The University Of Sheffield.

Tuning the properties of polymeric materials for applications in 'instant-effects' cosmetics

By:

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Declaration

The work described in this thesis was undertaken at the University of Sheffield under the supervision of Dr Sebastian Spain between October 2015 and September 2019 and has not been submitted, either wholly or in part for this or any other degree. All work is the original work of the author, except where acknowledged.

Signature: _____

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Abstract

This thesis describes the synthesis of polymeric materials for potential use in anti-aging cosmetic applications. Firstly, polymeric microparticles were synthesised from polyvinyl acetate by heterogeneous free-radical polymerisation. The emulsion polymerisation using poly(vinyl alcohol) as a steric stabiliser produced particles with diameters up to 500 nm. The suspension polymerisation using poly(vinyl alcohol) or poly(vinyl pyrrolidone) as steric stabilisers produced particles with mean diameters of 22.4 µm and approximately 2 cm respectively. In order to better stabilise the suspension polymerisation and hence produce particles with diameters more analogous to pore and wrinkle depth, a new copolymer was synthesised. Butyl methacrylate and methacrylic acid were copolymerised by semi-continuous free radical solution polymerisation to produce a water-soluble suspension stabilising agent P(BMA-*stat*-MAA). Poly(vinyl acetate) microparticles of defined sizes were synthesised by systematic variation of copolymeric stabiliser concentration and stirring parameters, eventually achieving round particles of 4.2 µm diameter as determined by optical microscopy. When subjected to simple degradation and cosmetic formulation tests, particles were not found to be appropriate for the application with only 7% degradation after 30 days.

The second results chapter explores the synthesis and analysis of poly(ester urea) (PEU) microparticles derived from the amino acids alanine and glycine. Diester-diamine toluenesulfonic acid salts, glycine-hexane-1,6-diester and alanine-hexane-1,6,-diester, were synthesised by esterification of the amino acid with 1,4-butanediol or 1,6-hexanediol. Purified salts were polymerised by reaction with triphosgene to produce the respective poly(ester ureas), GLY-HEX and ALA-HEX. Addition of an oil soluble stabiliser, Span 85®, or water-soluble P(BMA-*stat*-MAA) enabled the synthesis of the PEUs in the form of particles. SEM analysis showed the particles to be rough, angular shapes. Degradation studies with lipase CaLB showed minimal degradation over 28 days. Inclusion of glycine-hexane PEUs into cosmetic formulation had a blurring effect and filled pores according to industrial tests.

Due to the application-focussed nature of this work, the synthesis of polymeric microparticles was ended in 2017 with the introduction of government legislation banning their use in cosmetic products. Instead, Self-immolative poly(ethyl glyoxylate)s (PEtG)s, with intended use as film-forming agents in cosmetics, have been synthesised by anionic polymerisation. Acetyl chloride, benzoyl chloride and bromo-isobutyrylbromide have been utilised as end-cappers to ensure stability at room temperature. Glass-transition temperatures (T_g) of the polymers were increased by introducing terephthaloyl units into the polymer backbone. Atom transfer radical polymerisation of styrene onto PEtG macroinitiators produced solid copolymers with multiple T_gs by DSC indicating block copolymer phase separation. Degradation of the PEtG homopolymers was found to be rapid without using any enzymatic degradation catalyst. PEtG-PS copolymers did not degrade over 28 days.

Conferences

Oral presentation – Centre for Doctoral Training in Polymers, Soft Matter and Colloids annual Summer School, July 2016, Sheffield, UK

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Poster presentation – Croda PhD poster days, May 2018 and May 2019, Cowick Hall, Snaith, Goole, UK

Acknowledgments

A lot has changed since I began this research project back in 2015. Aside from the somewhat absurd election of the current POTUS and the even more peculiar 51.89% deciding to isolate us from the rest of the EU, in 2018 the term 'single-use' became the Collins English Dictionary 'word of the year'. This has had two distinct outcomes – the ending of one potential route of research and the interesting discussions I've had about my research with people I've taken to calling the 'plastic police'. Despite the hurdles, I've managed to reach an end-point and I'd therefore like the thank the following people for getting me here.

Firstly, I'd like to thank Seb for providing the ideas and support to help follow them through, but for most importantly trying to keep me motivated over the four years. The rest of the Spain group, Emma, Dom, Tom, Shallcross, Jasmine, Marissa, Anna, Laila and the newbies, Josh, Ellen and Sam, thanks for understanding that sometimes a Friday trip to the pub is completely necessary. Thanks to the rest of the F-floor hoard, especially Naomi and Reb for a good chin-wag and to Byard for constantly feeding us incredible cake. And thanks to the Crazy Ladies, Kat and Bry, for being generally fab and pushing me through the harder days with copious amounts of tea (or gin).

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"*it's the fine balance of caffeine and alcohol that bookends my days*" – Tim Minchin

List of Abbreviations

^{1}H NMR	proton nuclear magnetic resonance
AIBN	azobisisobutyronitrile
ALA-BUT	bis-L-alanine butane-1,4-diester
ALA-HEX	bis-L-alanine hexane-1,6-diester
ASTM	American Society for Testing and Materials
ATR FTIR	attenuated total reflectance Fourier-Transform infra-red
ATRP	atom-transfer radical polymerisation
BIB	α-bromoisobutyryl bromide
BSA	bovine serum albumin
BxTy	PEtG end capped with x w/w% benzoyl chloride and linked with y w/w% TereCl
DLS	dynamic light scattering
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
DP	degree of polymerisation
DPBS/ PBS	(Dulbecco's) phosphate buffered saline solution
DSC	differential scanning calorimetry
EtG	ethyl glyoxylate
GLY-BUT	bis-L-glycine butane-1,4-diester
GLY-HEX	bis-L-glycine hexane-1,6-diester
GPC	gel permeation chromatography
IPA	isopropyl alcohol
ISO	International organization for standardization
M_n	number average molecular weight
M_w	weight average molecular weight
M_w/M_n	dispersity
OECD	Organisation for economic co-operation and development
P(BMA-*stat*-BzMA)	poly(butyl methacrylate-*stat*-benzyl methacrylate)
P(BMA-*stat*-MAA)	poly(butyl methacrylate-*stat*-methacrylic acid)
PBMA	poly(butyl methacrylate)

PCL	polycaprolactone
PET	poly(ethylene terephthalate)
PEtG	poly(ethyl glyoxylate)
PEU	polyesterurea
PGA	poly(glycolic acid)
PLA	poly(lactic acid)
PLGA	poly(lactide-*co*-glycolide)
PMAA	poly(methacrylic acid)
PMDETA	*N*,*N*,*N*′,*N*′′,*N*′′-Pentamethyldiethylenetriamine
PMMA	poly(methyl methacrylate)
PS	polystyrene
PS-*b*-PEtG-*b*-PS	polystyrene-*b*-poly(ethyl glyoxylate)-*b*-polystyrene ABA triblock copolymer
PVA	poly(vinyl alcohol)
PVAc	poly(vinyl acetate)
PVP	poly(vinyl pyrrolidone)
rpm	revs per minute
SD	standard deviation
SDBS	sodium dodecylbenzene sulfonate
SDS	sodium dodecyl sulfate
SEM	Scanning electron microscopy
T_c	ceiling temperature
TEM	transmission electron microscopy
TereCl	terephthaloyl chloride
T_g	glass transition temperature
THF	tetrahydrofuran

Contents

Chapter 1: Introduction

1.1.Polymers

1.1.1.General polymer science

Polymers are macromolecules synthesised from repeating units of covalently bonded small molecules known as monomers. They occur naturally as biomolecules such as DNA and proteins, or can be synthetic such as polyethylene or poly(vinyl alcohol). Since the mid-20th century, synthetic polymers have been ubiquitous to everyday life and have been specifically designed to replace existing materials. Earliest mentions of synthetic 'polymers' include nitrocellulose; later modified to produce celluloid cinematic film, Bakelite; a thermoset polymer synthesised from phenol and formaldehyde and poly(vinyl chloride), a flexible material which resembled leather.[1] However, it wasn't until the late 1920s that the term 'polymer' to describe such macromolecules was eventually accepted, a definition which was first introduced by Henry Staudinger who later won the Nobel Prize for Chemistry in 1953 for his work in this area. Before this, macromolecules were assumed to be aggregates of smaller molecules with some scientists believing that it was simply impossible for a molecule to be over 5000 Da in molecular weight.[2]

Discussed within this thesis is a wide range of polymerisations with different growth mechanisms, reaction conditions and initiator types. The first chapter considers chain-growth, initiated with species which produce free radicals in heterogeneous conditions. The second chapter involves interfacial polymerisation, a type of step-growth polymerisation, performed heterogeneously. The third and final experimental chapter considers anionic chain-growth polymerisation in the solution state and a type of 'living free radical' polymerisation, atom transfer radical polymerisation in a bulk system. Therefore, this next section highlights the key aspects of these types of polymerisation.

1.1.2.Polymerisation type: step-growth or chain-growth

Monomers can be joined together in a number of ways. Firstly, step-growth polymerisation, by addition or condensation polymerisation, is a process where monomers react with each other to form dimers and oligomers and these then react together or with more monomer to form progressively longer oligomers and polymers. The chemistry is often simple organic chemistry between reactive functional groups; however, the monomers have to be bifunctional or multifunctional in order for repeat reactions and hence polymerisation to occur. The term addition polymerisation is specifically used for when molecules are added together without the release of any small molecules and occurs when monomers are not fully saturated, for example, polyurethane synthesis by reaction of an alcohol with an isocyanate. Condensation polymerisation, such as polyesterification, refers to the evolution of one small molecule, usually water, per addition step.[3]

Chain-growth polymerisation requires an external initiation source for polymerisation to occur. The monomers react with each other due to the formation of a reactive propagation site which will move along the polymer chain as it grows. For linear polymers it is usually only the end unit of the polymer chain that is reactive enough to react with another monomer, hence growing the polymer in a chain like fashion.

The reaction kinetics of the two types of polymerisation vary greatly. In step-growth polymerisations, high monomer conversion is required in order to achieve higher DP polymers. At the beginning of the polymerisation, molecular weights are low, but reactions are fast due to the large concentration of reactive species present. As more reactions occur and the concentration of functional groups available to react decreases, the rate decelerates. However in chain growth, high DP polymers are present at low monomer conversion and M_w increases only gradually with monomer conversion.[4,5]

1.1.3.Polymerisation mechanism: free-radical, anionic or cationic

Chain polymerisations are initiated by a reactive species such as a free-radical, a cation or an anion. However, not all polymers can be initiated by all species. Unsaturated polarised groups such as carbonyls are more likely to polymerise by an ionic rather than a radical initiator due to their polarised nature. However, carbon-carbon double bonds can be polymerised by both ionic and radical initiators, meaning the majority of vinyl monomers can be polymerised by a range of methods. The substituents attached to this double bond determine its reactivity and stability. For example, phenyl substituents can stabilise by delocalisation a positive charge formed from using a cationic initiator. Electron-withdrawing substituents however, are better for stabilising the negative charge formed from using an anionic initiating species. Thus, polystyrene is readily synthesised by cationic chain polymerisation and polyacrylonitrile by anionic chain polymerisation (figure 1-1). Delocalisation of radicals occurs with almost all substituents and therefore a wider range of polymers, i.e. both polystyrene and polyacrylonitrile, can be prepared using free-radical initiators. Examples of commonly used initiator species include Lewis acids such as aluminium trichloride, nucleophiles such as butyl lithium and peroxides for cationic, anionic and free radical systems respectively.[6]

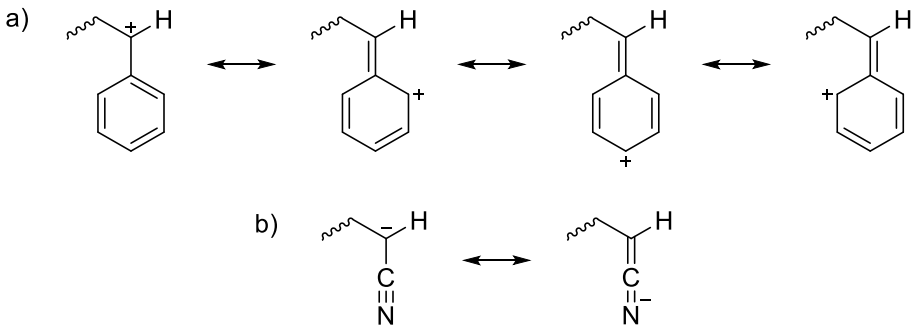


Figure 1-1. The delocalisation of a) the positive charge over the propagating chain end of polystyrene and b) the delocalisation of the negative charge over the propagating chain end of polyacrylonitrile make the polymers suitable for initiation by cationic and anionic species, respectively.

1.1.4.Synthesis of polymer particles

Heterogeneous polymerisations

Polymerisations can be carried out in bulk, in solution or heterogeneously. Bulk polymerisations do not include a separate continuous phase (solvent), instead the monomer provides a medium to allow the contact between reagents for the polymerisation to occur and therefore the reactions are often rapid due to the high concentration of monomer. Solution phase polymerisations include the use of a solvent to dissolve the reagents and the resulting polymer, the loci of the polymerisations are within the solvent which also often acts as a sink for heat released during the polymerisation. Heterogeneous polymerisations describe systems where there are multiple phases due to not all reagents and resulting products being soluble in the same phase. They are an appropriate method of particle formation for some polymers. Depending on the solubilities of the different components, including the eventual polymer product, the polymerisation can be described as a suspension, emulsion or dispersion.

Suspension polymerisation

In suspension polymerisations, the monomer, initiator and the polymer product are all insoluble in the polymerisation medium. A polymeric stabiliser or a surfactant is also used to stabilise the monomer droplets and help produce a small particle size distribution.

Oil-in-water (O/W) suspensions are commonly used in industry in the direct production of spherical particles of vinyl polymers.[7] The size range for this technique is generally from 10–2000 µm in diameter and can be readily controlled by the stirring speed, monomer concentration and the stabiliser used. For suspension polymerisation to occur, the initiator must be soluble within the monomer droplets and thus once initiated, this is where the loci of the polymerisation are situated (figure 1-2). Once initiation has occurred, the monomer droplet feeds the growing polymer chain eventually producing particles with sizes similar to the size of the initial droplets. It is because of this that the shear applied during the polymerisation has a large effect on the eventual polymer particle size.[8]

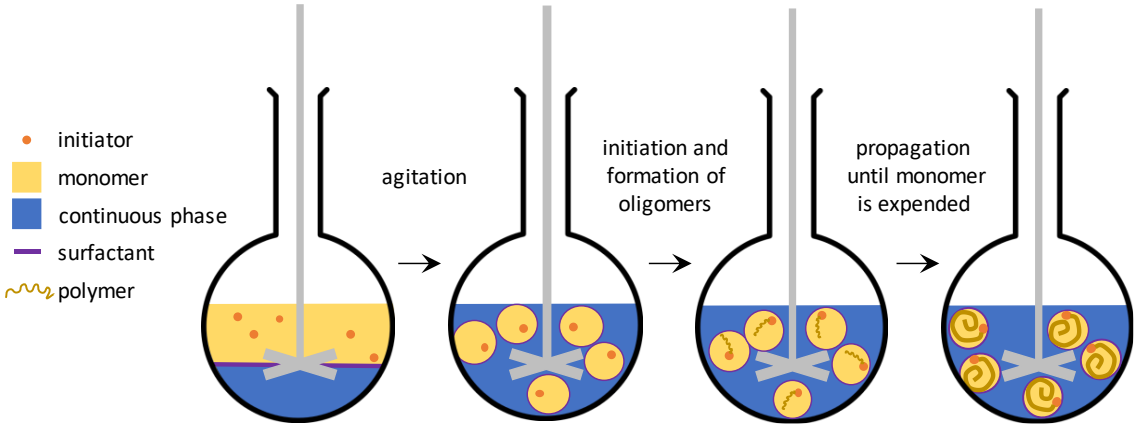


Figure 1-2. The steps of a typical free radical suspension polymerisation.

A monomer diluent can be added into the system to synthesis porous particles. The diluent is often a volatile solvent which when evaporated leaves behind small holes within the solid polymer. This technique is used in the preparation of porous polystyrene packing columns for chromatography as, by considering the solubility of the diluent, the size of the pores can be readily controlled.[9]

Each individual particle that is formed can be thought of as a micron-scale bulk polymerisation which occurs within an external medium which acts as a heat sink. Therefore, the kinetics of the polymerisation are often comparable to the equivalent bulk polymerisations. By addition of a monomer diluent, the heat transfer during the polymerisation can be improved and the kinetic profile mimics that of a solution polymerisation instead.[10]

Emulsion polymerisation

Emulsion polymerisations include an initiator which is insoluble in the monomer but is soluble in the reaction medium. A surfactant is used which interacts at the interface between the two phases to produce stabilised micelles. In a typical free radical emulsion polymerisation, the initiation occurs in the reaction medium producing oligomeric radicals which are absorbed by the micelles (figure 1-3). The polymerisation then occurs within the micelles which are fed by monomer droplets until all monomer is consumed. Micelles then grow gradually until all monomer has been consumed producing particles of typically 50–500 nm in size.[10]

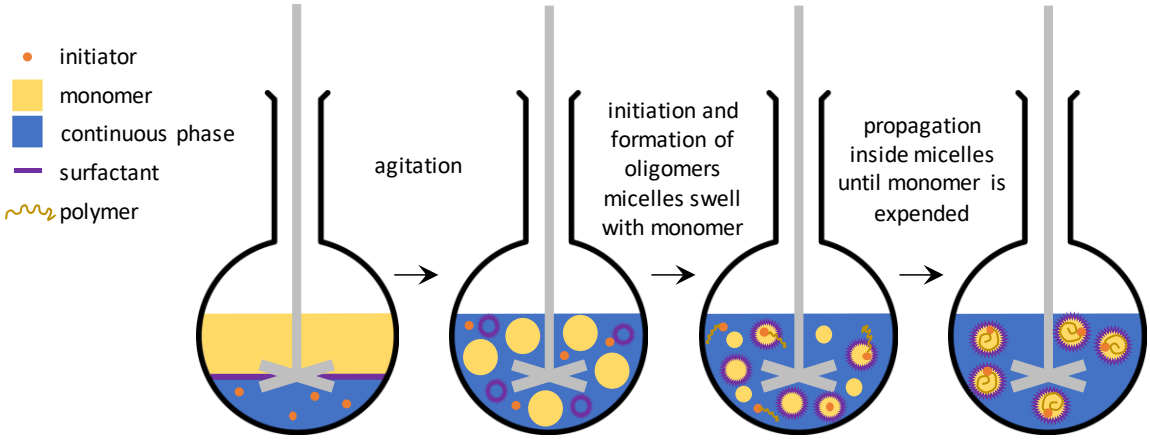


Figure 1-3. The steps of a typical free radical emulsion polymerisation.

In order to target specific particle sizes with narrow size distributions, emulsions can be seeded with a pre-formed polymer latex. The seed latex particles are added at the beginning of the reaction with the aim of avoiding any new particle nucleation. This technique can be used in the synthesis of block copolymers and also to produce beads with sizes that far exceed those usually achieved by emulsion polymerisation.[11]

Dispersion polymerisation

A dispersion polymerisation begins as a homogeneous solution where both the monomer and the initiator are soluble in the medium. However, once polymerisation occurs, the resulting polymer becomes less soluble leading to phase separation until eventually all monomer is consumed and solid polymer particles remain dispersed in the medium. The resulting particle size is usually 100 nm – 10 µm diameter, somewhere between those synthesised by suspension and emulsion polymerisation. When no stabiliser or surfactant is used, this is better described as precipitation polymerisation, where the particles coagulate producing large irregular shapes.[10]

Post-polymerisation manipulation

Top-down methods where large materials are broken down into smaller particles can be utilised for polymers which are less trivially synthesised by heterogeneous methods.[12] This is the case for most natural polymers such as polypeptides and celluloses and also usually the method for

synthesising particles from the poly-α-esters.[13] This particular group of polymers are normally synthesised by polycondensation or ring-opening polymerisation before they can be prepared as particles in aqueous dispersions via a top-down approach. Such approaches include salting out, nanoprecipitation and emulsification methods.[14]

1.1.5.Particle analysis

For the analysis of polymer particles, the two main techniques are light-scattering and microscopy. Light scattering such as dynamic light scattering (DLS) and laser diffraction can be used quickly to gather information about the particle size and size distributions but they are only really useful for round particles and can be affected by sedimentation. Microscopic methods can be used determine the morphology of particles and examine the surface detail.[15]

Optical microscopy (OM) or light microscopy is the cheapest and easiest technique to implement but has the lowest resolution. Most microscopes are now fitted with digital cameras which show the image directly on a monitor and can record real-time video without the need for eyepieces. Higher image resolutions can be achieved by increasing the magnification of the objective lens with maximum magnification of about 1000× being achievable by most microscopes. This makes OM suitable for imaging large particles in the micron scale but not useful for gaining information about particles smaller than about 500 nm (figure 1-4a).[16]

For imaging smaller particles, electron microscopy can be used whereupon a beam of electrons rather than a source of visible light is fired through a vacuum chamber towards a sample. Scanning electron microscopy (SEM) scans the surface of a sample supplying the atoms with energy. This energy exchange results in either reflection of electrons by elastic scattering, emission of secondary electrons by inelastic scattering or the emission of radiation. Each of these effects can be used to give different information about the material.

Samples are prepared by fixing to a support or stub and sputter coating with gold or a gold/palladium alloy if the material is non-conducting such as a polymer or biological species.

