80 at concentrations in the range 13 – 20 wt/v%., but those containing MMA were unstable even at such high concentrations of surfactant. It has been found that both amphiphilic PEGMA-b-THPMA diblock copolymers synthesised by us (chapter 3) were not capable of stabilising w/o HIPEs with volume fraction of water 0.8 at 2 wt/v% copolymer in the oil phase under the conditions studied. A remarkably strong effect of the nitrogen gas treatment on the emulsion viscosity and stability has been observed. Even after several minutes of treatment, the emulsions became viscous and very stable against sedimentation and coalescence. Further experiments are needed to reveal the reasons for the strong effect of nitrogen gas treatment on the emulsion stability observed. Influence of the nitrogen gas treatment on the morphology and mechanical properties of the polymerised foams has also been detected. The polymeric foams from HIPEs prepared with nitrogen treatment had smaller pore sizes separated by thinner walls. These findings are of practical importance because they demonstrate that the stability of HIPEs templates and the morphology of porous polymeric scaffolds produced could be tuned by a careful optimisation of the nitrogen gas treatment.

6.2 Future work

Further investigation needs to be undertaken to control the conditions for synthesis of PEGMA-THPMA diblock copolymer and to optimise their composition.The influence of the nitrogen gas treatment on the HIPE properties needs further investigation in order to reveal the reasons for the strong effect observed. Further studies should also be carried out to investigate and explore the effect of nitrogen gas treatment on the morphology of porous polymeric foams obtained from HIPE templates.

References

1. J. Palanuwech and J. N. Coupland, *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, 2003, **223**, 251-262.
2. L. Shui, A. van den Berg and J. C. T. Eijkel, *Lab on a Chip*, 2009, **9**, 795-801.
3. L. Z. Hong, G. Q. Sun, J. G. Cai and T. Ngai, *Langmuir*, 2012, **28**, 2332-2336.
4. H. F. Zhang and A. I. Cooper, *Soft Matter*, 2005, **1**, 107-113.
5. C. J. Morales, U. Riebel, N. M. Guzman and M. Guerra, *Latin American Applied Research*, 2011, **41**, 105-112.
6. L. L. Schramm, E. N. Stasiuk and D. G. Marangoni, *Annual Reports Section "C" (Physical Chemistry)*, 2003, **99**, 3-48.
7. W. Hua and T. Liu, *COLLOID. SURFACE. A.*, 2007, **302**, 377.
8. M. Chatterjee, M. K. Naskar, B. Siladitya and D. Ganguli, *J. MATER. RES.*, 2000, **15**, 176.
9. M. K.Naskar, A.Patra and M.Chatterjee, *J. COLLOID. INTERF. SCI.*, 2006, **297**.
10. D. Rousseau, *Food Research International*, 2000, **33**, 3-14.
11. D. Chiaramonti, M. Bonini, E. Fratini, G. Tondi, K. Gartner, A. V. Bridgwater, H. P. Grimm, I. Soldaini, A. Webster and P. Baglioni, *Biomass and Bioenergy*, 2003, **25**, 85-99.
12. B. P. Binks and e. Tommy S. Horozov, *Colloidal Particles at Liquid Interfaces*, Cambridge University Press, 2006.
13. B. P. Binks, in *Modern Aspects of Emulsion Science*, The Royal Society of Chemistry, 1998, pp. 1-55.
14. D. G. A. L. Aarts, H. N. W. Lekkerkerker, H. Guo, G. H. Wegdam and D. Bonn, *Physical Review Letters*, 2005, **95**, 164503.
15. T. F. Tadros, in *Emulsion Formation and Stability*, Wiley-VCH Verlag GmbH & Co. KGaA, 2013, pp. 1-75.
16. J. W. Nicholson, in *Chemistry of Polymers (3)*, The Royal Society of Chemistry, 2006, pp. 1-22.
17. F. W. Harris, *Journal of Chemical Education*, 1981, **58**, 837.
18. H. Mori and A. H. E. Müller, *Progress in Polymer Science*, 2003, **28**, 1403-1439.

19. L. Zhang and A. Eisenberg, *Journal of the American Chemical Society*, 1996, **118**, 3168-3181.
20. N. Hadjichristidis, M. Pitsikalis and H. Iatrou, in *Block Copolymers I*, ed. V. Abetz, Springer Berlin Heidelberg, 2005, vol. 189, pp. 1-124.
21. G. Theoni, in *Nanobiomaterials Handbook*, CRC Press, 2011, pp. 1-29.
22. O. W. Webster, W. R. Hertler, D. Y. Sogah, W. B. Farnham and T. V. Rajanbabu, *Journal of the American Chemical Society*, 1983, **105**, 5706-5708.
23. O. W. Webster, *New Synthetic Methods (Advances in Polymer Sci)*, 2004, **167**, 1-34.
24. J. Raynaud, Y. Gnanou and D. Taton, *Macromolecules*, 2009, **42**, 5996-6005.
25. S. Forster and M. Antonietti, *Advanced Materials*, 1998, **10**, 195- 217.
26. Z. H. Lu, S. Y. Lee and S. H. Goh, *Polymer*, 1997, **38**, 5893-5895.
27. F. Chécot, J. Rodríguez-Hernández, Y. Gnanou and S. Lecommandoux, *Biomolecular Engineering*, 2007, **24**, 81-85.
28. S. D. Kimmins and N. R. Cameron, *Advanced Functional Materials*, 2011, **21**, 211-225.
29. A. Vilchez, C. Rodriguez-Abreu, J. Esquena, A. Menner and A. Bismarck, *Langmuir*, 2011, **27**, 13342-13352.
30. N. R. Cameron, D. C. Sherrington, L. Albiston and D. P. Gregory, *Colloid and Polymer Science*, 1996, **274**, 592-595.
31. A. Barbetta and N. R. Cameron, *Macromolecules*, 2004, **37**, 3188-3201.
32. N. R. Cameron, *Polymer*, 2005, **46**, 1439-1449.
33. P. Pakeyangkoon, R. Magaraphan, P. Malakul and M. Nithitanakul, *Macromolecular Symposia*, 2008, **264**, 149-156.
34. M. T. Gokmen and F. E. Du Prez, *Progress in Polymer Science*, 2012, **37**, 365-405.
35. G. N. Taylor, L. E. Stillwagon, F. M. Houlihan, T. M. Wolf, D. Y. Sogah and W. R. Hertler, *Chemistry of Materials*, 1991, **3**, 1031-1040.
36. A. B. Lowe, N. C. Billingham and S. P. Armes, *Macromolecules*, 1998, **31**, 5991-5998.
37. C. I. Park, W. G. Cho and S. J. Lee, *Korea-Australia Rheology Journal*, 2003, **15**, 125-130.
38. E. M. Christenson, W. Soofi, J. L. Holm, N. R. Cameron and A. G. Mikos, *Biomacromolecules*, 2007, **8**, 3806-3814.

39. A. Menner, R. Powell and A. Bismarck, *Soft Matter*, 2006, **2**, 337-342.

40. A. Barbetta, R. J. Carnachan, K. H. Smith, C.-t. Zhao, N. R. Cameron, R. Kataky, M. Hayman, S. A. Przyborski and M. Swan, *Macromolecular Symposia*, 2005, **226**, 203-212.

Appendix I ^{1}H NMR

Appendix II GPC Chromatograms