One advantage of SEM is that is can be used to analyse particles in the solid state unlike OM and transmission electron microscopy (TEM) which usually require the use of dilute dispersions. A further advantage is that multiple samples fixed to the same sample stage can often be analysed without the need for evacuating the vacuum chamber to change samples, making the technique significantly quicker than TEM.[17]

TEM offers the highest magnification and can be used for nanoparticles. In this technique, electrons are transmitted through the sample, usually suspended on a carbon-coated palladium grid. The image formed represents the interactions which occur between the electrons and the sample. Samples are prepared as a thin film upon the grid support, meaning that particles often have to be in the form of dispersions at a very low concentration. For polymeric materials this is usually a preparation of 0.1 w/w% sample dispersed in water to ensure particles are substantially separated in a uniform layer. Staining materials such as uranyl acetate or osmium tetroxide are often used to increase the contrast of materials which have a low scattering power. Due to the signals being detected from electrons which have passed through a sample, TEM offers valuable information on the inner structure of the sample, such as crystal structure, morphology and any encapsulated material as a one-dimensional image.[15] SEM however is more useful for information on the sample's surface and its composition, and provides two-dimensional images (figure 1-4b and c).[16,18]

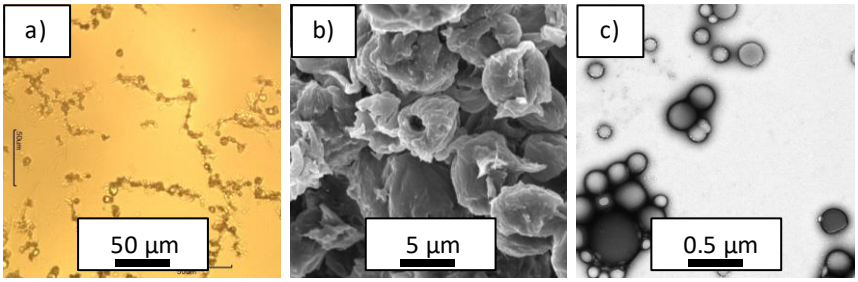


Figure 1-4. Images of polymer particles obtained by a) OM, b) SEM and c) TEM respectively

1.1.6.Polymer coatings and films

The term coating generally refers to a layer of polymer applied onto the surface of another material or substrate.[19] Polymers are used as coatings for a wide range of materials as a method for altering the surface properties for specific applications. For instance, they have been shown to improve resistance to abrasion and chemicals[20,21] and to improve wettability for applications such as drug delivery and food preservation.[22–24] Aside from being cast as the sole coating material, polymers can also be included into formulations to improve the flexibility of the dried product, a technique which is used in paints and cosmetics.[25]

Mechanism of film-forming

Polymer coatings can be cast from solution or from dispersions.[26] Solution films simply involve the complete dissolution of the polymer within a volatile solvent and then the evaporation of the solvent to leave the solid as a residual layer on the surface. The thickness of this film produced depends on the ambient temperature, the concentration of the polymer solution and the volatility of the solvent.[27] Films from aqueous dispersions mean that potentially toxic volatile organic solvents are not required as the continuous medium. This has both an economical benefit and an environmental benefit.[28] It also means that higher concentrations of polymer can be used as dispersions typically do not increase in viscosity with increasing concentration in the same way as polymer solutions. The dispersions can be applied in the same way as solutions but the mechanism of the film-formation is different and depends more on the properties of the polymer rather than the solvent. The individual polymer spheres must interact and interpenetrate in order for a continuous film to be formed and this occurs at temperatures above the minimum film forming temperature (MFFT) (figure 1-5). The MFFT is the lowest temperature at which the particles will uniformly coalesce and depends on the particle size and is usually higher than the polymer glass transition temperature, T_g.[29] The chemistry behind the coalescence of the polymer spheres is complex but is known to be affected by the radius of the particles and the viscosity and surface tension of the latex.[30]

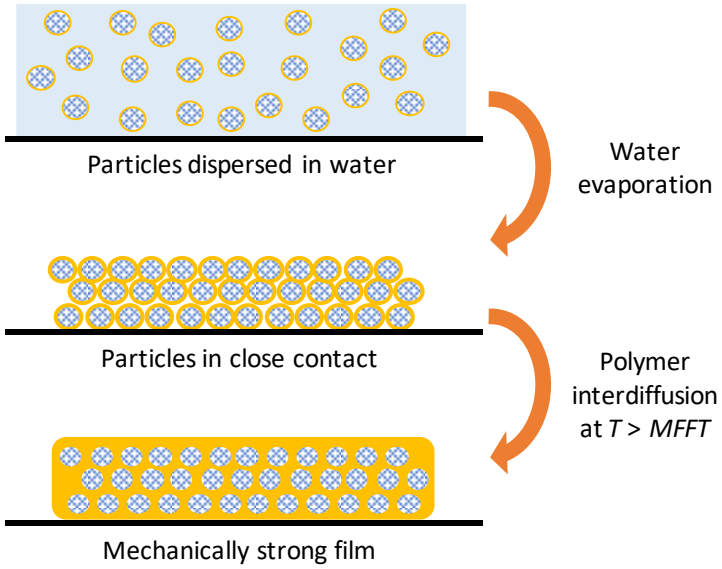


Figure 1-5. The mechanism of film forming from polymer dispersions

Differential scanning calorimetry of polymers to obtain the T_g

The thermal properties of a polymer can be analysed by techniques such as thermogravimetric analysis, dynamic mechanical analysis and differential scanning calorimetry, all of which measure the material's response to either being heated or cooled. Regardless of the technique used, the information produced is used to determine the connection between temperature and the properties of the material.[31]

Differential scanning calorimetry determines the amount of heat absorbed or given out by a sample during specific thermal transitions such as melting or crystallisation. Measurements are made with respect to a reference sample of air in an empty pan. The types of transition that can be analysed from amorphous or crystalline polymers include the glass-transition temperature, T_g, crystallisation temperature T_c, melting temperature, T_m, and latent heats of fusion or crystallisation ΔH_f and ΔH_c (figure 1-6).[32]

The T_g of a polymer is not a single value but in fact the transition occurs over a range of temperatures which depend on the rate of heating or cooling. Therefore, from DSC analysis, an estimate of the temperature can be obtained by taking the value at the point half-way through

the transition (i.e. at half the height of the incline). Because this method is subject to a significant amount of human error, values can change from one measurement to another and therefore should be taken as estimates rather than exact. Typically, when using DSC, the transition temperatures are obtained from a second heating or cooling cycle to account for any solvent loss that may occur during the first cycle or for any thermal lag that may occur due to insufficient contact between the polymer and the aluminium pan.

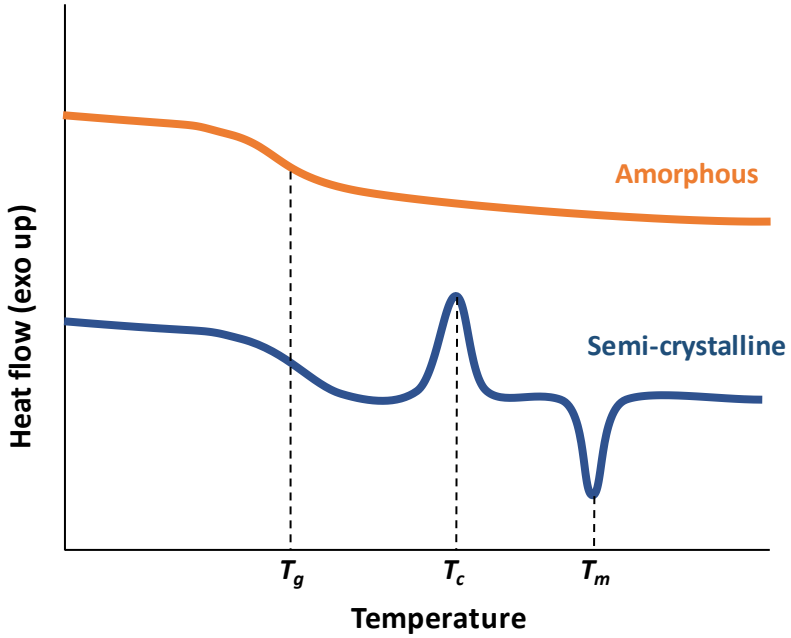


Figure 1-6. Typical DSC thermogram displaying the thermal transitions that occur in amorphous or semi-crystalline polymers. Exotherms are shown by increases in heat flow (exo up)

1.1.7.Biodegradable polymers

Due to the exponentially increasing global population there are two main arguments for the more conscientious use of plastic materials. Firstly, large amounts of plastics are sourced from crude oil, a resource which is quickly running out and needs to be conserved for other uses. Secondly, the majority of plastics that have been produced are extremely durable compared to the natural materials they were originally designed to replace and therefore are contributing to serious environmental pollution if they cannot be recycled efficiently. Therefore, both the sources of polymers and their environmental fate have in recent years become some of the

most important factors to consider in their design.[13,33,34] Some examples of synthetic biodegradable polymers are outlined below.

Polyesters

The term polyester describes any polymer which has a backbone consisting of ester linkages and no other heteroatoms. They are not all readily biodegradable in the environment, for instance, poly(ethylene terephthalate), one of the plastics most commonly used in packaging, can only be degraded by very specific bacteria and makes up about 5% of solid waste plastic in Western Europe.[33]

Aliphatic polyesters such as poly(lactide) (PLA), poly(glycolide) (PGA), poly(ϵ-caprolactone) (PCL) and poly(β-butyrolactone) (PβBL) are known biodegradable polyesters that have been shown to degrade in both the human body and the environment (figure 1-7).[35,36] Linear PLA is synthesised by ring-opening polymerisation of lactide. Due to the chirality of the lactide group, it exists as PLLA, PDLA and as copolymers of both the L and D units isomers usually written as PDLLA, all of which have different physical properties.[35]

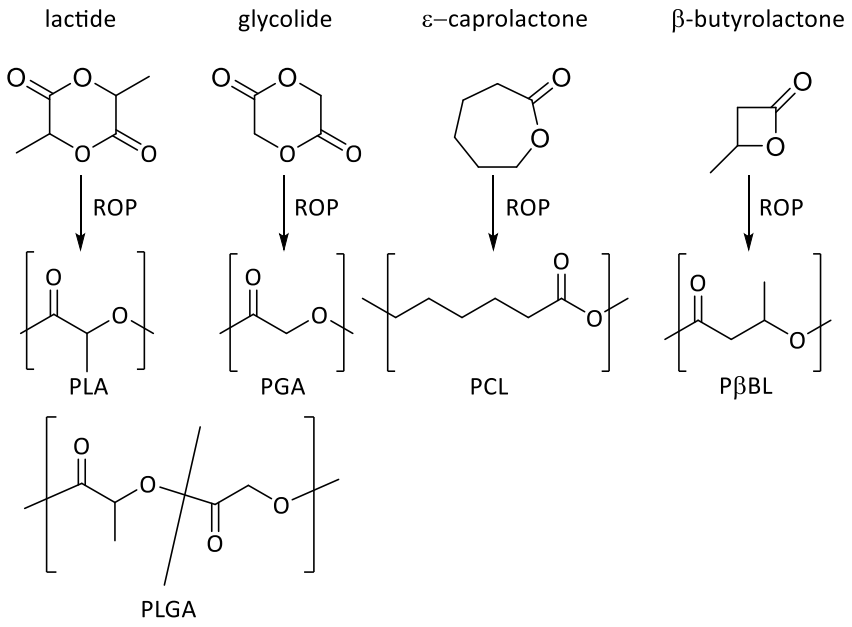


Figure 1-7. Examples of biodegradable aliphatic polyesters that can be synthesised by ring-opening polymerisation (ROP).

PGA is the simplest of the aliphatic polyesters and is also copolymerised with PLA to form poly(lactide-*co*-glycolide) PLGA copolymers. The ratio of G to L units in the polymer has been shown to impact the overall biodegradability of the polymer, with G units generally degrading more quickly.[37] PLGA is one of the most commonly used polyesters in biomedical engineering due to its tuneable degradation profile and good biocompatibility.[38,39] Nanoparticles of PLA, PGA and PLGA have been synthesised for their use as encapsulants in intracellular drug delivery producing by-products that are water-soluble and generally non-toxic.[40,41]

PCL is synthesised from the seven-membered ϵ-caprolactone ring and therefore has more carbon atoms per repeating unit than the PLA and PGA polymers. This means there are fewer accessible ester units per chain. Alongside its significantly more crystalline structure, this is likely the explanation for why its degradation rate is quite a lot slower than PLA and PGA.[42] Copolymers with other aliphatic esters generally were found to degrade more rapidly than their parent homopolymers due to morphological differences such as reduced crystallinity, making the reactive ester groups more available for cleavage.[42,43]

PβBL is a polyhydroxyalkanoate, which when it is naturally produced by microorganisms such as *Bacillus* megaterium it is better known as polyhydroxybutyrate (PHB), can also be obtained synthetically by a multistep process.[44] In comparison to the aliphatic polyesters already mentioned, they are less susceptible to hydrolysis. They have also been used in a range of biomedical applications, alone and alongside PLGA scaffolds where they show promise due to their low inflammatory response.[45]

Polymers containing other heteroatoms in their backbone

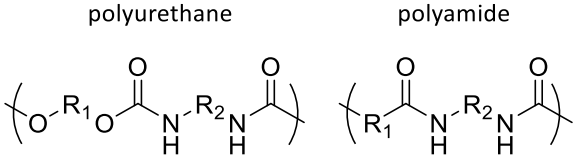


Figure 1-8. The general structure of a polyurethane and polyamide

Polyurethanes (PUs) are used in the medical, automotive and industrial markets as adhesives, fibres and paddings.[20,23,46,47]They generally have increased tensile strength, resistance to degradation by water and can be readily produced in the form of foams of low density and high flexibility.[48] They are typically synthesised by the reaction of isocyanates with hydroxyl-functional molecules and are made up of 'hard' and 'soft' segments with respect to the flexibility each segment imparts on the overall polymer structure. For biodegradable PUs, these hydroxyl-functional molecules are often polyester or polyether macrodiols. *In vivo* they degrade to nontoxic urethane and urea fragments as well as the ester or ether segments, therefore the aliphatic polyesters outlined previously are often used in the synthesis of biodegradable PUs for medical applications.[46] Generally, the longer the units between the urethane linkages, the less crystalline the polymers and the more accessible to biodegradation.[33]

Polyamides exhibit strong resistance to enzymatic degradation due to their highly ordered structure arising from the strong hydrogen bonds formed between chains.[49] However, this tough structure enables them to provide useful properties to other polymers such as polyesters by copolymerisation. Polyester amides are a recently researched family of materials which combine the degradability of the polyester with the good thermal and mechanical properties of the polyamide.[50]

Polymers containing carbon-only backbones

Poly(vinyl alcohol) (PVA) is one of the few carbon-backboned polymers that is known to be biodegradable.[33] It is synthesised from poly(vinyl acetate) because vinyl alcohol monomer tautomerises spontaneously and therefore cannot be polymerised directly. Instead, the isolated poly(vinyl acetate) (PVAc), usually produced by free radical polymerisation, is readily deprotected to form poly(vinyl alcohol) chains. The hydrolysis of the acetate groups is normally performed either in methanol with sodium hydroxide or with hydrazine.[51,52]

Poly(vinyl acetate) particles can been synthesised directly in heterogeneous polymerisations for a wide range of applications.[53] Without hydrolysing it, one of the most common uses for PVAc is in coatings for enhanced adhesive and mechanical properties.[54,55] PVA on the other hand is used in cosmetics, medicines and food[25,56,57] Due to its biocompatibility and water solubility, it has been used in biomedical research as an embolic agent where it was investigated for both treatment of the kidneys and central nervous system.[58]

A proposed two-step biodegradation process for PVA is illustrated in figure 1-9. A PVA dehydrogenase has also been isolated and is shown to carry out the same process – implying that degradation is not limited to the presence of a particular microorganism or enzyme. The biodegradation of PVA solutions is a two-step process. Firstly, the secondary alcohol oxidase (SAO*)* oxidises the hydroxyl groups to produce a β-diketone. Then, a β-diketone hydrolase (BDH) causes cleavage of the C-C bond producing a carboxylic acid and β-hydroxyketone.[56] This is just one specific mechanism which has been proposed, but in general the enzymes capable of the degradation of PVA are oxidases, dehydrogenases or hydrolases.

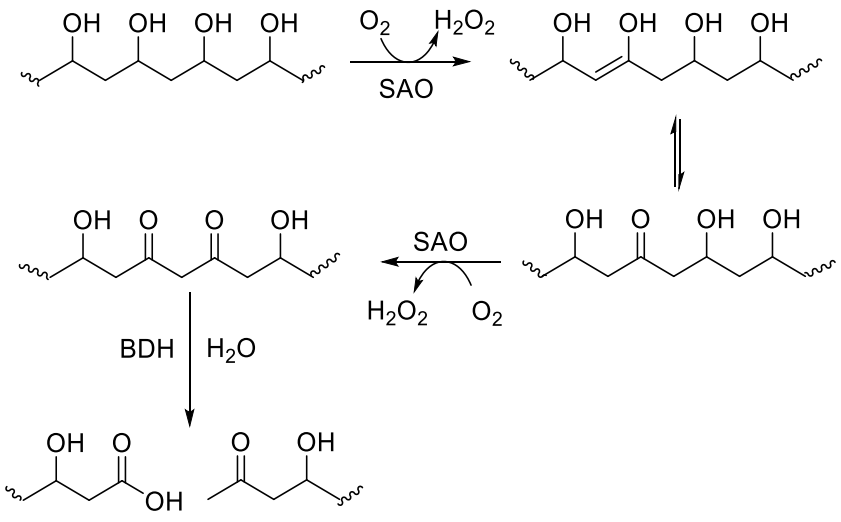


Figure 1-9. Two-step biodegradation pathyway of PVA.

1.1.8.Biodegradation testing of materials

Although there are now many materials which have been studied for with respect to their specific biodegradation mechanisms, the main testing method used by industry to analyse the

overall fate of a material in the environment is the 'ready biodegradability test', defined in OECD 301 B.[59] This is also known as a 'modified Sturm test', a test derived from a method originally used to predict the rate and ultimate biodegradation of nonionic surfactants.[60] It measures mineralisation by the amount of carbon dioxide that is evolved over a set time period, usually every day or couple of days up to at least 28 days. A composted matter or 'activated sludge' is used to mimic the conditions of a highly biotic environment such as in a sewage treatment plant. The amount of CO_2 collected is measured by titration and this is then compared to the amount of CO_2 that theoretically could be evolved if 100% of the carbon-based species in the material had been mineralised.[61]

1.2.Skin

1.2.1.Structure

It is often forgotten that the skin is the largest organ in the human body and that it carries out a variety of vital functions.[62] As well as being a protector for everything inside the body, it regulates the body temperature and excretes toxins by producing sweat, and it works as a sensor for detecting any other changes in the external environment (figure 1-10). Different parts of the body are covered with skin of different thicknesses and structures, but it can be generally described as consisting of two main tissue types. These are the epidermis and the dermis, both of which are made up of layers with each individual layer serving a unique purpose.[62,63]

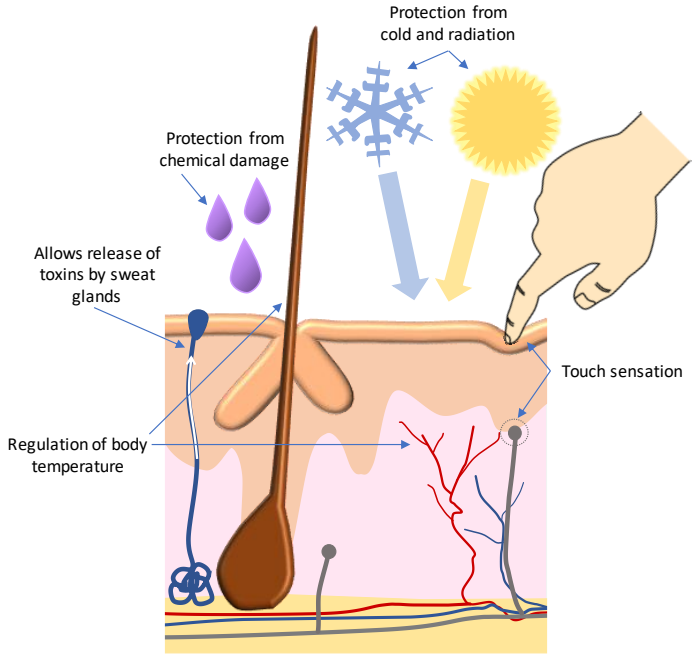


Figure 1-10. A cross-section of skin showing briefly the important functions it plays. Blood vessels and fine hairs enable regulation of body temperature, sweat glands allow the release of toxins, the strong outer layer protects from chemical, cold and radiation damage and the nerve endings under the skin allow the sensation of touch.

As an example of the important roles that each layer plays, consider the outermost layer of the epidermis, the stratum corneum. This layer provides an ultimate barrier to water whilst keeping the rest of the skin hydrated.[64] Its structure has been likened to a brick wall where corneocytes are the tough building blocks held together by a lipid matrix which controls the skin's barrier properties. Skin hydration is important in order for toxins to be removed through sweat glands and for the transport of nutrients between the cells beneath the surface. There is a constant flux of moisture at the skin's surface which is required in order for the skin's outermost cells to shed. Therefore, poor hydration is detrimental to the stratum corneum's performance and can lead to skin being overly dry and flaky.[65] Another consequence of poorly hydrated skin is the formation of deep, wide wrinkles as the skin ages.[66]

1.2.2.Skin aging

Over time all skin undergoes intrinsic, or chronologic, aging processes which cannot be prevented. Extrinsic aging, also known as photoaging, also occurs but this is heavily dictated by the lifestyle of the individual. For example, common behaviours which increase the rate of

photoaging are smoking, excessive alcohol consumption and sun exposure. In areas of the skin which are most often exposed to the sun's UV rays, in particular the face, skin ages even more rapidly.[67] The simplest way to slow down this aging would be to decrease sun exposure but sun exposure actually carries with it many benefits to both physical and mental health and is not easy to avoid.[68–70]

One of the most obvious signs of aging is the formation of wrinkles and widening of pores, particularly on the face around the eyes and mouth. Three main types of wrinkle have been described in the literature: crinkles, glyphic wrinkles and linear facial wrinkles, all of which are a consequence of aging but occur due to different changes in the skin's structure. Crinkles or temporary wrinkles which appear in all areas of the skin are the smallest and will disappear on stretching of the skin. These are formed by the breaking down and disorganisation of collagen fibres and the diminishment of elastin. In turn, this causes the smoothing of the dermal/epidermal junction, weakening the connection of the dermis and epidermis. (figure 1-11). Glyphic wrinkles are specifically due to sun exposure and are permanent, deep wrinkles that typically appear on the back of the neck or cheeks. Linear or expressional wrinkles are those that appear due to movement of skin, such as frown-lines on the forehead or crow's feet around the eyes. In youthful skin these generally will disappear on relaxing of the facial muscles but become more permanent with age. [71]

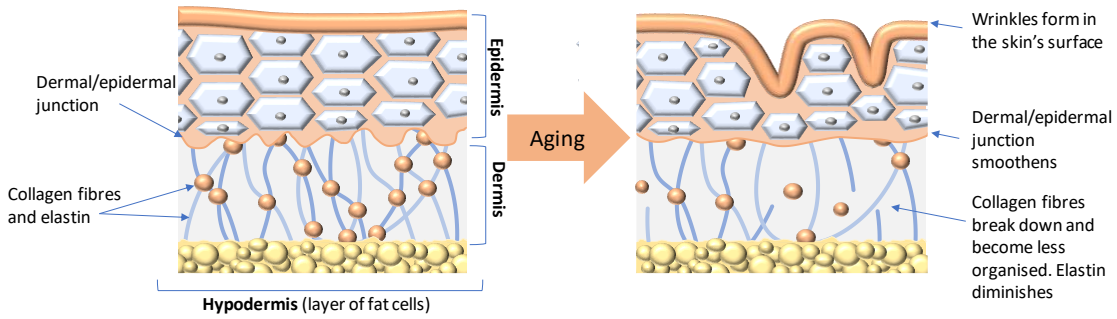


Figure 1-11. The effect that the aging process has on the top layers of the skin.

There have been many attempts to quantify the size and depth of wrinkles and how these are related to the age of the skin. Skin measurements can be carried out using a variety of techniques such as corneometry to measure hydration,[66,72] cutometry to measure elasticity[66,73,74] and optical coherence tomography to visualise the skin's surface on a micron scale.[75,76] High definition photography achieved using a Visioscan® can be used to visualise the skin in incredible detail to correlate parameters such as UV damage, hydration and skin roughness.[66] These techniques are all used in the cosmetics industry to enable the production of products to help combat the signs of aging.

As well as the development of wrinkles, aged and dehydrated skin can appear rough and dull. This is caused by deceleration of the cell cycle and the build-up of dead cells on the skin surface. Environmental factors such as smoking, drug use and excessive sun exposure can accelerate this negative effect on the structure and appearance of skin. One of the most effective ways to prevent aging would be to avoid these aging accelerators, however photoaging caused by sun exposure is ultimately unavoidable.

1.2.3.Anti-aging

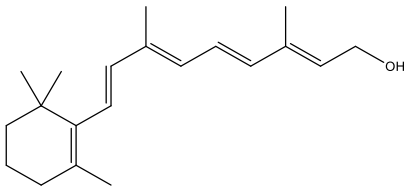
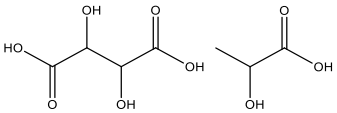
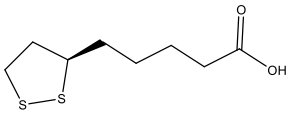
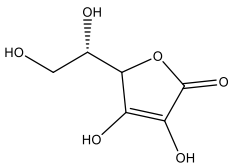
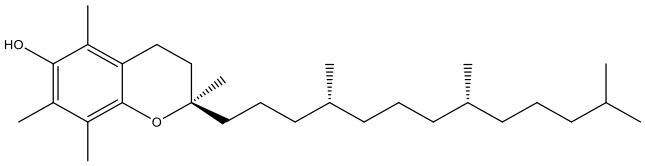
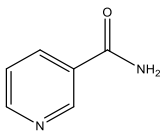
The subject of aging is not just a biological matter but also an interesting sociological matter. There are many people who will allow their skin to wrinkle naturally without any attempt at disguising their age. Conversely, some people will spend vast amounts of money both preventing and covering up wrinkles and other signs of aging such as sagging skin. In a psychology study carried out at the University of Kansas, the opinion of the participants below the age of 25 was that the use of common anti-aging treatments by an older generation is almost deceitful and can be compared to acting as an imposter.[77] This is because appearing more youthful is of high value in western society with older people supposedly being viewed as less attractive and therefore inferior. The common stereotype of 'loss of physical attractiveness' is what influences older women specifically to make themselves appear younger.[78] Because of this it is common for people to undergo anti-aging treatments from as early as in their 20s, with

some dermatologists claiming that collagen and elastin begin to decrease by the age of 25 and this therefore being the ideal age to begin treatments.[79]

One of the earliest mentions of anti-aging procedures dates as far back as ancient Egypt when it is said that Cleopatra would bathe in donkey's milk to help preserve her youthful skin.[80] Nowadays it is common for adults to use a wide range of remedies to combat the signs of aging, from nutritional supplements to topical formulations and invasive surgical procedures.[81] Most of these techniques either deliver a gradual decrease in the signs of aging with daily use, or an instant effect which wears off over time and then needs reapplication. There are plenty of over-the-counter products that incorporate 'actives' to enhance the replenishment of collagen and elastin in the skin with the overall effect being the slowing of wrinkle formation, however there are few 'actives' that are actually able to penetrate far enough into the dermis to significantly improve the skin's appearance.[82,83] There are generally few products on the market which are able to offer an overall instant positive effect and those that are available are usually expensive and therefore not widely used by the general public.

In order to improve the rejuvenation of cells, active ingredients such as retinoids and α-hydroxy acids are added into anti-aging formulations. UV radiation damages the skin by the production of reactive oxygen species and therefore antioxidants such as vitamin E are often recommended to prevent aging caused by sun damage by scavenging free-radicals that are formed. Another method of treatment is the stimulation of collagen growth, often by oligopeptides which can imitate its peptide sequences. Some of the most commonly used anti-aging actives are outlined in table 1.2.3-1.

Table 1.2.3-1. Commonly used anti-aging actives, their biochemical method of action and the visual effect that is observed with their sustained use.[84]

Name and structure	Action	Observed effect
Retinoids 	Normalise the life cycle of skin cells and control pigmentation	Skin has fewer lesions and blemishes such as liver-spots
Alpha-hydroxy acids 	Promote cell growth	Skin appears brighter and smoother
Alpha-lipoic acid 	Antioxidant	Acts as an anti-inflammatory giving smoother skin
L-ascorbic acid 	Antioxidant and can stimulate collagen synthesis	Acts as an anti-inflammatory and can reduce blemishes
Vitamin E 	Antioxidant	Moisturises the skin and reduces reddening caused by photo-aging
Niacinamide 	Reduces trans-epidermal water loss	Improves skin elasticity and reduces wrinkle formation

1.3.Introduction to cosmetic science

1.3.1.General cosmetic science

The term cosmetic applies to any substance applied to the body or face which is designed to improve its appearance and covers everything from hairspray to foot creams. Topical lotions and creams are usually formulated as a simple oil in water (O/W) or water in oil (W/O) emulsion, with each providing a unique set of properties. For everyday face creams and moisturisers, it is more common to use O/W systems as they are more able to penetrate the skin to deliver actives and also have a lighter, less greasy feel.[85] The following section gives a brief review of recent technologies in cosmetic science, specifically referring to methods to improve the appearance of facial skin.

1.3.2.Examples of cosmetics designed to have an effect on the appearance of skin

A large range of cosmetic products focus on the mattifying of the skin's texture. 'Mattifying', 'improving mattitity' or 'matting' is described as reducing the shininess of the skin, often to treat greasy skin in particular where an excess of sebum is produced. However, many inventions which make these claims also claim to improve the appearance of fine-lines and pores. For instance, the use of PTFE microbeads in a mattifying composition showed the decrease in the greasy appearance of the skin but also an ability to camouflage microreliefs, wrinkles, fine lines and pores. The particles were 0.5-15 μm in diameter and when used in formulation against a control formulation, they greatly improved the 'dullness' at an inclusion level of only 1.5 w/w%.[86]

A further such example of mattifying uses cellulose microbeads such as Cellulo Beads D-5® or Covabeads CLO® in a cosmetic composition. The beads outperform the sensory feel and skin adhesion of powders of talc, starch, or polymers such as polyethylene and PMMA. These inferior materials also generally have a long-term dehydrating effect on the skin. The use of cellulose is said to have better physiological compatibility due to their natural origin and they have a soft feel with good moisturising power due to their water absorbing capacity. They are

monodisperse and spherical, and generally synthesised by a viscose-phase-separation method, based on the phase-separation phenomenon between cellulose xanthate and water-soluble polymers in water.[87]

In a direct comparison between two different sized cellulose beads and a silica bead with similar particle diameters, the better mattifying effect was seen with the smallest of the cellulose beads with number average diameters of 10 µm. This effect is measured by gonio reflectometry where the ratio, *R*, of the specular reflection to the diffuse reflection depends on the shininess of the skin. The lower the value of *R*, the better the mattifying effect.[88] Therefore, the use of cellulose microbeads could be a potential way forward in the formation of biodegradable materials for instant effects cosmetics.

Cross-linked silicone particles have also been used to disguise skin shininess.[89] Silicones are often used in cosmetics due to their advantage of conferring good application properties, they feel soft to the touch and spread well across the skin, efficiently filling in the 'hollows' which are present. The synthesis of the particles used a platinum catalyst to crosslink two different siloxane components. Using similar chemistry, elastomeric films can be produced directly onto the skin's surface to instantly improve its elasticity.[90] A crosslinked polymer layer (XPL) is formed by a two-step process. Firstly, a layer of a formulation comprising of a reactive blend of polysiloxanes is applied and allowed to dry. Then, a second formulation is applied which contains a platinum catalyst at 0.02 w/w% and causes the room temperature hydrosilylation crosslinking reaction *in situ*. Nylon microparticles with a refractive index to match that of skin are also included in the second phase to ensure transparency of the final film. The blend of siloxanes used was optimised to give the best effect i.e. one which restores the natural skin aesthetic and exerts stresses on the skin which cause it to tighten. The chosen blend has since been formulated into a product, formerly marketed by Living Proof as Neotensil but since has been bought by Shiseido group (figure 1-12).[91] Second-skin approaches to anti-aging are

becoming more popular with two more products being launched in recent years from Kao[92] and Lily Bioceuticals,[93] both using completely different chemistry but providing similar effects. The main drawback with all of these products is that they can cost anywhere between £100-500 per 100ml, which is expensive especially as they only offer immediate effects and need to be reapplied daily.

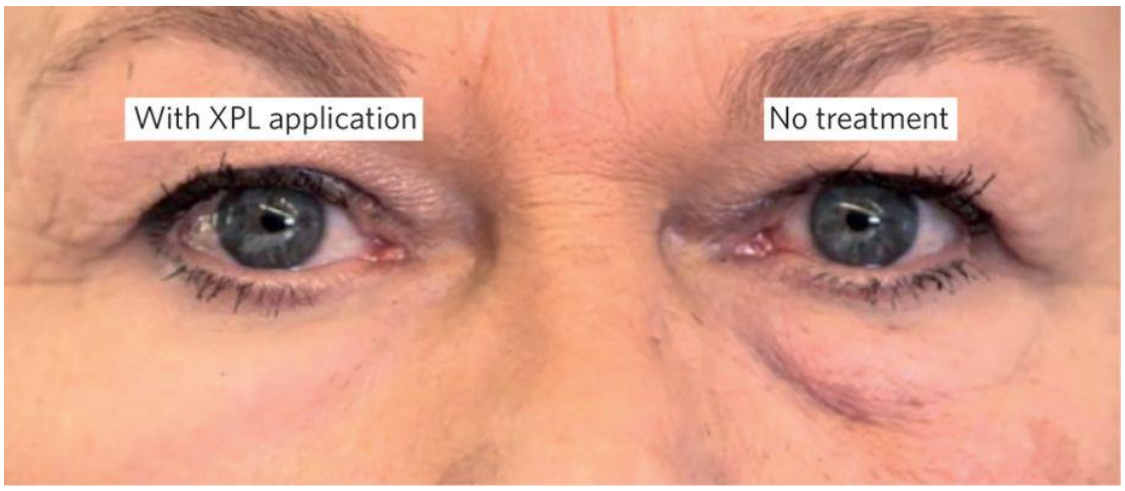


Figure 1-12. Image taken from 'An elastic second skin' shows the improvement to eye-bags after application of the crosslinked polymer layer.[90]

A cosmetic composition that provides an immediate skin-tightening effect on the skin is described in US patent 2019105254 from L'Oreal. It claims that their invention gives long-lasting improvements to the skin for the treatment of a range of aging signs, including wrinkles and eye-bags. The typical composition of the cosmetic disclosed in the patent includes a sodium silicate film former, a second film forming polymer such as PVP, polyacrylate, polysaccharide or mixtures of these, a polyvalent silicate, an anionic associative polymeric thickener such as a behenth-25 methacrylate copolymer and a third film former, usually in the form of a vinyl pyrrolidone/vinyl acetate copolymer. It is not unusual for a composition to contain this many different polymers, however it must be noted that none of the polymers included fall under the description of 'biodegradable polymers'.[94]

Another polymer, PMMA or 'methyl methacrylate crosspolymer' appears in a lot of the patent literature on cosmetic formulation as it is FDA approved and is therefore also deemed

completely safe for use as both a film-former and as a viscosity-increasing agent.[95] A PMMA powder designed to deliver a wrinkle-improving active material is described in European patent EP1321136.[96] The powder is produced by dissolving the anti-wrinkling active in a PMMA solution and then encapsulating by cross-linking the polymer. The capsules contain a significant amount of liquid and therefore their surface is then treated using a hydrophobic polymer to enable better formulation into a powder. The overall powder composition contains this PMMA material dispersed in a large amount of silicone powders which provide the desired sensory feel. This is a novel technique of delivering anti-wrinkle actives to improve skin-elasticity through the use of powder-based cosmetics, however, it does not offer the 'instant-effect' of wrinkle camouflage.

A further use of PMMA is as a dermal filler, another commonly used anti-aging technique.[97] Dermal fillers work by two actions, directly increasing the tissue volume for a short period of time or by causing a foreign body reaction which induces collagen formation for a long period of time. Fillers which work-best in the direct approach often contain a large amount of an animal-based collagen or hyaluronic acid (HA). Due to their natural origin, these materials can exhibit superior biocompatibility, however, they are expensive and often are degraded and consumed by the body very quickly. Ideally, a filler will rapidly exhibit its effects and will also be able to maintain them for a long as possible. A filler composed of PMMA and a cross-linked dextran is shown to rapidly increase the volume of tissue at the application site and is not easily degraded or absorbed. The PMMA stimulates the fibroblasts to produce collagen and is then encapsulated by this collagen leading to some filling of recessed areas such as wrinkles. This material is also said to be 10–30 times cheaper than HA-based fillers. This is important as synthetic polymers are often significantly cheaper than natural products and therefore can be used to make products which are accessible to a wider demographic.

A styrene/acrylic copolymer often used in paint formulations, trade-name ROPAQUE^{TM} has been repurposed for the blurring of skin-defects such as wrinkles and pores. The copolymer particles

are 0.1–5 µm in diameter and only need to be included at 0.5–5 w/w%. Its use in a moisturising composition means that other filler powders can be used in smaller amounts than usual leading to a less powdery appearance on the skin. The use of less powder also means that the composition has a better sensory feel during application and causes less irritation to or drying of the skin. A range of products can be used as filler powders from starch powders such as DRY-FLO to silicone-resin microbeads such as Tospearl*, either of which can contribute to the sensory feel but do not help to unify the complexion.[98] Although not a biodegradable polymer, the relatively simple polymeric structure does prove the concept that polymeric microbeads can be successfully applied as blurring agents.

As a response to the recent trend of removing non-biodegradable solids from cosmetics, a recent patent has described the use of biodegradable microbeads for use as exfoliants.[99] A known polymer which is degraded by a number of different organisms and has polysaccharide-based degradation products is polygalacturonic acid or pectin.[100,101] It is readily synthesised by oxidation of polysaccharides derived from fruit and vegetables. The polymer is then made into abrasive particles by dissolution and crosslinking in solution whilst encapsulating the core substance of a water-insoluble mineral such as a sand, calcium salt or a bio-glass. The polymeric shell is cross-linked lightly enough to degrade quickly and is hydrophilic to allow for sufficient dispersion in the exfoliant formulation. The microbeads have a half-life of one month in an ambient environment but are stable enough to allow an adequate shelf-life for the end-product. On degradation, the mineral core is either readily dissolved by water or in the case of sand, released into the environment without any toxic side-effects to oceanic life. There are many examples of the use of pectin as an encapsulant for fragrances and drug-delivery but not as a solid, spherical micro-particles.[102,103] Synthesis of microparticles could therefore provide a potential novel material for use in the instant-effect cosmetic, providing suitable control could be obtained over the size and sphericity of the particles.

Sederma, a member of Croda International Group, have many patents highlighting the use of natural materials as actives in the anti-aging field.[104,105] Firstly, an acetylated oligoglucuronan, an oligomeric compound of D-glucaronic acid, has been shown to combat the reduction in the elastic properties which occurs as skin ages and to increase the dermo-epidermal cohesion. The DP of the oligomer is typically 18–19 (n = 15–16 in figure 1-13). The degree of acetylation is shown to be most effective when between 8.7 and 9.2%. Without the acetylation, oligoglucuronans do not show any improvement in the skin elasticity.[106,107] Intensive studies on the effects of these materials showed positive results for stimulation of HA synthesis, stimulation of laminin synthesis, an anti-glycation effect and an overall increase in skin elasticity. Laminin is a complex which helps to anchor and stabilise the dermal-epidermal junction, hence reducing the flattening effect which occurs as skin ages and leads to the formation of wrinkles.[108]

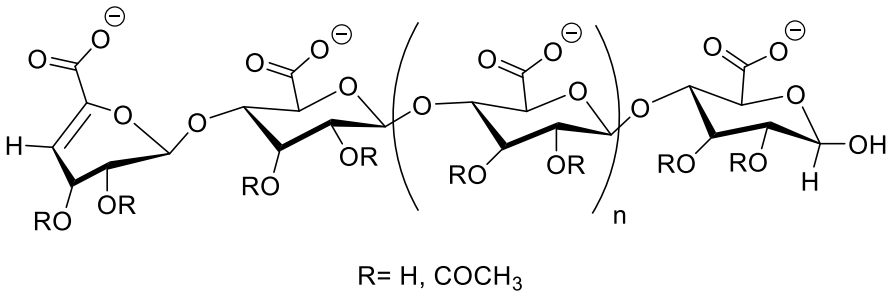


Figure 1-13. The oligoglucuronan sequence responsible for a large range of anti-aging effects where n =15–16 and R = H or $COCH_3$ depending on the degree of acetylation.[104]

Peptides have been shown to have anti-aging activity by acting on the main molecules that constitute the extracellular matrix (ECM), which also decrease with age.[105] A novel peptide which has been said to beautify skin by stimulating the synthesis of these molecules contains a very specific sequence of amino acids. Activity, particularly pro-collagen activity, occurs from inclusion at only a few ppm. The peptides are 3–10 amino acids in length and contain at least one sequence (K*(Ac))GH where K*(Ac) refers to an acetylated lysine, ornithine, diaminobutyric acid or diaminopropionic acid unit (figure 1-14), or a hydroxylated derivative of any of these. Providing this sequence appears in the peptide, the remainder of the peptide can be made from any other of the 20 commonly occurring amino acids.

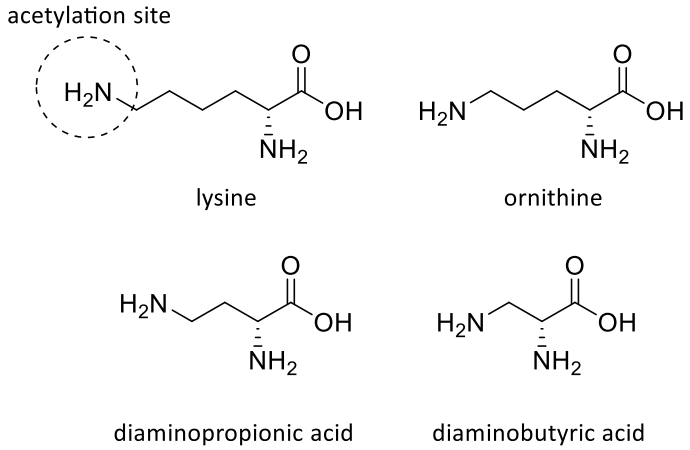


Figure 1-14. The amino acids labelled as K, or K* when acetylated in the specific sequence required for the stimulation of ECM molecules.[105]

Other naturally occurring molecules, such as essential oils, silk fibroins and sugars derived from fungi have been shown to have anti-wrinkle properties.[109–112] One of the most challenging aspects of delivering anti-aging actives is the transdermal absorption through the stratum corneum barrier. Often, actives are minimally absorbed or expressed and therefore claims of repair of the skin from below the surface cannot be made. *Ganoderma lucidum*, an oriental fungus, produces a polysaccharide which can be reacted with ferulic acid to produce the corresponding ester. This has been shown to have an anti-aging and anti-wrinkle effect when combined with a skin probiotic and a suitable synthetic transdermal absorbent material, specifically an *N*-alkyl pyroglutamate[113], which allows the active to repair the skin from the inside. Skin probiotics are used to help regulate the skin flora as their deregulation can lead to functional immunodeficiency and the slowing of repair to damaged cells. This is observed by a decrease in the visible effects of improved micro-undulation and smoothness of the skin.[109]

An eye-mask specifically for reducing the fine-lines around the eyes known as 'crow's feet' can be made from modified silk fibroin/alginate composites.[112] Many life factors such as excessive screen use and irregular work and rest patterns can lead to abnormal protein synthesis in the skin. This cause the skin of the eye to decrease in elasticity and produces crow's feet. This invention claims to provide both a quick and long-lasting effect which outperforms other

materials which can only claim one of these. The mask also uses an epidermal cell growth factor to promote the growth and division of cell tissues and peptides to block the nerve conduction of acetylcholine, relaxing the eye muscles and eliminating dynamic wrinkles. The silk fibroin is combined with sodium alginate and poly(ethylene glycol) diglycidyl ether to produce the film-forming component which makes these actives applicable as an eye-mask. Masks which did not contain the natural silk fibroin faded the crow's feet less than those which did. This invention therefore shows how the use of natural ingredients alongside polymers can boost anti-aging effects.

1.4.Plastic pollution

1.4.1.The problem with plastics

The term plastic has become synonymous with the term polymer, despite the latter covering any material which is made up of smaller molecular units. Plastics are specific types of polymer which are malleable and can therefore be moulded into solid objects for many applications. Unfortunately, the properties which they were originally invented for, such as their longevity, have become their short falling as there is now a growing concern about the huge quantities of plastic that exists and is contributing to the pollution of the planet (figure 1-15).[49]

A substantial amount of research has been carried out into the persistence of plastic in the environment, particularly microplastics in the oceans. Marine-life such as barnacles and sea cucumbers have been known to ingest microparticles and it is believed that these particles can then pass into and accumulate in organisms higher in the food chain such as ourselves. Most of the microplastics obtained from studies on the sea-bed can be traced back to the degradation of larger plastic items such as car-tyres, from degradation of polymer-based paints on ocean-vessels and from laundry fibres produced when clothes are washed. Figure 1-16 shows data collected in 2011 regarding the number of microparticles per mL found on 18 shorelines.[114] Since these findings, world-wide ocean clean-up operations have been initiated[115] and many people are making the conscious decision to reduce the amount of plastic that they use.[116]

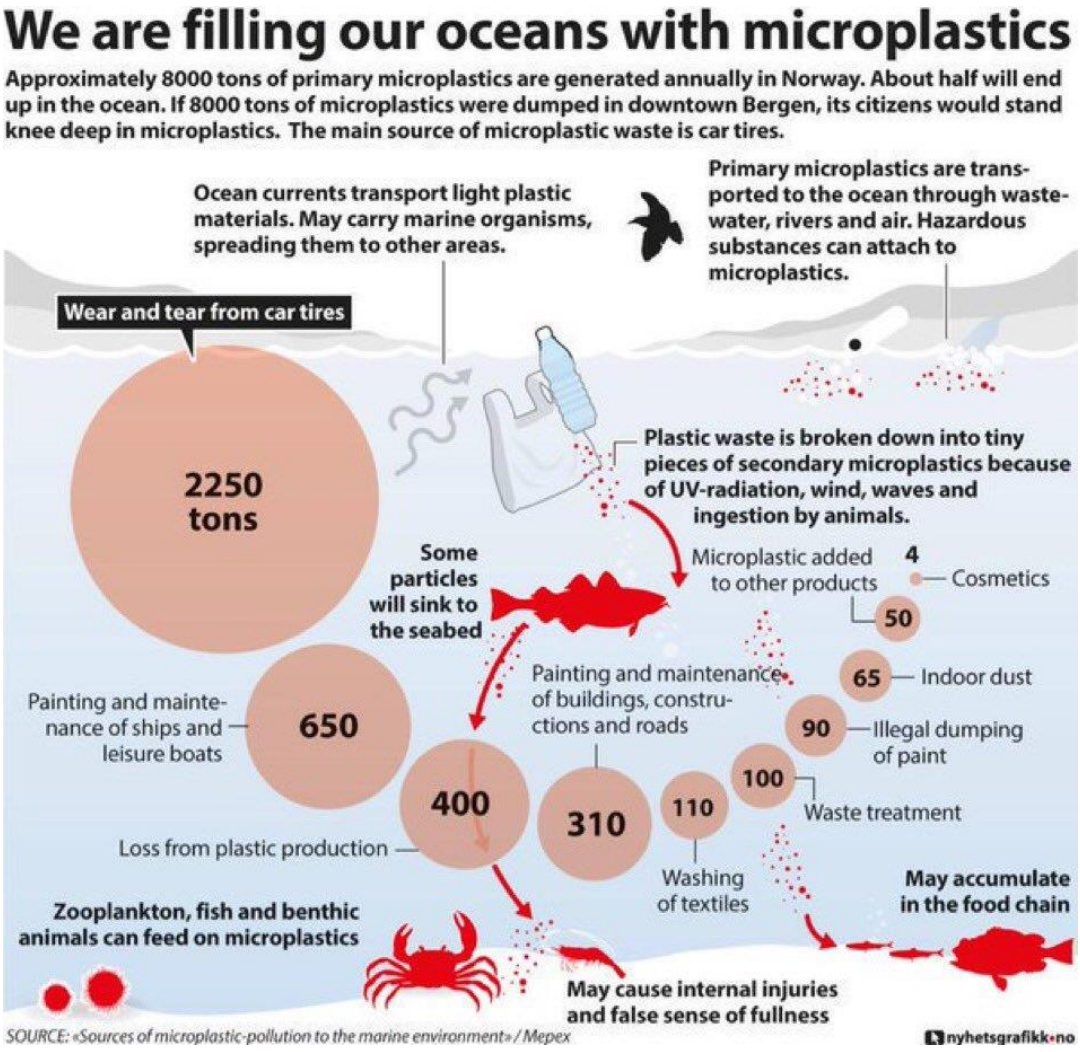


Figure 1-15. Graphic taken from 'Sources of microplastic-pollution to the marine environment'[117]

Figure 1-16. Graphic taken from 'Accumulation of Microplastic on Shorelines Worldwide: Sources and Sinks'.[114]

1.4.2.Microbeads in cosmetics

Currently, microbeads in personal care formulations are mainly made of silicones, polyethylene and polypropylene, materials which do not readily biodegrade and have been found in lakes and oceans throughout the world.[118,119] However, only a very small amount of plastic in the oceans can be traced back to facial cleansers, potentially as little as 0.05%.[117] The 'Beat the Microbead' act aims to increase awareness of the materials that are present in cosmetic products, specifically rinse off products such as exfoliating cleansers. They offer a mobile application which when used to scan a product will indicate whether it contains a material which they deem as a problematic microbead which will contribute to the 'Plastic Soup swirling around in our oceans'.[120]

1.4.3.Solutions

Although increasing awareness of the microplastics that people use is a positive initiative, there needs to be suitable alternatives to these materials in order to completely remove them from products for this to completely successful. Natural, degradable materials such as cellulose[87,88], chitosan[14,121] or peptides[122] would be a simple replacement if the chemistry can be tailored to suit the needs of the cosmetic application. One study on the toxicity to marine life of secondary microplastics (particles that have formed from larger plastic), primary microplastics (specifically designed beads) and natural particles such as kaolin clay has determined that the natural materials and spherical beads are not problematic, but the larger, misshapen secondary microplastics do elevate the fatality rate of the plankton.[123] This result implies that although the cosmetic microbeads do accumulate in the ocean, they are not as hazardous as the other sources of plastic which are in much higher abundance. Arguably, the removal of microbeads from cosmetic products is a futile attempt at tackling a much bigger problem.

1.5.Thesis Aims

The overall aim of this work is to synthesise a material which is appropriate for use in an instant-effect cosmetic product and that will pass an externally performed 'ready biodegradability' test.

This will be approached from two perspectives, particles to fill pores and wrinkles or films to tighten skin, both of which should give an overall anti-aging effect.

Firstly, the synthesis of monodisperse polymeric microparticles with diameters similar to pore and wrinkle sizes will be attempted. Ideally, particles will be spherical and monodisperse. Polyvinyl acetate (PVAc) is a polymer which has a wholly carbon-based backbone yet is known to be biodegradable in specific enzymatic environments. It will be synthesised in the form of microparticles by heterogeneous polymerisation with use of a polymeric stabiliser. Polymeric stabilisers are preferred to ionic surfactants due to the product stability in cosmetic formulation being an important factor to consider. A range of different polymeric stabilisers will be used including a synthesised statistical copolymer. The particle size and shape will be optimised by varying polymerisation parameters such as stirring speed and reagent concentrations.

Polyesterureas (PEUs) will also be synthesised as possible candidates for biodegradable microparticles. Their synthesis will be via interfacial polymerisation using diester salts synthesised from common amino acids and diols. Polymerisation parameters will again be optimised. Particles will be analysed by SEM to determine their morphology. The best particles from both PVAc and PEU will be formulated and applied to some in vitro test methods. Their biodegradation will also be analysed to determine their suitability for use in the cosmetic application.

Polyethyl glyoxylate, a self-immolative polymer, will also be synthesised and analysed for its film-forming capabilities. The polymerisation method will be optimised to enable high monomer conversions and polymerisation reproducibility. The T_g for this material is known to be around -30 °C and therefore attempts will be made to increase this to make it suitable for application onto skin. This will be done by addition of functional groups to stiffen the polymer chain and by copolymerisation with a known high-T_g polymer such as polystyrene. Differential scanning calorimetry will be the method of T_g analysis. A range of synthesised polymers with different

structures will be compared for biodegradation to determine a balance between increased T_g and suitable degradation rate.

Chapter 2: Polymeric stabilisation of poly(vinyl acetate) microparticles

2.1.Introduction

2.1.1.Poly(vinyl acetate)

The synthesis of poly(vinyl acetate) (PVAc) was first established over 100 years ago and is now multi-million tonne industry. This is largely due to its use as an adhesive and in coatings in the paper and textiles industries because of its good adhesive and mechanical properties.[54,55] PVAc is also the precursor for poly(vinyl alcohol) (PVA), a known biocompatible polymer used in cosmetics, food and medicine such as in embolic agents for treatment of the kidneys and central nervous system.[7,58] Consequently, the synthesis of PVAc has been heavily studied to optimise it for a wide range of applications.[58,124]

Vinyl acetate is most often polymerised by free-radical polymerisation in bulk, or, due to its low water solubility, it is also prepared by emulsion polymerisation with a suitable water-soluble stabiliser. Generally, in such heterogeneous polymerisations there are a wide range of available stabilisers that can provide colloidal stabilisation of the monomer droplets through either steric and/or electrostatic interactions. Steric stabilisation as opposed to electrostatic stabilisation is more useful for the present application to ensure that there is little interaction between the stabilised particles and the charged additives used in a typical cosmetic formulation.

2.1.2.Steric stabilisation using polymers

Amphiphilic molecules and polymers are often used as steric stabilisers in both heterogeneous polymerisations and in post-polymerisation particle formation techniques.[125] They are added to aid dispersion of droplets by causing an increase in the viscosity of the reaction medium and by forming a protective layer around the monomer droplets preventing coalescence during the polymerisation.[126] PVA and poly(vinyl pyrrolidone) (PVP) are both common polymeric stabilisers

used in heterogeneous polymerisations.[127,128] Both of these polymers are generally water-soluble and therefore are applicable to the polymerisation of water-insoluble monomers such as vinyl acetate, styrene and acrylic monomers in both emulsion and suspension polymerisation systems.[129,130]

PVA is produced from PVAc by the hydrolysis of acetate functional groups. It is less easily synthesised from vinyl alcohol monomer due to its enol-keto tautomerisation.[131] It is generally inexpensive and commercially available in a range of molecular weights and degrees of hydrolysis. The resulting properties of the PVA such as solubility and degradability rely heavily on the degree of hydrolysis. The term 'partially hydrolysed' is used to describe PVA where hydrolysis of the acetate is less than 90% and therefore at least 10% of the acetate groups from the PVAc precursor remain. It is this copolymer-like structure of PVA that makes it suitable as a stabiliser in polymerisations with a hydrophilic reaction medium. The alcohol functional groups on the PVA extend into the hydrophilic solvent and the acetate groups interact with the hydrophobic monomer. Generally, the structure of partially hydrolysed PVA, formed by reaction of PVAc with NaOH as opposed to hydrazine, is blocky and it is the blocks of acetate groups that anchor themselves onto the monomer (figure 2-1). Consequently, 'fully hydrolysed' PVA is often less efficient as a polymeric stabiliser due to its inability to interact adequately with the monomer. The mechanism of stabilisation of PVA has been studied via investigation into the extent of grafting of PVA onto vinyl acetate and it is found that only a very small amount of PVA chemically grafts onto the PVAc chain, most stabilises the growing polymer chain but is rinsed off in the polymerisation work-up.[132,133]

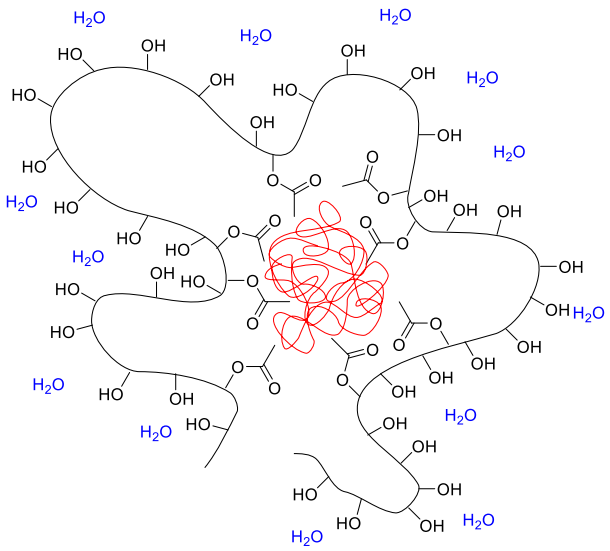


Figure 2-1. Partially hydrolysed PVAc is able to stabilise water insoluble polymers in an aqueous environment due to its amphiphilic character

There is a lot of evidence of the use of PVP as a stabiliser because it is suitable in the synthesis of particles for biological applications. It can be bought in a range of molecular weights, generally available from 10,000 to 360,000 g mol^{-1}. In the dispersion polymerisation of methyl methacrylate (MMA) it was found that, by using a PVP with a higher molecular weight, an increase in the viscosity of the solution arises leading to smaller poly(methyl methacrylate) particles. It was also found that increasing the concentration of PVP causes the formation of smaller particles, which is expected as the presence of a larger quantity of stabiliser molecules enables stabilisation of an overall larger surface area.[134] In the dispersion polymerisation of styrene and butyl acrylate to form polystyrene and poly(butyl acrylate) particles, PVP with molecular weights of 40 kDa and 360 kDa were studied.[127] In this study, it was found that a small proportion of the PVP grafts onto the polymer and the remaining dissolved PVP, especially in the case of the 360 kDa PVP, is able to be recycled and stabilise a second batch of polymer particles in a seeded dispersion. The evidence for grafted PVP was demonstrated by performing NMR and IR spectroscopy on the washed particles.

There are many other examples of polymeric stabilisation, some of which involve water-insoluble stabilisers such as polydimethylsiloxanes which can be used in the polymerisation of

hydrophilic monomers in solvents such as hexane.[135,136] However, another water-soluble stabiliser system which has been used in the suspension polymerisation of styrene is based on copolymers of acrylic acid and MMA.[126] The molecular weight and the composition of these copolymers were shown to affect the size of polystyrene particles produced. An increase in molecular weight of a polymeric stabiliser is said to produce a thicker layer of stabilisation around the monomer droplets resulting in better protection from coalescence. The hydrophobic-hydrophilic balance of the stabiliser also affects the suspension stability by dictating the extent of stabiliser adsorption at the water/droplet interface. The batch process resulted in methacrylate-concentrated polymers due to the high reactivity ratio of MMA ($\simeq$2) in comparison to acrylic acid ($\simeq$0.3) and these spontaneously precipitated out of solution at low overall monomer conversions. Therefore, changing the copolymerisation from a batch process to a semicontinuous feed of MMA produced copolymers with more evenly spaced out MMA groups. These more balanced copolymers were 100% soluble in water and had the strongest adsorption at the interface between the styrene monomer droplets and the aqueous continuous phase.[124]

As mentioned previously, there are many variables of a heterogeneous polymerisation which control the size and dispersity of the particles formed. As well as changing the concentration and composition of the stabiliser, another of the easiest variables to control is the stirring speed of the polymerisation.[10] The suspension polymerisation of styrene was investigated with respect to both of these variables with the following conclusions: at low levels of stabiliser, an increase in stirring speed decreased the particle size initially but at the highest speeds the particle size increased drastically, possibly attributed to coalescence of the particles due to inadequate surface coverage of the stabiliser. Contrastingly, with higher stabiliser concentrations there was more uniform surface coverage and particles remained stable at the highest speeds.[137]

2.1.3.Chapter Aims

The aims of this chapter are to use polymeric stabilisers such as PVA and PVP to stabilise the aqueous heterogeneous polymerisation of vinyl acetate. Both emulsion and suspension polymerisations will be attempted in order to achieve a particle diameter of roughly 1-5 µm, a size usually achieved by dispersion polymerisation. A further range of butyl methacrylate/methacrylic acid copolymers will be synthesised and their performance as steric stabilisers will be investigated. The two key reaction parameters which will be explored in order to optimise the size and dispersity of the particles produced are the concentration of stabiliser and the stirring speed. Once isolated, it is important that the particles are stable in water as the majority of skincare formulations are water-based. Ideally this means the particles will disperse readily in water and will not be prone to aggregation. Particles which are of the desired size will be degradation tested by a standardised test and will be formulated and compared to some benchmark materials.

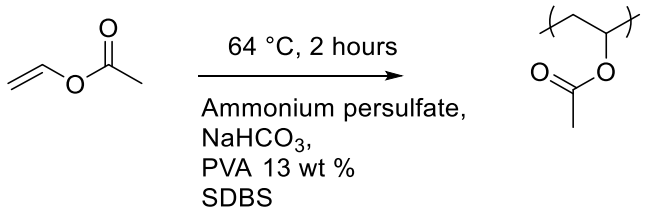
2.2.Results and Discussion

2.2.1.PVA stabilised PVAc particles by emulsion and suspension polymerisation

Initially, PVA with a molecular weight of approximately 67 kDa and a degree of hydrolysis of approximately 88 % (Mowiol 8-88) was selected for the emulsion polymerisation of vinyl acetate as it has been shown in the literature to be an effective stabiliser.[132] Vinyl acetate is a hydrophobic monomer and will spontaneously form droplets in water. This makes it applicable to emulsion and suspension polymerisations techniques but not to dispersion polymerisation as this requires a water-miscible monomer. Dispersion polymerisation of vinyl acetate has been shown in the literature to produce micron-sized spheres in solvents such as ethanol or propanol/ water mixtures.[138,139] Here, water was chosen as the reaction medium as the particles produced would need to be stable in water once formulated. Typically, the standard emulsion polymerisation of vinyl acetate has been shown to have an upper limit of around 500 nm

diameter,[140] and suspension a lower limit of approximately 10 µm.[141] For this work it was predicted that utilising a variety of common and novel stabilisers alongside finely tuned reaction conditions, it should be possible to access larger particles by emulsion polymerisation or smaller particles by suspension polymerisation.

The initial syntheses using PVA as a stabiliser were developed from previously reported emulsion and suspension polymerisations.[7,141,142] The method used for the emulsion polymerisation is one which has been developed to investigate the effects of reactor conditions on the final PVAc particles.[7] The reaction temperature was set to 64 °C and kept at this throughout all vinyl acetate polymerisations due to the vinyl acetate/water azeotrope having a boiling point of 66 °C.[53] Alongside the PVA stabiliser, sodium dodecylbenzenesulfonate (SDBS) was used as an additional surfactant and ammonium persulfate was used as the water soluble initiator (scheme 2-1). The method used was a semi-continuous process which are commonly used in industry. It utilised an initial charge of monomer and initiator, followed by the remainder of these being fed into the reactor at a steady rate. This was used instead of batch emulsion polymerisation in order to attempt control over the growing particles. At the end of the addition, the polymerisation was allowed to proceed for a further two hours and was then terminated by exposing to air and removing from the heat source.



Scheme 2-1. The emulsion polymerisation of vinyl acetate in the presence of Mowiol 8-88 PVA stabiliser, SDBS surfactant and $NaHCO_3$ buffer

In order to determine the particle size, dynamic light scattering (DLS) and transmission electron microscopy (TEM) were performed. For both of these techniques a 0.1 wt% solution in water is required. DLS was performed on a sample straight from the reaction at completion and on a

sample after washing. The washed sample was collected by filtration which results in partial drying. DLS and TEM of this sample shows aggregation of the particles and this is likely due to the tacky drying effect of PVA.[143] Nonetheless, the TEM and DLS data both before and after washing gives particle size of less than 500 nm, with aggregated masses of approximately 2.5 µm (figure 2-2). This size generally correlates with the upper limit of particle size that can be achieved by emulsion polymerisation of vinyl monomers.[10]

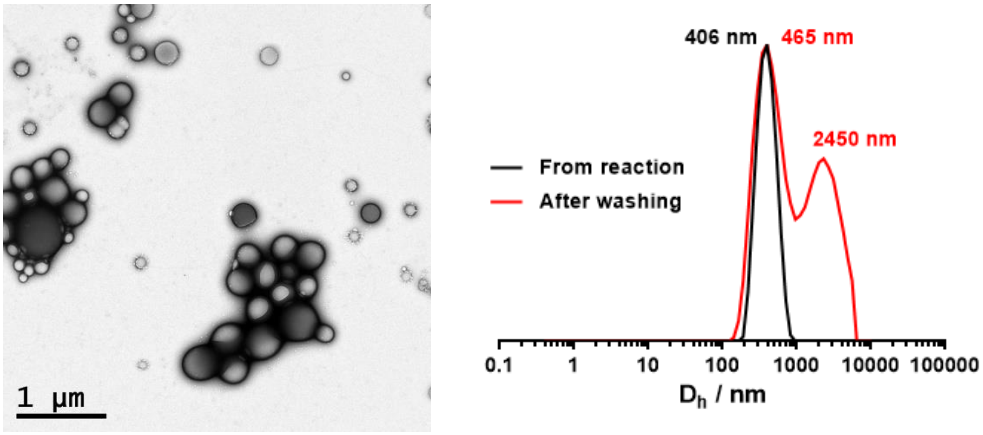
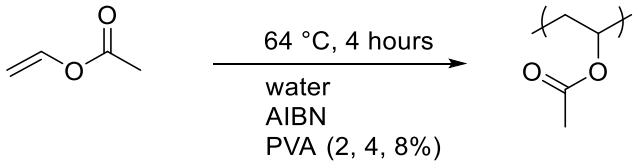


Figure 2-2. TEM image of the particles synthesised by emulsion polymerisation with PVA as a steric stabiliser, alongside the intensity-weighted DLS particle size distribution for particle samples from the reaction and from the washed and redispersed particles.

Dispersion polymerisation is normally used to prepare particles within the diameters that are required for this project's application, however due to the water immiscibility of vinyl acetate this cannot be carried out with water as the reaction medium. Therefore, in order to increase the size of the PVAc particles, suspension polymerisation was employed. The main difference between the emulsion and suspension polymerisation is the solubility of the initiators used. For suspension polymerisation, a water-insoluble but monomer-soluble initiator is required, and in these experiments AIBN has been used. The method was modified from a report by Murakami et al. who examined the effect of a PVA-borax stabiliser system and achieved particle sizes throughout the micron range.[141] In their work, particles with sizes less than 10 microns were synthesised using a solution of PVA at 2% and borate at 0.08 mol dm^{-3}. Reproduction of these conditions gave a tacky, gel-like product, likely due to the extensive cross-linking that occurs

between PVA and borax.[144] Therefore, the borax was omitted from the polymerisations and a range of experiments using solely PVA as the stabiliser were performed. Three reactions with PVA at 2, 4 and 8 wt% were performed, with AIBN as a monomer soluble initiator, to determine the effect of stabiliser concentration on the particle size (scheme 2-2).



Scheme 2-2. The suspension polymerisation of vinyl acetate with Mowiol 8-88 PVA stabiliser at 2, 4 and 8 % to form PVAc particles.

The increase in concentration of PVA caused the particle size, the standard deviation and the size range of the particles in the sample to decrease (table 2-1 and figure 2-3).This is characteristic of heterogeneous polymerisations where an increased stabiliser concentration is able to stabilise an increased surface area and therefore produces smaller particles.[10] The particles were redipsersed for imaging by addition of 1 wt% of sodium dodecyl sulfate (SDS), a surfactant often used in heterogeneous polymerisations. This was to prevent any reaggregation in order to achieve clear images that would allow particle sizing. A control image of the 4 wt% PVA stabilised sample was taken of a dispersion without SDS and this shows particles of similar sizes which are heavily aggregated (figure 2-4). Even with the presence of SDS, the particles of the smallest size were prone to aggregation. This is likely due to insufficient removal of the PVA stabiliser causing a tacky outer coating on the particles, despite multiple washes using warm water being carried out.

Table 2-1. The increase in concentration of PVA causes a decrease in both the mean and standard deviation (SD) of the particle diameter. Numerical data has been calculated from measurement of 100 particles by optical microscopy.

[PVA] / wt %	Mean / μm	SD	Range / μm
2	22.4	17.2	5-100
4	13.4	6.8	2-50
8	8.4	2.9	2-20

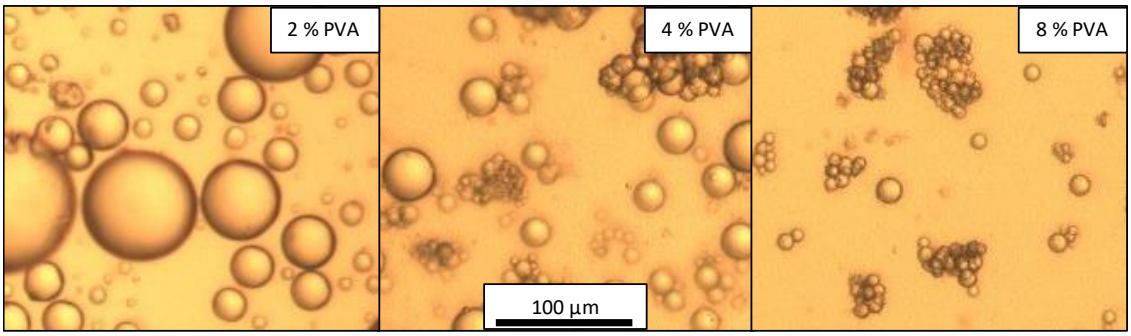


Figure 2-3. Optical microscopy images of the particles formed in the suspension polymerisation of PVAc using PVA as a steric stabiliser and SDS to aid dispersion.

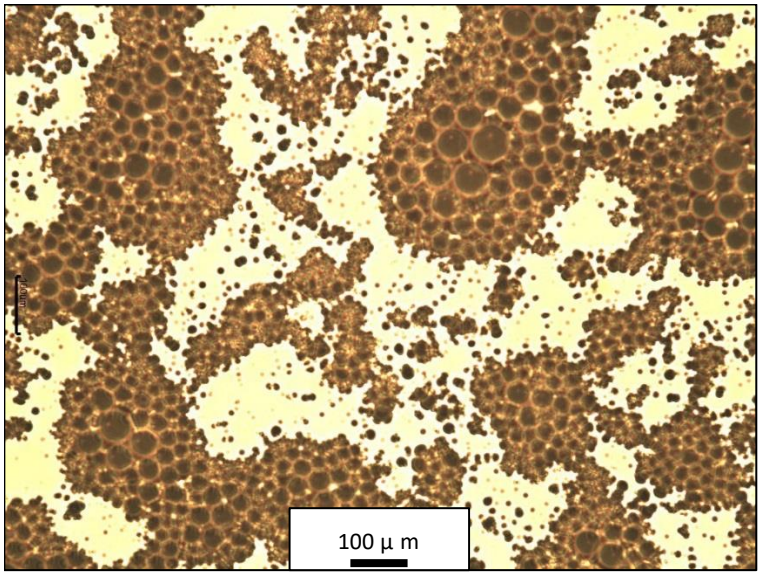


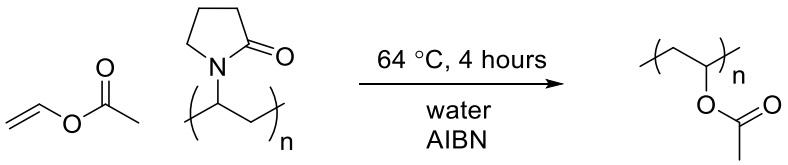
Figure 2-4. Optical microscopy images of a sample of 4 % PVA stabilised PVAc particles without any extra SDS added.

As a control reaction, the polymerisation of vinyl acetate using the same reaction conditions but without any stabiliser has been carried out. A large mass of insoluble but flexible polymer was produced around the stirrer with no particles present.

2.2.2.PVP stabilised PVAc particles by suspension polymerisation

Even with addition of surfactant to help disperse the particles in water, the PVA-stabilised particles are too aggregated to redisperse fully. The cosmetic application requires a smooth dispersion without aggregated lumps in order to spread evenly on the skin. Therefore, PVP was investigated for its effectiveness as a stabiliser in place of PVA in the suspension polymerisation. As it has been shown to stabilise particles synthesised from other vinyl monomers, it was

predicted to be a suitable stabiliser for this synthesis. PVP with a molecular weight of 10 kDa was used at a concentration of 2 wt % in a similar reaction to the PVA stabilised reactions. This was in order to determine if PVP would cause less aggregation of the particles. The stirring speed used was 500 rpm and temperature set at 64 °C as before (scheme 2-3).



Scheme 2-3. The polymerisation of vinyl acetate carried out with PVP (M_w = 10 kDa, 2 wt %) as a steric stabiliser to yield PVAc particles.

After removing from the heat and ceasing agitation, large polymer beads settled at the bottom of the vessel leaving a transparent supernatant. Centrifugation was not required to isolate the particles as the liquid could simply be decanted off. The beads were washed with warm water and left to dry in air which caused some aggregation, especially with the smaller particles, but these could be separated with agitation. NMR spectroscopy in $CDCl_3$ showed the three characteristic peaks of PVAc at 1.8, 2.0 and 4.9 ppm with no evidence of unreacted vinyl groups suggesting complete monomer conversion in the particles (figure 2-5). There is also no presence of PVP in the NMR spectrum, implying that the washing of the beads removed all free stabiliser and any grafting of stabiliser to the PVAc is minimal. This gives rise to the decrease in aggregation that occurred with use of PVP in contrast to the PVA stabilised particles.

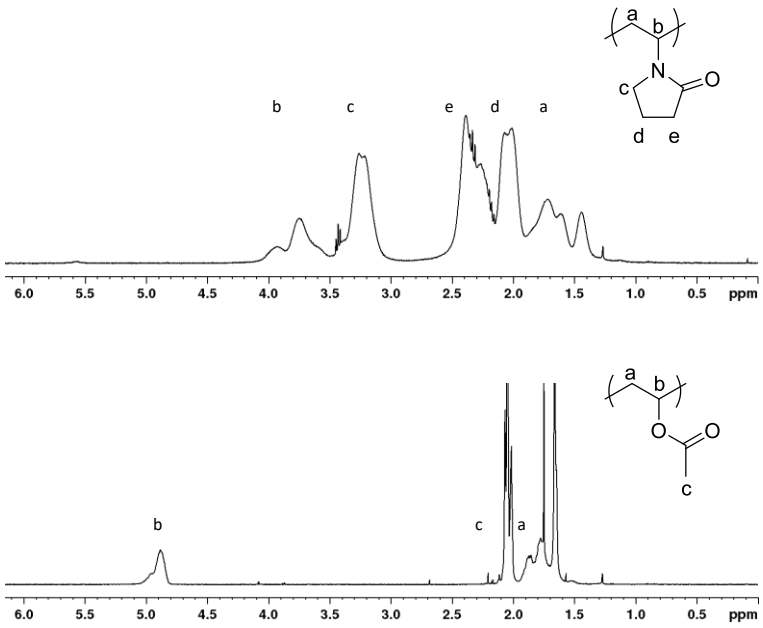


Figure 2-5. The ^{1}H NMR spectrum of commercial PVP and PVP-stabilised PVAc beads in $CDCl_3$.

In order to decrease the size of the beads and induce better stabilisation, the PVP concentration was increased to 4%. Following the same polymerisation protocol, beads which appeared slightly smaller in size were produced at some loss of uniformity in shape. Again, NMR analysis after washing did not show any PVP to be present. In both of these reactions at 500 rpm, there was a large amount of coagulum in the form of solid PVAc on the stirrer rod and around the sides of the vessel, likely to be caused by splashing. Following this, the reactions were repeated at 300 rpm in order to attempt to reduce this coagulum. Coagulum was decreased but the beads were very random in shape and size with some being long and wire-like (figure 2-6). This would suggest that in order for round particles, more agitation is required. At 500 rpm and 8% PVP, particles did not form, instead a large aggregated mass of polymer was produced around the stirrer which could not be broken apart. This could be a result of beads coalescing during the reaction or by the build-up of layers of polymer on the stirrer itself.

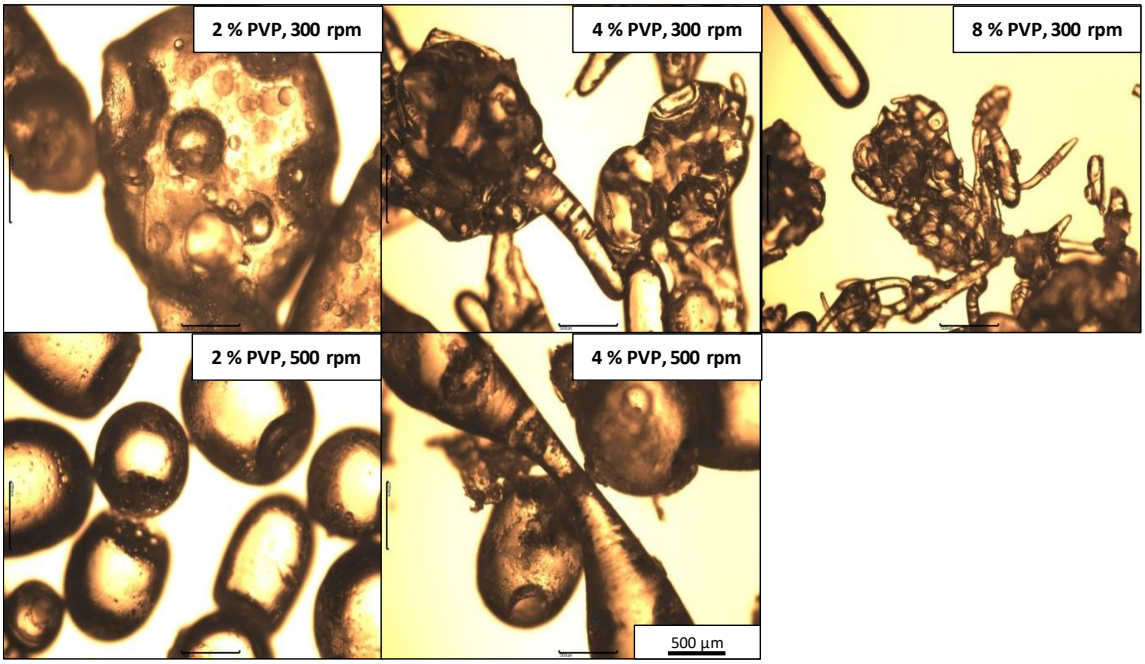


Figure 2-6. Optical microscopy images of polymerisations of vinyl acetate using PVP as a stabiliser.

Although synthesis at 300 rpm decreased the amount of coagulum and hence led to a larger yield of stabilised, individual particles, the particles produced were not as spherical as those synthesised at 500 rpm with 2 % PVP. Under contrasting conditions, at 8 % PVP and 300 rpm, the particles had random shapes and were highly aggregated. Loss of sphericity at high concentrations of stabiliser and less agitation could be due to bridging flocculation, a phenomenon where a stabiliser molecule is able to stabilise two adjacent particles, causing them to join irreversibly. PVPs are often used as flocculating agents in industry due to this behaviour.[145] Ultimately, the increase in PVP concentration did decrease the particle size but only by a small amount, implying that it is not an efficient stabiliser. After filtration of the particles, the solids content of the reaction medium filtrate as obtained by moisture analysis was slightly less than the solids content of the PVP solution used in each case. This implies that some of the PVP had irreversibly reacted during the polymerisation, likely in grafting to the PVAc particles, but at low concentration that was not visible by NMR spectroscopy. Generally, grafting of PVP or PVA onto the polymer is undesirable, as both of these stabilisers will increase the tackiness and therefore likelihood of aggregation on drying. Although they are effective

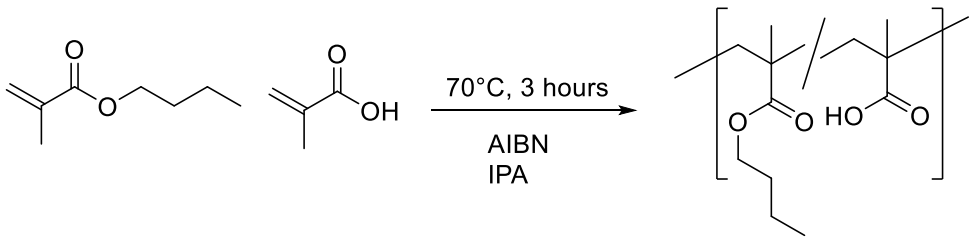
stabilisers in the polymerisation, they are likely more suited to applications where the particles will remain in a wet state and not for redispersion as dry particles in cosmetic applications, specifically where the use of an added dispersant is undesirable.

*2.2.3.Synthesis of poly(butyl methacrylate-*stat*-methacrylic acid) copolymer*

Design of a polymer for use as a water-soluble steric stabiliser has been carried out in order to overcome the problems of aggregation on drying. Amphiphilic copolymers can be used as steric stabilisers in heterogeneous polymerisations due to their ability to interact with both the monomer and reaction medium. Poly(butyl methacrylate) (PBMA) is water immiscible but will interact with vinyl acetate monomer, and poly(methacrylic acid) (PMAA) is water soluble. Therefore, a random or statistical copolymer of the two should enable steric stabilisation of vinyl acetate in water analogous to the action of partially hydrolysed PVAc. If required, deprotonation of the methacrylic acid should increase the stabiliser's water solubility and also may aid stabilisation by contributing some charge effect alongside the steric effect.

The synthesis of a statistical copolymer (P(BMA-*stat*-MAA)) has been carried out by free-radical polymerisation in isopropyl alcohol using AIBN as an initiator at 70 °C (scheme 2-4). The copolymerisations have reached high conversions as shown by NMR and have been carried out in batch processes. The reactivity ratios of the monomers are similar so no compositional drift would be expected to have occurred.[146] Three different but analogous stabilisers have been synthesised; two different compositions, 60:40 and 70:30 BMA to MAA respectively, have been targeted and the initiator concentration has been varied to see effects of both the overall solubility and overall chain length on the effectiveness of stabilisation. Details of the polymers are given in

table 2-2.



Scheme 2-4. The free radical polymerisation of butyl methacrylate and methacrylic acid to synthesise a statistical copolymer suitable for use as a polymeric stabiliser.

Table 2-2. Monomer feed and initiator concentrations for the synthesis of P(BMA-stat-MAA)

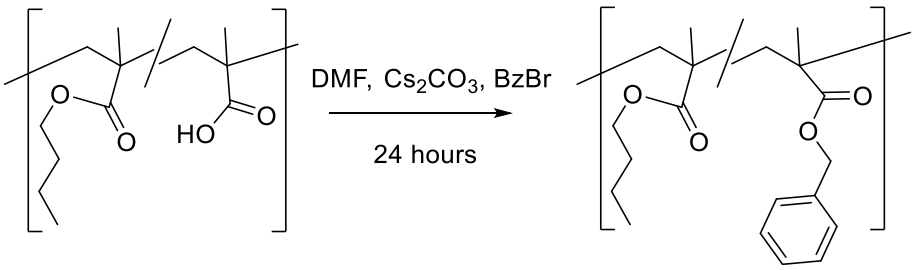
Stabiliser	Mole fraction BMA	Mole fraction MAA	[AIBN] / wt %
1	0.6	0.4	1.0
2	0.7	0.3	1.0
3	0.6	0.4	0.5

After reaction for 3 hours at 70 °C, NMR spectroscopy showed overall high conversions of monomer to polymer by disappearance of the vinyl signal. Polymers were then precipitated from petroleum ether to remove any residual monomer. The overall ratio of methacrylic acid to butyl methacrylate in the copolymers could not be determined from ^{1}H NMR spectroscopy directly due to overlapping of the backbone signals of the two different types of monomer unit.

*2.2.4.Alkylation of the P(BMA-*stat*-MAA) copolymer*

In order to determine the composition, substitution of the acid group with a benzyl group has been carried out by an alkylation process. The successful alkylation causes the addition of benzyl signals into the NMR spectrum in a region of the spectrum where the peaks are not contaminated by any residual solvent signals or any other signals from the butyl methacrylate units of the chain. Only a small sample of each stabiliser was alkylated, enough to enable analysis by GPC and NMR spectroscopy. The alkylation reaction was carried out at room temperature under N_2 and only required simple solvent removal and washing steps to isolate the solid poly(butyl methacrylate-*stat*-benzyl methacrylate) (P(BMA-*stat*-BzMA)) (scheme 2-5). Often, a

similar analysis of copolymer composition is carried out by methylation of the carboxylic acid, however this process is potentially hazardous due to the use of volatile and highly toxic trimethylsilyldiazomethane.[147] From these results, alkylation with benzyl bromide appears to be efficient and it has the added advantage of being a far safer reaction. The composition of the polymer was then calculated from the ratio of the integrals of the aromatic signals at 7.4 ppm and the signal representing the protons nearest to the carbonyl on the butyl chain at 4.0 ppm (figure 2-7). A further method used to assess the extent of alkylation is ATR FTIR spectroscopy. The O-H stretch of the acid group disappears from the spectrum after the alkylation process, suggesting that all acid groups have been substituted (figure 2-8).



Scheme 2-5. The alkylation of P(BMA-stat-MAA) stabilisers occurs via a substitution reaction with caesium carbonate and benzyl bromide. The resulting polymer is a P(BMA-stat-BzMA) of the same degree of polymerisation

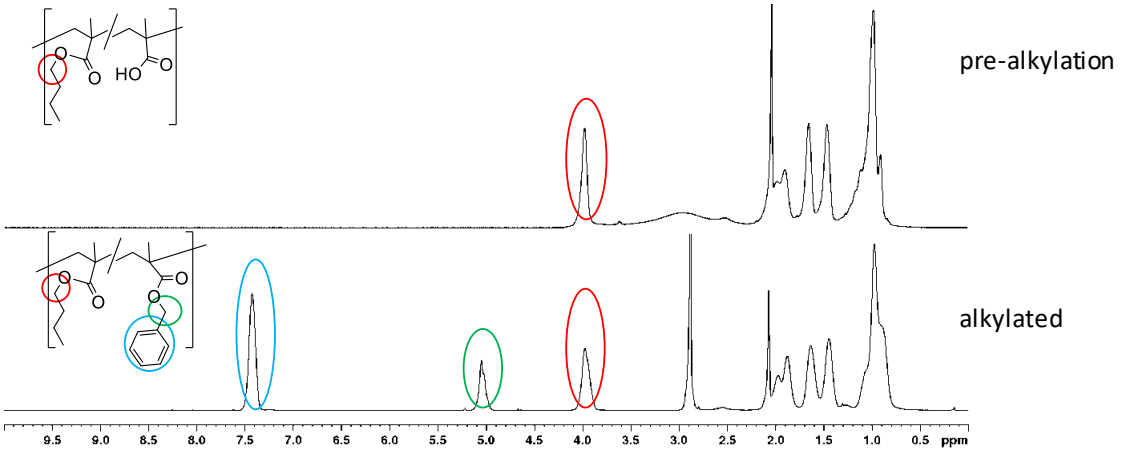


Figure 2-7. ^{1}H NMR spectra of P(BMA-stat-MAA) stabilsier 3 in deuterated acetone before and after alkylation. The clear addition of two peaks at 7.4 and 5.0 ppm has occurred implying successful alkylation. The target composition for this stabiliser was 60:40 BMA to MAA. In order to find the actual composition, the integrals for the peaks at 4.0 and 7.5 are required.

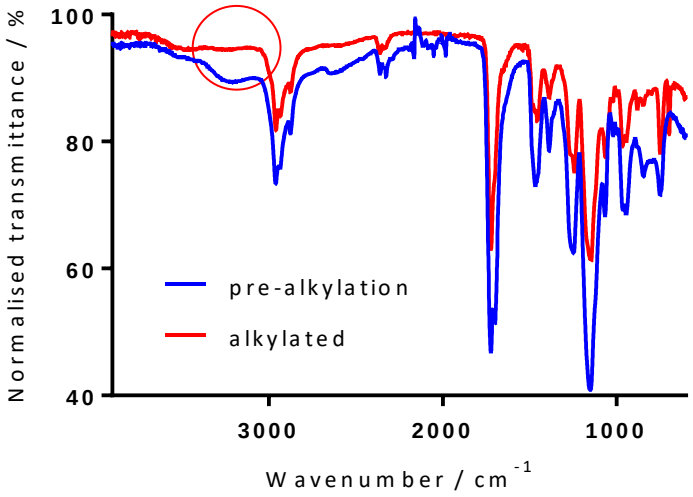


Figure 2-8. ATR FTIR spectra of P(BMA-stat-MAA) before and after alkylation. The spectra are very similar, with the main difference being the loss of acid functionality shown by the disappearance of the O-H stretch at 3100 cm^{-1}

The molecular weights of the polymeric stabilisers have been determined by gel permeation chromatography (GPC) in DMF relative to PMMA standards (figure 2-9). The composition of the polymers is summarised in table 2-3. Usually when polymers are acidic, a mixed solvent system GPC is required in order to prevent too much interaction of the acid functional groups with the GPC column. In this case, the alkylated analogues of the stabilisers have been used on a standard DMF GPC, however, measurements on an acetic acid/THF GPC system would have been more appropriate if the polymer had been soluble in that particular solvent system. This is because the substitution of acid groups for benzyl groups may affect the calculated molecular weight of the polymer. As well as this, any trace acid functionality may cause interaction with the chromatography columns which could in turn compromise the accuracy of the results. Due to the use of PMMA standards, the M_n values calculated from the GPC chromatograms will not be true representations of the p(BMA-*stat*-MAA) polymer molecular weights. The difference in molecular weights between stabilisers 2 and 3 show that the decrease in initiator has enabled polymerisation of longer polymer chains as expected.[6] The M_w/M_n values (molecular weight distributions) are typical of batch free-radical polymerisations because they can terminate freely by multiple mechanisms leading to a large range of molecular weights being produced. Finally,

all successfully isolated stabilisers were dried under vacuum and stored in the fridge to be used in polymerisations.

Table 2-3. Compositions and molecular weights of the three P(BMA-stat-MAA) stabilisers synthesised by free-radical polymerisation. Compositions have been calculated from NMR data of the alkylated polymers and DMF GPC with PMMA standards has been used to calculate M_n and M_w/M_n, a measure of the polydispersity.

Stabiliser	Targeted BMA:MAA	Calculated BMA:MAA	M_n / g mol^{-1}	M_w/M_n
1	60:40	60:40	14,100	2.41
2	70:30	70:30	12,400	2.47
3	60:40	60:40	22,100	2.54

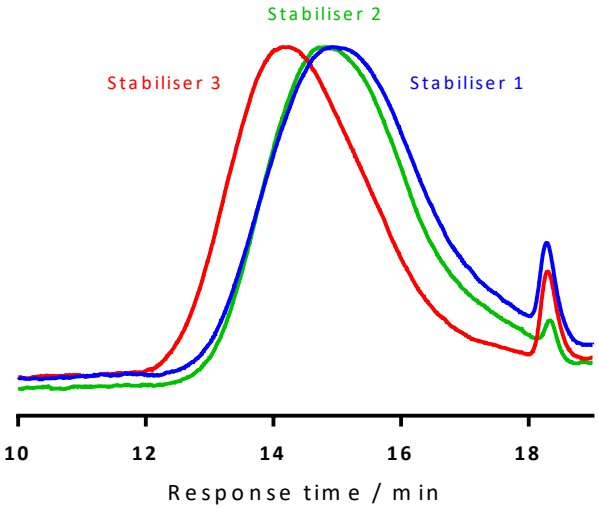
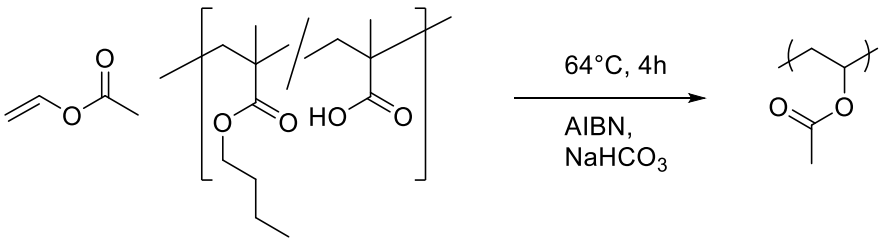


Figure 2-9. Normalised GPC chromatograms (RI detection) for the alkylated P(BMA-stat-MAA) stabilisers. All are monomodal with respect to polymer peaks. The peak at 18 minutes is the flow-rate marker added to the eluent.

2.2.5.P(BMA-stat-MAA) stabilization of PVAc particles by suspension polymerisation

In all of the following polymerisations, all conditions have been kept constant except for the stirring speed and the concentration of stabiliser. Stabiliser 1 was used in scoping experiments to find the best conditions for production of 5 µm diameter particles with narrow size distributions. AIBN has been used as the initiator and $NaHCO_3$ has been used to enable dissolution of the stabiliser in water (scheme 2-6).



Scheme 2-6. The polymerisation of vinyl acetate with P(BMA-stat-MAA) stabiliser to synthesise PVAc particles.

The apparatus set-up for the reactions involved the same anchor-shaped stirrer and all polymerisations have been performed at the same scale. Reactions were terminated after 4 hours and PVAc beads were collected by centrifugation and washed 3 times with warm water to remove excess stabiliser and initiator. Particles were readily dispersed in water for microscopy, which primarily showed that stabilisation by P(BMA-*stat*-MAA) was sufficient enough to allow dispersion of the dried particles, unlike PVA or PVP. Microscopy images have been used to calculate average particle sizes from measurements of at least 100 particles. The results are displayed in figure 2-10 alongside representative microscope images for each sample.

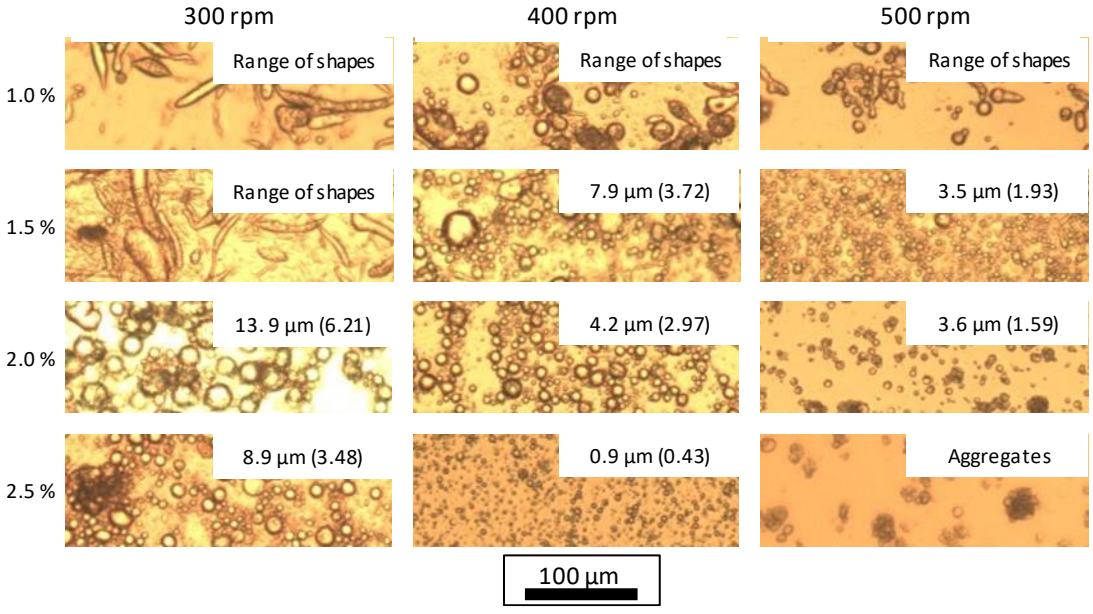


Figure 2-10. Optical microscopy images of PVAc particles synthesised with a P(BMA-stat-MAA) stabiliser at different stirring speeds and stabiliser concentrations. Average sizes and standard deviations have been calculated from measurement of 100 particles.

As before, an increase in stabiliser concentration led to smaller, more spherical particles. An increase in the stirring speed generally had the same effect, although at 500 rpm the particles with 2.0 and 2.5% stabiliser became aggregated and would not separate readily. With respect to the aims of the experiment, the reaction conditions that gave the best results were 2.0% at 400 rpm and 1.5% at 500 rpm. In these reactions, the particles had fairly narrow size distributions and were closest to the target particle size of 5 µm. Further fine-tuning of stirrer speed and stabiliser concentration should therefore lead to the formation of particles even closer to the desired properties.

These particles have been analysed by ^{1}H NMR spectroscopy after washing and drying to ensure that all unreacted stabiliser, monomer, $NaHCO_3$ and initiator have been removed, and also to examine the extent of reaction with the stabiliser. A representative NMR spectrum is given in figure 2-11 which shows the presence of the stabiliser in the form of butyl methacrylate and stabiliser polymer backbone signals at 4.0 and 1.0-1.5 ppm respectively. This suggests that the stabiliser has reacted with the growing PVAc chains hence why the stabilisation is so effective.

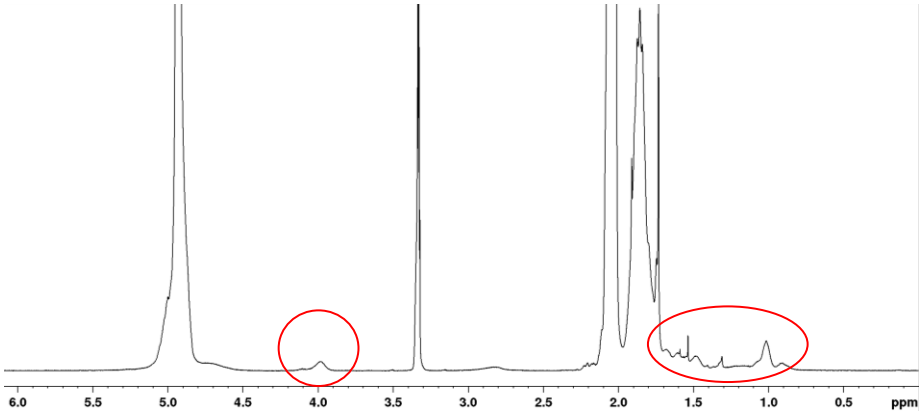


Figure 2-11. The NMR of PVAc particles synthesised with P(BMA-stat-MAA) stabiliser at 2.0% stirred at 500 rpm.

Kinetic analysis was carried out on a polymerisation with stabiliser 1. With suspension polymerisation, reaction progress can be monitored by measurement of solids content using a moisture analyser. As the reaction proceeds, volatile monomer is converted to polymer chains which will remain intact when heated under the conditions of the moisture analyser. This device

reaches a maximum temperature of 150 °C which causes any unreacted monomer and the reaction medium to boil off. Therefore, the mass that remains after heating will be proportional to the amount of polymer that has formed once the mass of initiator, stabiliser and $NaHCO_3$ has been accounted for and an approximate conversion can then be calculated.

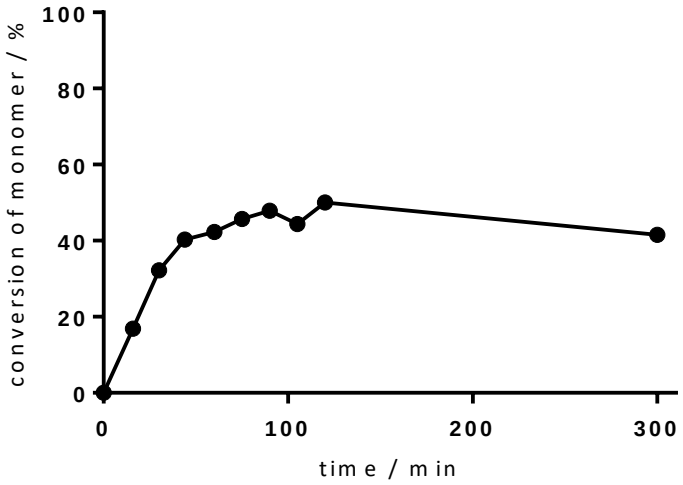


Figure 2-12. The conversion of monomer to polymer during the reaction calculated from moisture analysis measurements.

As shown in figure 2-12, the conversion increases rapidly in the first 30 minutes of the reaction which is typical heterogeneous free radical polymerisations where the concentration of monomer and initiator are both at their highest and therefore the production of short-chain polymer radicals is very fast. As the supply of initiator and free monomer decreases, the reaction slows down until after 120 minutes where polymerisation appears to stop. However, it is unusual for the conversion to have halted so early as AIBN has a 10h half-life at 65 °C[148] and therefore should still be present in the reaction. It is possible that the low boiling point of vinyl acetate is the cause for the deceleration of the reaction as, even with condensers applied, sampling and poor sealing of the reactor vessel could mean that a large amount of monomer has leaked out during the polymerisation. Therefore, it is likely that actual monomer conversion is higher than shown by the moisture analysis technique. Analysis of each sample by NMR or gas

chromatography to detect residual vinyl acetate levels would provide sufficient evidence for this.

So far, the suspension polymerisations have been carried out with stabiliser 1. The stabilisers with targeted compositions of 60:40 (stabilisers 1 and 3) dissolve readily in water with the addition of $NaHCO_3$ at twice the concentration. However, stabiliser 2 with composition 70:30 did not dissolve in water and instead formed a cloudy dispersion, even with addition of $NaHCO_3$. It is possible that this polymer is not hydrophilic enough to dissolve fully in water, therefore making it inappropriate for use in the suspension polymerisation when the reaction medium is water. In an attempted polymerisation using a cloudy dispersion of stabiliser 2, no particles were formed and instead a thick layer of PVAc coagulum was present after 2 hours.

The effect of increasing the molecular weight of the stabiliser on the stabilisation has also been investigated, with predictions that this copolymer (stabiliser 3) would provide more effective stabilisation and therefore decrease the particle size further. This is because the longer polymer chain should increase the thickness of the stabiliser layer surrounding the droplets and therefore better protect the droplets from coalescence. Reactions at 400 rpm with concentrations of 1.5, 2 and 2.5% produced mainly round particles, however at the lower stabiliser concentration, a large amount of irregular shapes were also present in the sample. In comparison with particles made at the same stirring speed but using stabiliser 1, the particles made with stabiliser 3 are very similar in both shape and size (figure 2-13). This implies that although there is a reasonable increase in molecular weight between stabilisers 1 and 3, the increase is not large enough to induce extra stabilisation as predicted. Therefore, there appears to be no extra stabilisation gained from using the higher molecular weight polymer in this case.

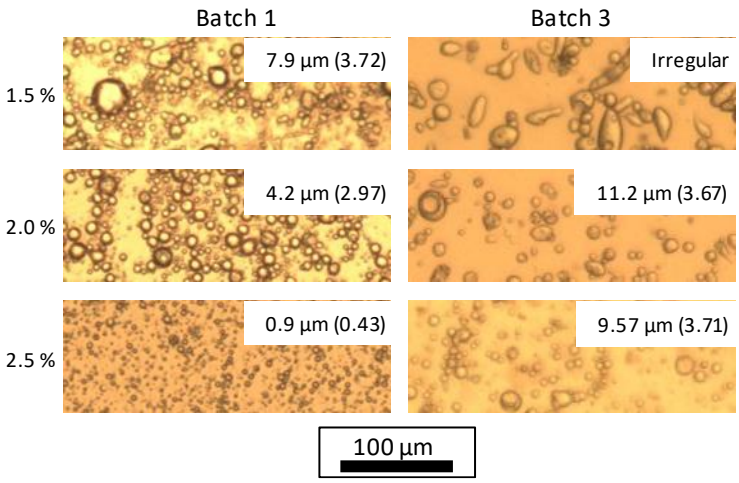


Figure 2-13. Optical microscopy images of P(BMA-stat-MAA) stabilised PVAc particles at different stabiliser concentrations with stabiliser 1 and 3 respectively. Average sizes and standard deviations have been calculated from measurement of 100 particles.

2.2.6.Biodegradation testing

A polymerisation scale-up was successfully carried out at 2% stabiliser concentration and 400 rpm with an aim of producing approximately 50 g of solid PVAc particles. There were no extra changes required for the scale up other than the use of a 1 L vessel in place of the 250 mL used previously and the particles produced were analogous in size to the smaller scale product. From this batch, a 40 g sample of dried PVAc particles was sent for biodegradation testing at Chemex Environmental International Ltd where a 'ready biodegradability' test was carried out. This test method was originally developed by R. N. Sturm in 1973 and considers the total degradation of a compound to be its ultimate conversion to H_2O and CO_2.[60] This method is the Organisation for Economic Co-operation and Development (OECD) and the International Organization for Standardization (ISO) standard for measurement of the degradability of a non-volatile compound or one with poor water solubility.[59] The test involves the dispersion of the compound in a buffer solution inoculated with a mixed population of micro-organisms. The bottles are sealed with a headspace of air, allowing for aerobic respiration. The CO_2 from the degrading compound is passed through an alkali trap, typically NaOH, and collected as 'inorganic carbon' i.e. carbonate. The traps are then removed and the total inorganic carbon is calculated. It is important that the carbon content of the test compound is known to a high degree of accuracy

in order to be able to compare the calculated result with the theoretical maximum result and hence calculate the degree of biodegradation as a percentage. In a typical test, measurements are taken every 3-5 days for 28 days and the test is ended if degradation does not seem to be accelerating at this point.

The full report from the testing can be found in the appendices. In summary, the degradation of the particles was slow with only approximately 7% degradation occurring over the 28 day test (figure 2-14). The sodium acetate and toxicity controls used for comparison showed degradation of 90% over the same time period. Subsequently, the final conclusions of the study state that the PVAc particles are not readily biodegradable in an aerobic aqueous environment and hence failed the OECD test required for the particles to be classed as biodegradable.

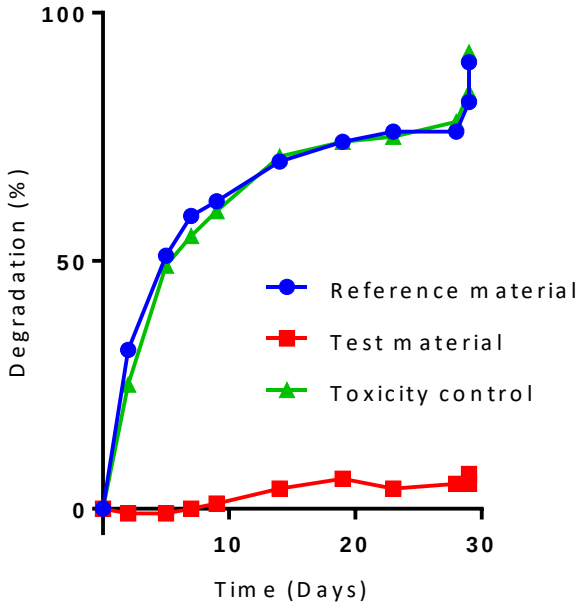


Figure 2-14. The results of the 'Ready biodegradation' of PVAc particles 2% P(BMA-stat-MAA) CO_2 evolution test performed by CHEMEX Environmental International Limited.

2.2.7.Formulation testing

Alongside this biodegradability testing, particles from the same large-scale batch were sent to CRODA for testing in formulation. In this test, particles are introduced into a base emulsion at 10 wt% and applied to their in-house benchmarking tests which are used to compare the competitor materials to their own. The study director of these tests was unable to smoothly incorporate the particles into the formulation and was consequently unable to perform any of the required benchmarking tests. It is likely that the particles were too agglomerated to be able to disperse fully, giving the emulsion a rough, lumpy texture unsuitable for use in the cosmetic product. These tests will be further discussed in the following chapter where they were used successfully.

2.3.Conclusions

The aim of this chapter was to synthesise PVAc spheres with a diameter of 5 µm by heterogeneous polymerisation by incorporating a steric stabiliser. Three different polymers were used as stabilisers, each with a different effect. Emulsion polymerisation with PVA as a stabiliser produced nano-sized particles which were prone to aggregation on drying. Suspension polymerisation with PVA enabled production of larger particles due to the difference in loci of polymerisation; however, these were also prone to aggregation. It was thus concluded that PVA is not a suitable stabiliser in this application where solid, dry particles are required. PVP was then investigated as a stabiliser and was found to be required at higher concentrations than PVA in order to synthesise micron-sized particles; however, at 8% PVP the smallest sizes were reached but aggregation was a problem. Therefore, PVP has also been deemed ineffective as a stabiliser in the polymerisation of vinyl acetate under the described conditions where the production of micron-sized particles is desired. Both PVA and PVP are known to produce tacky materials and overall this has shown to be problematic in the drying and dispersion of the

particles. This is possibly due to the small amount of grafting that occurs, causing the washed particles to still have tacky coatings which cause this irreversible aggregation.

A series of P(BMA-*stat*-MAA) stabilisers were synthesised using free-radical polymerisation. The initial batch of P(BMA-*stat*-MAA) stabiliser with a composition of 60:40 BMA/MAA was used to determine the concentration and stirring speed required to synthesise PVAc particles of 5 µm. A strong dependence of particle size on both of these reaction conditions was found, leading to tuning of particle size to close to the desired size at 2.0% and 400 rpm or 1.5% and 500 rpm. The next challenge in this synthesis would be to attempt to decrease the particle size distribution. A homogeniser can be used to pre-mix the monomer within the stabiliser solution, hopefully leading to the formation of monomer droplets with a narrow particle size distribution. This in turn should induce the production of more uniform particles on polymerisation. If this is not sufficient to increase particle uniformity, homogenisation during the polymerisation may be employed using a different reaction vessel. However, due to the poor performance of the particles in the ready biodegradation and formulation tests, the synthesis was not optimised further.

A further idea which could be attempted to further investigate the stabilisation could be the synthesis of a different statistical copolymer stabiliser. For instance, the work described by Vilchis uses acrylic acid and methyl methacrylate[126] and found that with increased hydrophobic content the stabilisation improved. Although in this work different compositions and molecular weights of the P(BMA-*stat*-MAA) stabiliser were synthesised, no work was attempted using different monomers. Therefore, the stabilisation effectiveness could potentially be tuned further by experimenting with other monomers. Attempts at a semi-continuous synthesis of the P(BMA-*stat*-MMA) would likely also tell us more about how the structure of the statistical copolymer effects its interaction with the monomer droplets.

Overall, due to the material failing in both formulation testing and biodegradability, this work on PVAc particles was ended. Although the work on tuning the properties was successful and enabled the production of particles in the correct size range, ultimately the properties of the particles were inferior to both current benchmarks and the biodegradation qualities that are required. There is also the issue of poor monomer conversion in these reactions, something which is undesirable on an industrial scale and therefore makes vinyl acetate suspensions impractical for large scale reactions. However, the P(BMA-stat-MAA) stabiliser could be used in future particle production as it shows good promise at stabilising suspension polymerisations in water.

Chapter 3: Synthesis and degradability of polyester urea microparticles from amino acid starting materials

3.1.Introduction

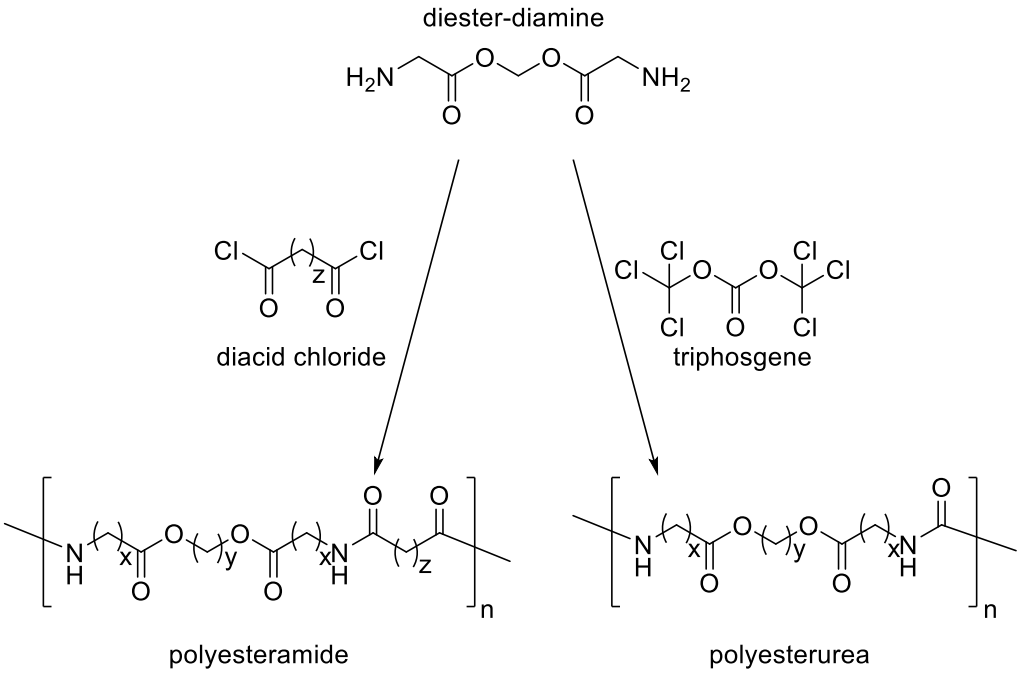
3.1.1.Biodegradability of polymers

There are a wide range of commercially available synthetic biodegradable polymers. Some of the most commonly reported are aliphatic polyesters such as polycaprolactone (PCL), poly(glycolic acid) (PGA) and poly(lactic acid) (PLA). This group of polymers is extensively used in biomaterials because they are readily degraded both hydrolytically and by esterases and lipases, enzymes that are in abundance in the biochemical environment. However, although technically classed as biodegradable, degradation by hydrolysis is very slow with half-lives of some polymers to be almost two years in DPBS.[36] Degradation of PLA in an organism-rich environment such as soil or compost is however shown to be faster[149] but still not fast enough to be considered truly biodegradable with respect to the legislative requirements for polymer microbeads included in rinse-off applications. These OECD 301B test requirements for a degradable material is 60% mineralisation to CO_2 and H_2O within 28 days and therefore these polyesters are not acceptable for use in this cosmetic application. Aside from this slow degradation, the by-products of these materials are also mildly acidic and are known to cause local inflammation when degradation occurs *in vivo*. This formation of acidic side-products does however increase the rate of the acid-catalysed hydrolysis. However, it potentially could be an issue if a similar effect occurs when applied topically to the often-sensitive organ that is human skin.

Incorporation of ester functional groups into the polymer backbone should offer a simple route to biodegradability due to their susceptibility to hydrolysis. However, there are other functional groups which may be integrated into the polymer chain such as amides or ureas which are also able to be hydrolysed but do often require harsher hydrolysis conditions. Polymers which can

be degraded enzymatically are often natural polymers or polymers synthesised from naturally occurring monomers such as amino acids. For example, polyester amides have been designed largely for biomedical applications because they offer good biodegradability, both hydrolytically and enzymatically, and also increased strength in comparison to both polyesters and polyamides alone.[150] This increase in physical strength is due to strong hydrogen bonding interactions that occur between chains. One method of their synthesis is the ring opening polymerisation of morpholine-2,5-dione derivatives[151] but a far wider range of polymer structures can be created by polyaddition techniques from amino acid starting materials.[50]

As well as polyester amides, amino acids have been used in the synthesis of polyester ureas (PEUs), a fairly newly designed range of polymers which also exhibit biodegradability and enhanced strength due to the hydrogen bonding between chains.[152] The Becker group have studied their synthesis from a range of amino acids and their applications in regenerative medicine. For example, phenylalanine based polyester ureas have higher elastic moduli than commercially available polyesters and have therefore shown promise for load bearing applications such as bone scaffolds.[153] Biodegradation studies of these polyester urea films when suspended in buffered saline have shown not only far faster degradation than PLA films but degradation which is tuneable by the diol length. Previous work on these materials also showed no local inflammation on implantation of a PEU device *in vivo*.[154] Polymers synthesised from tyrosine and valine have also been demonstrated by this group for other biomedical applications showing that the method of synthesis translates well to other amino acids.[155,156]



Scheme 3-1. A diester-amine can be used in the synthesis of polyester amides and polyester ureas by reaction with diacid chlorides or triphosgene respectively. This implies that by careful design of the precursor molecule, a huge range of complicated polymer structures can be synthesised.

3.1.2.Interfacial polymerisation

Interfacial polycondensation occurs at the interface between two monomers dissolved in two different phases.[157] A common example is the reaction which occurs in the 'nylon rope trick' experiment where the polymeric nylon which forms at the interface can be drawn out as a string continuously until one of the reagents is exhausted. This technique can be applied in the synthesis of many polymers, as long as the pairs of reagents are difunctional and react readily with each other. When synthesising polyester based copolymers such as polyester ureas and polyester amides, one method is to first synthesise a water-soluble diester-diamine salt by esterification of the amino acid using a diol. This monomer is then polymerised by use of a highly reactive diacid chloride or phosgene which joins segments together forming a urea or amide linkage respectively. Triphosgene, a crystalline, more stable analogue of phosgene is often used and is first dissolved into chloroform to prepare the organic phase. The polymerisation then occurs spontaneously at the interface between the water and organic phases and could, if required, be drawn out as a film in the same way as nylon. Depending on the structure of the

amino acid and length of the diol used in the synthesis of the diester-diamine, the resulting polymer chain often is made up of flexible 'soft' segments and rigid 'hard' segments. Hence, this method of synthesis can be applied to create polymers with a range of mechanical properties.[154]

Manipulation of this chemistry into the formation of particles can be achieved by addition of a suitable stabiliser.[158] Depending on the solubilities of the reagents in the two phases and on the stabiliser used, the microspheres synthesised can be hollow or particulate. In order to produce solid particles, it is important that the oligomers that form at the interface are highly soluble in the droplets formed from stabilisation. For instance, if the stabiliser used produces an oil in water emulsion, it is important that the growing polymer is soluble in the oil phase. This way, the initial droplets become trapped inside the oil droplets and therefore grow larger resulting in solid polymer particles in the aqueous phase (figure 3-1). In comparison to the nylon rope example, this polymerisation will also begin at the interface but this interface is on the surface of the droplets. The stabiliser used therefore will also potentially affect the reactivity of the two reagents as it will also be at the interface. Ideally, the stabiliser will offer separation between the two phases due to its amphiphilic behaviour but will not covalently interact with either phase and can hence be washed away afterwards. The previously synthesised amphiphilic P(BMA-stat-MAA) stabiliser will therefore be used in this section of work. Triphosgene should not be able to react with the BMA groups of the chain, however it could potentially enable the deprotonated methacrylic acid groups to form methacrylic anhydrides, crosslinking the polymer stabiliser and decreasing its solubility in water (scheme 3-2).

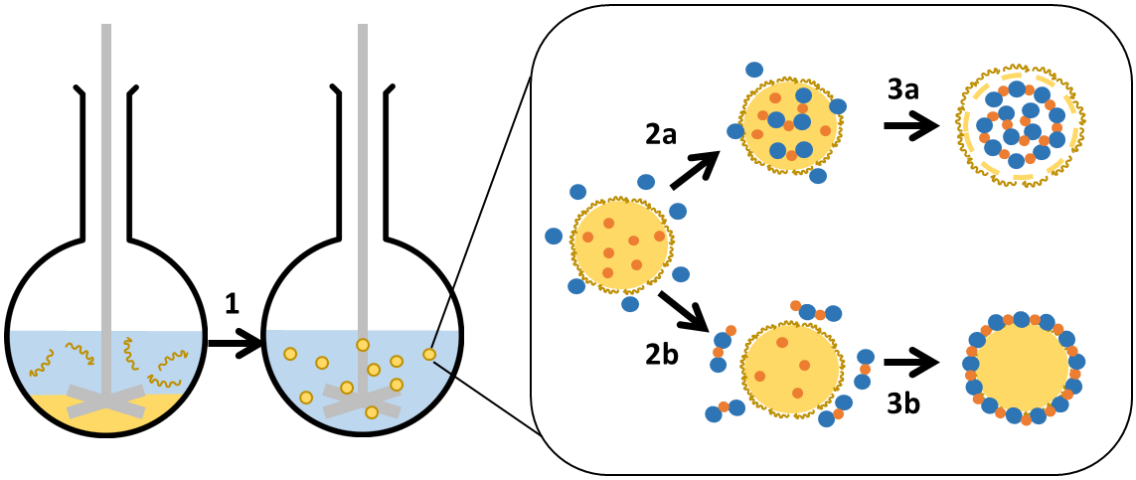
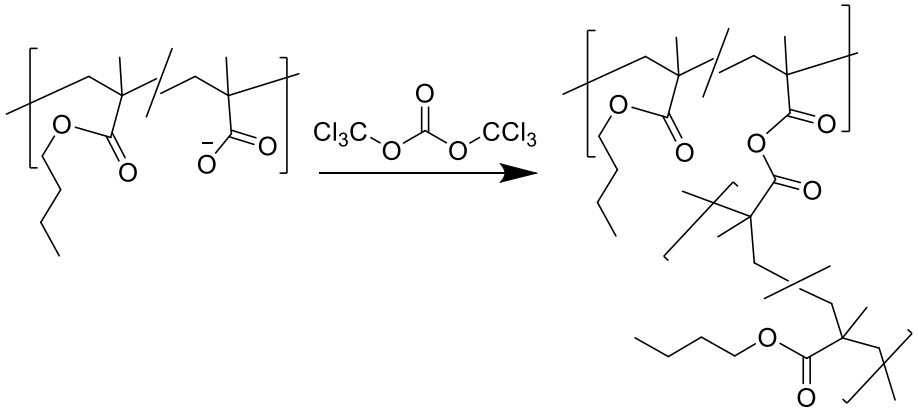


Figure 3-1. 1) Droplets form on stirring the two phases in the presence of a water-soluble stabiliser to produce an oil/water emulsion. 2a) Polymer is soluble in the droplet phase. Oligomers begin to grow within the droplet and become trapped. 3a) Solvent swollen microspheres produce solid polymer particles. 2b) Polymer is insoluble in the droplet phase. Oligomers grow in the continuous phase until they become insoluble and precipitate out. 3b) The polymer is deposited droplet interface producing a membrane and therefore hollow microspheres.



Scheme 3-2. The p(BMA-stat-MAA) stabiliser is first deprotonated in order to solubilise it in the aqueous phase. On reaction with the triphosgene-containing organic phase, any two deprotonated acid groups may react to form an anhydride, cross-linking the polymer chains. This may decrease their solubility and hence effect the polymer's ability to stabilise the droplet formation.

3.1.3.Degradation tests

The degradation of polyester materials is of worldwide interest at present due to the spotlight on plastic recycling and reducing the amount of plastic waste that is present on the planet. Previously reported analysis on the hydrolytic degradation of polyester ureas have been according to the ASTM standard F1635-11 where polymer films are submerged in PBS and measurements of mass lost and polymer molecular weight over time have been used to determine the biodegradability.[153] This test is a specific requirement for materials that are to be

implanted within the body. However, in the environment, even more so in landfill or sewage, waste plastic materials can potentially be attacked by a wide range of enzymes meaning this test would be an inaccurate representation of the materials' biodegradability. In a soil environment there are millions of microorganisms present[149,159] but only specific enzymes will act upon any material as they interact selectively with particular functional groups within a molecule.[33] The biodegradability of PLA in compost and soil environments has previously shown a considerable decrease in molecular weight by GPC after 14 days largely due to hydrolytic cleavage along the polymer backbone caused by a combination of penetrating moisture and high incubation temperatures. On further increase of temperature and on addition of lipase into the matrix the degradation rate increased showing the susceptibility of the PLA backbone to degradation by lipase.[149] One such study which involved the use of lipase in organic solvents to degrade poly(ε-caprolactone) showed that introduction of the enzyme to degrade the polymer had a very different effect to using acid hydrolysis alone. Although degradation did occur, once the solvent was removed the lipase was able to then catalyse the polymerisation of the residual oligomers overall reversing the degradation.[160]

3.1.4.Chapter Aims

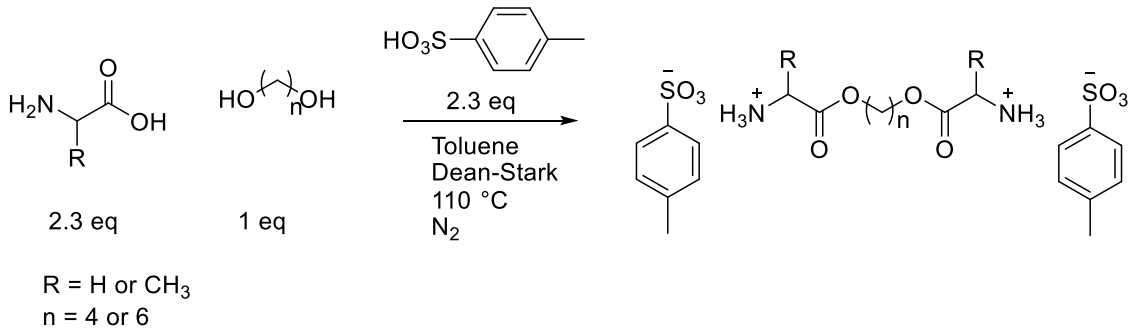
In this chapter, polyester ureas from the amino acids alanine and glycine will be synthesised in the form of particles. As with the previous chapter, the aim here is to optimise the polymerisation procedure by tuning the stabiliser concentration and stirring speed in order to produce spherical polyester urea particles. It is predicted that due to the hydrolysable ester functionality in the polymer backbone, the particles will be more readily degradable than the previously synthesised PVAc particles. These amino acids have been chosen as they are less hydrophobic than and therefore more readily attacked by water than phenylalanine based polyester ureas and hence degrade more rapidly. They should also be the simplest to work with as they don't contain any other acid or amide functionality which could react with the diol or triphosgene to produce unwanted side-products.

Simple degradation tests will be designed that can be applied to a wide range of materials made due to the previously used CO_2 evolution test being expensive and time consuming and requiring a very large amount of material. Instead, the polymers will be dissolved and cast as films to be submerged in DPBS and DPBS with lipase. Degradation products will be analysed by their appearance, SEM and GPC. Finally, the particles synthesised will be tested for their stability in a typical cosmetic formulation. This formulation is outlined by CRODA and consists of an oil in water emulsion at approximately pH 5 with the particles suspended within it. If successfully formulated, simple blurring and pore-filling tests will be carried out by CRODA to examine the effectiveness of the particles as a wrinkle-filling cosmetic additive.

3.2.Results and discussion

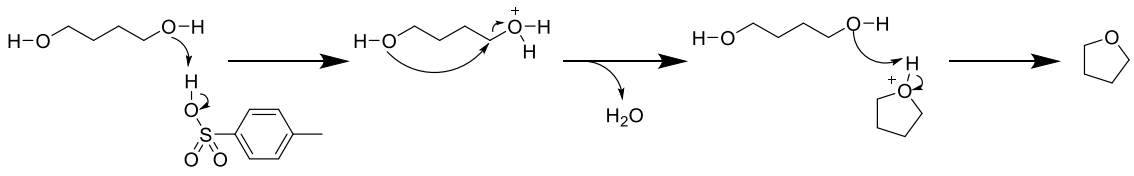
3.2.1.Synthesis of the diaster-diamine starting materials

The synthesis route outlined below (scheme 3-3) is adapted from the previously mentioned phenylalanine PEU and polyester amide work.[153,161] Alanine and glycine were reacted with 1,4-butanediol or 1,6-hexanediol in order to produce four different diester-diamines: ALA-BUT, ALA-HEX, GLY-BUT and GLY-HEX. Toluenesulfonic acid monohydrate was used to both catalyse the esterification and stabilise the end product in the form of a salt. Dean-Stark conditions were required to remove the water from the toluenesulfonic acid monohydrate and any water produced by the esterification. A third amino acid source, CRODA's 'crotein', a mixture of amino acids from keratin proteins, was also investigated in this synthesis. However, due to the crude nature of this starting material and the wide range of amino acids it contains, little control could be had over the selectivity of the diol for the specific carboxylic acid groups in order to produce the diamine-diesters and therefore no product was isolated.



Scheme 3-3. The general reaction scheme for the synthesis of the diester-diamine salts from alanine and glycine with 1,4-butanediol and 1,6-butanediol.

In early attempts, extra water was added into the reagents in order to fully solubilise them, however, it was later found that this simply caused the reaction to reach lower conversions as not all of this water was removed successfully by the Dean-Stark apparatus. Instead, it was observed that the reagents dissolved suitably in toluene at the reflux temperature of 110 °C and on removal of all solvent and cooling to room temperature a solid salt remained. For the glycine-butanediol reaction, no product was recrystallised. This has also been seen by other research groups for this particular salt where instead the toluene sulfonic acid salt of the amino acid is produced without any reaction with the diol.[161] In this case, 1,4-butanediol can cyclise to form the cyclic ether (tetrahydrofuran) which can occur spontaneously in the presence of water and with an acid catalyst meaning both reagents are no longer available to perform the esterification (scheme 3-4). Also, as the water is taken out of the reaction, the equilibrium for the cyclisation is shifted in favour of the cyclic product, therefore if esterification doesn't happen early on it is even less likely to occur once all water has been removed.



Scheme 3-4. The cyclisation of 1,4-butanediol that can occur in the presence of acid preventing the diol from reacting with the amino acid to form the diamine-diester.

Reaction of alanine with 1,4-butanediol was also attempted with successful isolation of a 5% yield of the diamine-diester product after 3 recrystallisations in ethanol. There are few reports of 1,4-butanediol being used in amino acid esterification for PEUs or PEAs and I believe its low yield and poor reactivity is the reason for this. Therefore 1,6-hexanediol was utilised under the same reaction conditions. On recrystallisation from ethanol, both the ALA-HEX and GLY-HEX diamine diester salts precipitated at room temperature to give high yields of the product. A summary of the reaction results is given below. The small increase in diol length should not have an adverse effect on the overall degradability of the product as the reactivity of the ester should be very similar.

Table 3-1. Record of diamine-diester salt syntheses.

Diamine-diester product targeted	Yield	Scaled up?
Glycine-butane-1,4-diester (GLY-BUT)	0%	No
Alanine-butane-1,4-diester (ALA-BUT)	5%	No
Glycine-hexane-1,6-diester (GLY-HEX)	74%	Yes
Alanine-hexane-1,6-diester (ALA-HEX)	87%	Yes

Analysis of the two hexane-based products has been carried out using ATR FTIR (figure 3-2) and ^{1}H NMR (figure 3-3) spectroscopies, melting point analysis and electrospray mass spectrometry. The transmittance spectra show the successful esterification of the starting reagents by introduction of the ester carbonyl stretch at 1800 cm^{-1}. The loss of the alcohol functionality at 3300 cm^{-1} and the carboxylate from the zwitterionic form of the amino acid at 1550 cm^{-1} can also be observed. The ^{1}H NMR spectra for the two salts and the melting point analyses show the products to be of high purity by the integration values and narrow melting point ranges respectively. Both sets of data also agree with analysis from the literature.[152,162] Time-of-flight electron-spray mess spectroscopy was carried out in both negative and positive mode to characterise the salt ions and the respective signals for the negatively charged and positively charged species were seen at high intensity. In the positive-mode electron-spray mass spectroscopy, peaks at 233.2 m/z and 261.2 m/z are seen for the GLY-HEX and ALA-HEX ions

respectively. Thus, successful syntheses were repeated on both a small scale and on a larger scale with analogous results. In conclusion, both hexane-diol-based salts have been successfully synthesised and their analyses have shown the product to be pure enough for use in the polyester urea syntheses.

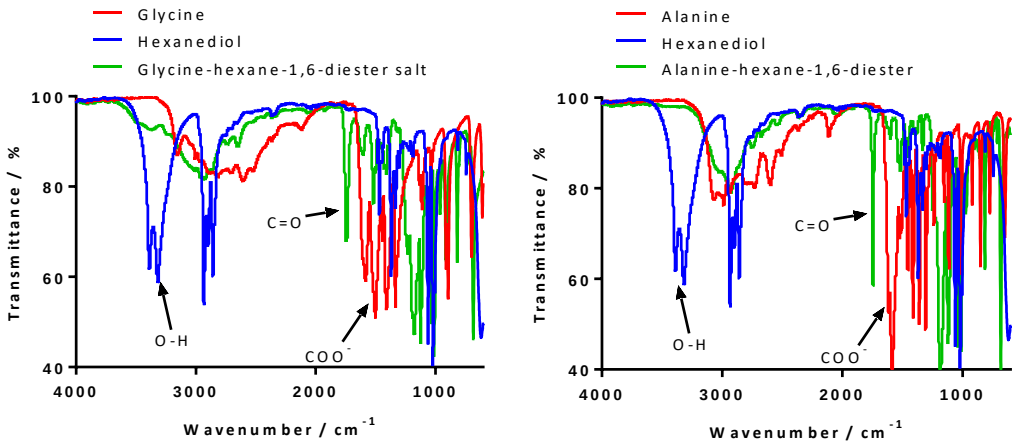


Figure 3-2. IR spectra of the GLY-HEX and ALA-HEX diester salts in comparison to their starting materials.

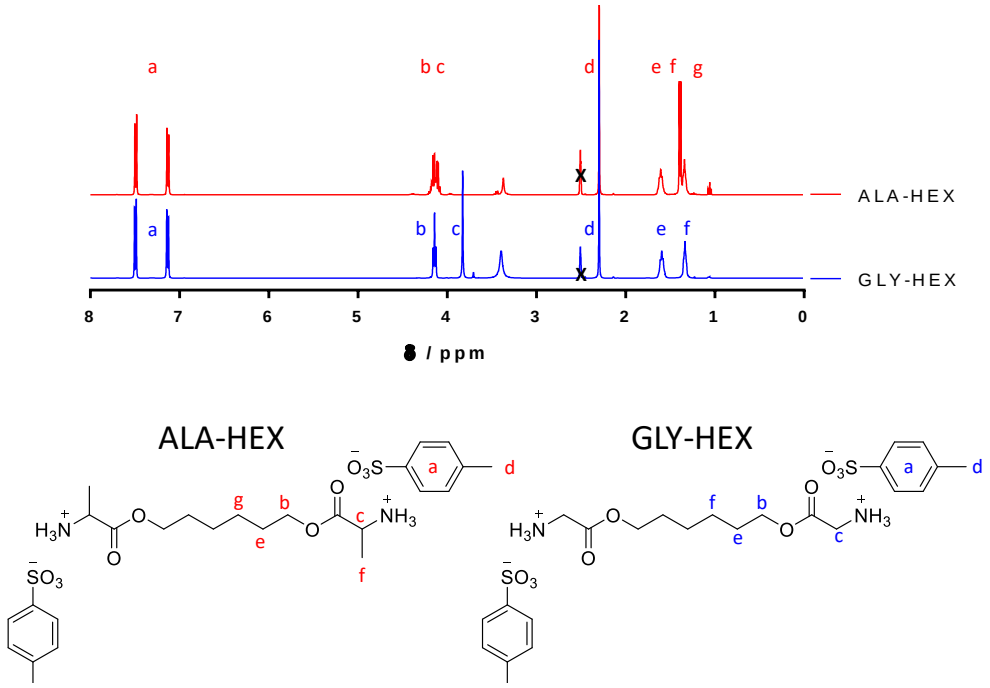
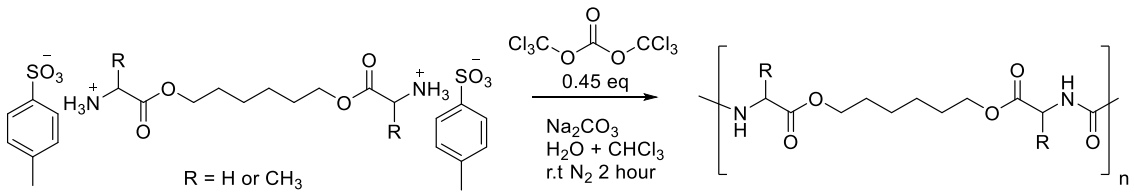


Figure 3-3. Assigned ^{1}H NMR spectra for both the toluenesulfonic acid alanine-hexane-1,6-diester and glycine-hexane-1,6-diester salts in d^{6}-DMSO. The peaks at approximately 2.5 ppm are the residual DMSO solvent peaks. Peaks at around 3.4 ppm are the NH_3 protons.

3.2.2.Interfacial polymerisation of diester-diamine salts with triphosgene to produce polyester ureas

The diester-diamine salts (1 equivalent) were dissolved in water along with sodium carbonate (2.1 equivalents) to neutralise the toluenesulfonic acid and deprotonate the amino groups. Triphosgene was dissolved in chloroform and kept in the freezer until ready to use. Due to the reactivity of triphosgene with water, the aqueous phase was cooled to 0 °C before triphosgene addition. These PEU syntheses without stabiliser were performed on a small scale with magnetic stirring. Due to the spontaneous nature of the interfacial polymerisation, this reaction is carried out at room temperature and reached high conversion after 2 hours. The polymers were isolated by washing with water to remove any salt by-products followed by slow precipitation of the organic phase into hot water (80 – 90 °C) to boil off the chloroform, producing the polymers as a sticky powder. These were then washed again with water and freeze-dried for analysis by GPC, IR (figure 3-4) and NMR (figure 3-5) spectroscopies.



Scheme 3-5. The interfacial polymerisations of both salts are performed under the same reaction conditions.

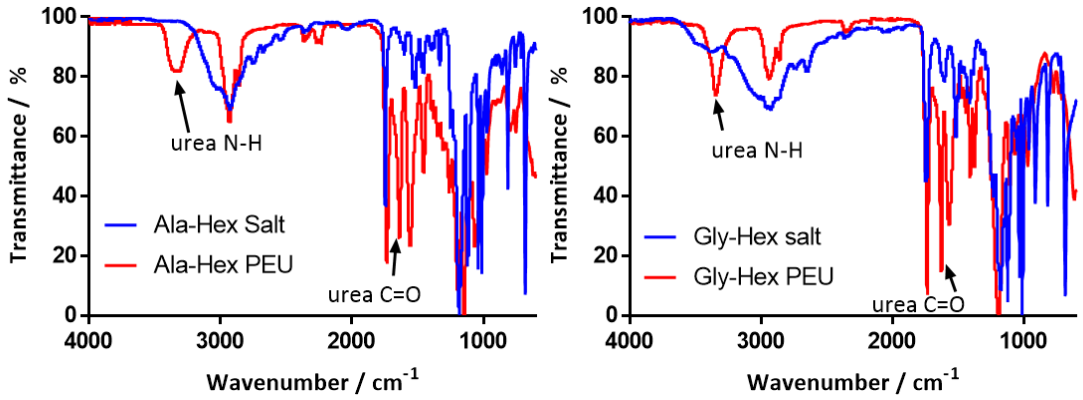


Figure 3-4. IR spectra show the introduction of the new N-H and C=O stretches which occur on polymeriation and formation of the urea bond.

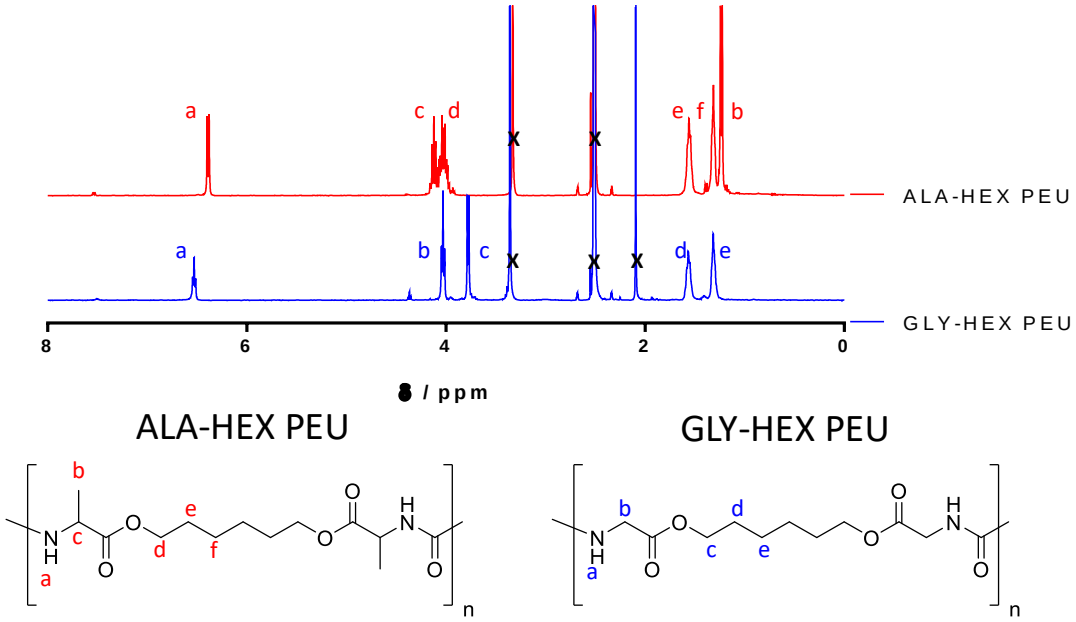


Figure 3-5. Assigned ^{1}H NMR spectra for both the ALA-HEX PEU and GLY-HEX PEU in d^{6}-DMSO. The peaks at approximately 2.5 ppm are the residual DMSO solvent peaks. Disappearance of the toluenesulfonic acid peaks show successful polymerisation.

3.2.3.Synthesis of particles using Span®85 stabiliser

PEU particles from both the ALA-HEX and GLY-HEX starting materials were synthesised by a W/O manipulation of the interfacial polymerisation using a common oil-soluble stabiliser, Span®85 (figure 3-6). There is a large range of span products which are based on sorbitan esters and are frequently used in both personal care and food production.[163,164] The 'natural' oleic acid chain on Span®85 is why this has been chosen over other sorbitan analogues.

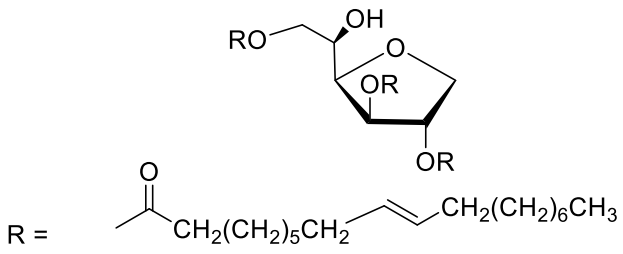


Figure 3-6. Span®85 – a Sorbitan Trioleate used commonly as a water in oil (W/O) emulsion stabiliser.

In order to include it into the polymerisation it was dissolved in a small portion of chloroform and subsequently added to the continuous phase at the same time as triphosgene. An immediate phase change was seen as a white emulsion was formed from the two immiscible

layers. Three different concentrations of Span®85, 0.15, 0.25 and 0.35 w/w% relative to overall reaction mass, were used in order to determine the amount required for sufficient stabilisation of the particles. Initially, a stirring speed of 150 rpm was used to minimise splashing. Attempts at 400 rpm resulted in a large build-up of coagulum around the vessel and the stirrer blade. Reactions were terminated after 2 hours at room temperature and the resulting white solids were washed by centrifugation and refreshing of the supernatant. Wet particles in the form of a paste were then dispersed in water and freeze-dried to give a fine white powder. Analysis by ^{1}H NMR spectroscopy (figure 3-7) implied the polymerisation to be successful by the presence of peaks corresponding to the diester salts without any trace of the stabilising toluenesulfonic acid. There was no evidence of any remaining stabiliser after isolation, but it was used at such small concentration that it would not likely be seen by NMR. Analysis by GPC (DMF eluent, PMMA standards) also shows successful polymerisation, however only relatively low molecular weights of between 4890 and 12000 g mol^{-1} were achieved (figure 3-8). The differing stabiliser concentration had further effect on the molecular weight but the reaction conditions do appear to be readily reproducible. This is implied by the set of molecular weights for each type of polymer (ALA-HEX or GLY-HEX) being very similar considering that the technique of interfacial polymerisation is not very refined.

The particles were examined by optical microscopy as a 0.1 w/w% dispersion in water (figure 3-9). Samples were prepared by sonication to break up any aggregated particles before pipetting onto the glass slide. Even with sonication, it was immediately obvious that the particles were prone to aggregation and that they were not regular in size or shape. Increasing the stabiliser concentration from 0.15 to 0.35 w/w% did not improve this. In all cases, the GLY-HEX particles dispersed more readily in water with smaller aggregates than the ALA-HEX particles presumably because they are less hydrophobic.

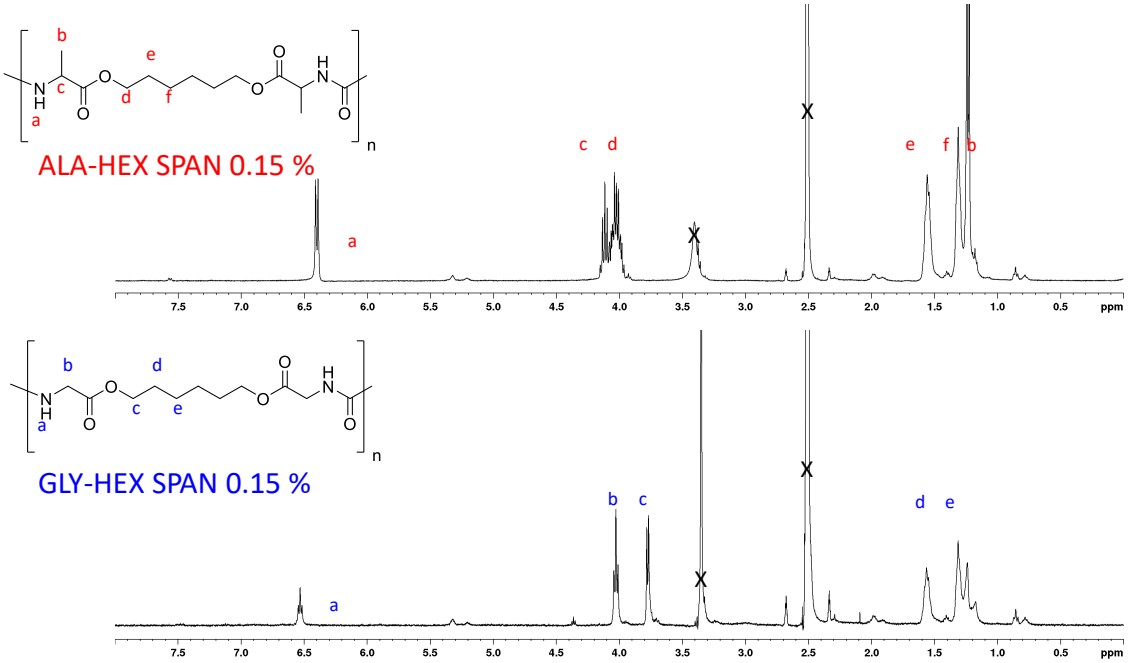


Figure 3-7. The assigned ^{1}H-NMR spectra in d^{6}-DMSO for the particles synthesised using 0.15% of Span®85 stabiliser. The loss of aromatic peaks at 7.0-7.5 ppm shows the successful polymerisation. Peaks at 3.3 ppm are residual water signals and at 2.5 ppm residual DMSO signals.

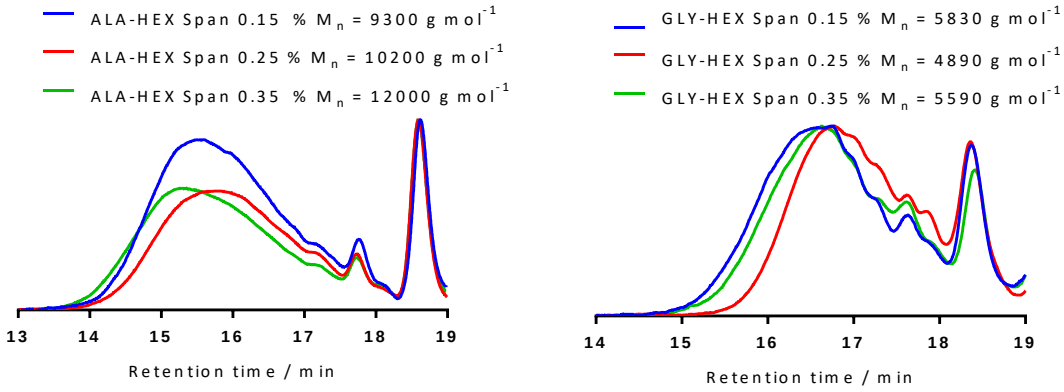


Figure 3-8. GPC chromatograms (DMF 0.1% LiBr). Values calculated relative to PMMA standards. Polymerisations using the same salt have similar molecular weights but the ALA-HEX reactions have reached higher DP.

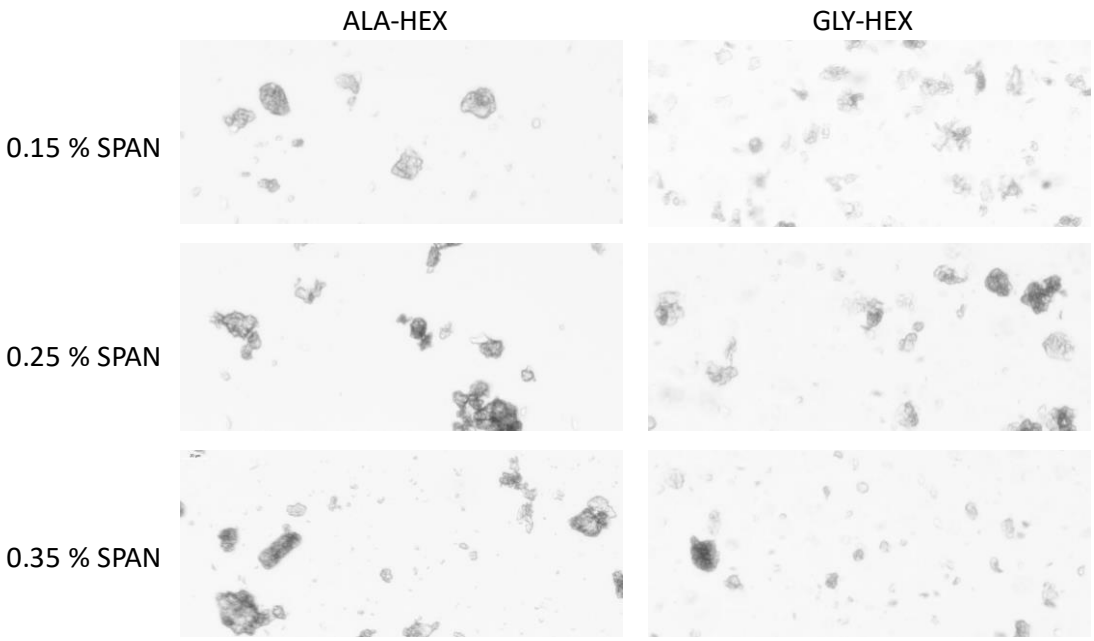


Figure 3-9. Optical microscopy images of Span®85 stabilised ALA-HEX and GLY-HEX PEU particles synthesised at stabiliser concentrations of 0.15, 0.25 and 0.35 w/w%.

*3.2.4.Synthesis of particles using a synthesised P(BMA-*stat*-MAA) stabiliser*

As the stabilisation by an oil-soluble additive was unsuccessful, the P(BMA-*stat*-MAA) synthesised previously was used as a water-soluble stabiliser (see page 21). A 4 or 2 w/w% stabiliser stock solution in water was used throughout all experiments to ensure that the concentration was adequately controlled. These were then diluted to 2 and 1 w/w% respectively when added to the other reagents for the polymerisation. As well as concentration, stirring speed and type of stirring (vortex or homogenisation) was varied to see the effect this had on the particle size and shape.

To begin with, 1 and 2 w/w% stabiliser solution were compared at stirring speeds of 150 rpm and 400 rpm using an anchor-shaped stirrer. Noticeable differences between particulate products occurred due to the increasing of the stirring speed. Little difference in particle size and shape was observed at the increased concentration of stabiliser. At 150 rpm and 2 w/w% stabiliser the particles were most uniform. At 400 rpm, large aggregates were present, likely due to coagulum formed on the stirrer and around the vessel caused by splashing. With

homogenisation at 3000 rpm, particles were more uniform but there is also an interaction seen between particles which is not seen in the other samples. It is possible that, due to the more intensive mixing caused by homogenisation, the stabiliser polymer chains are stabilising multiple particles, hence linking them together (figure 3-10). To compare, homogenisation of the Span®85 stabilised polymerisation was also carried out but this resulted in a thick layer of coagulum with no microparticles formed.

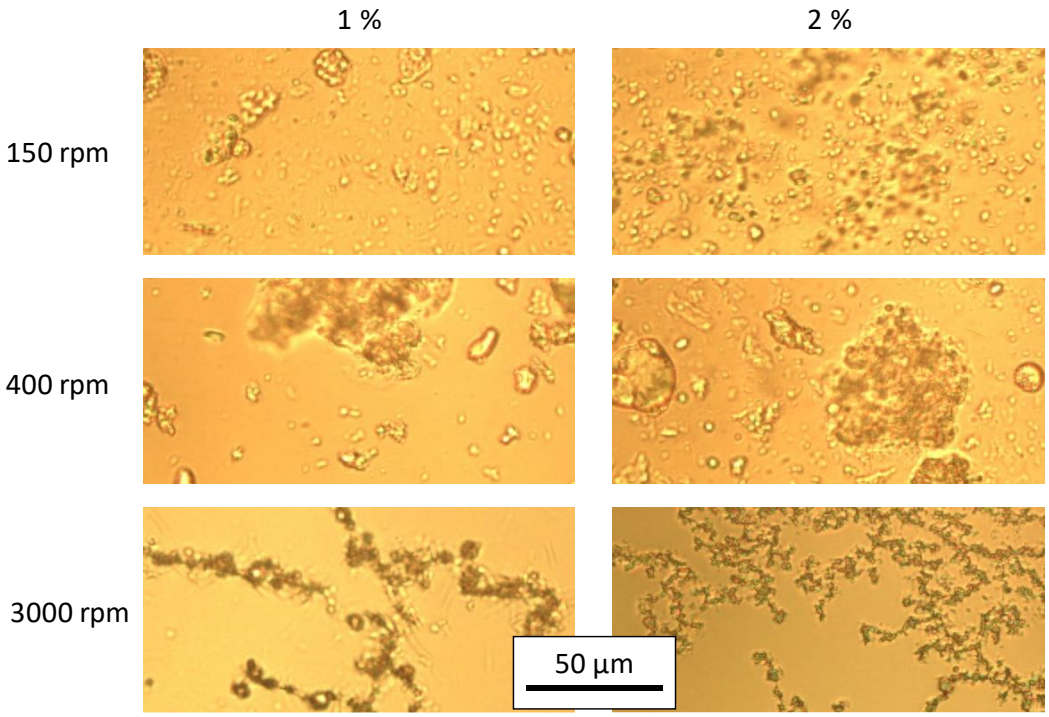


Figure 3-10. Optical microscopy images of P(BMA-*stat*-MAA) stabilised ALA-HEX PEU particles at stabiliser concentrations of 1 and 2 w/w% and at 150 and 400 rpm stirring or 3000 rpm homogenisation.

To investigate these particles further, scanning electron microscopy (SEM) was used. As this technique requires dry material, particles were freeze-dried after multiple washes to ensure they were cleaned of unreacted material. From SEM, it can be seen that the particle structures are not uniform and in fact very few rounded particles were present in each sample. This was seen with all polymers in this series. Analogous reactions using the GLY-HEX starting material

have also been carried out with similar results: an overall lack of uniformity and sphericity of particles (figure 3-11 and figure 3-12).

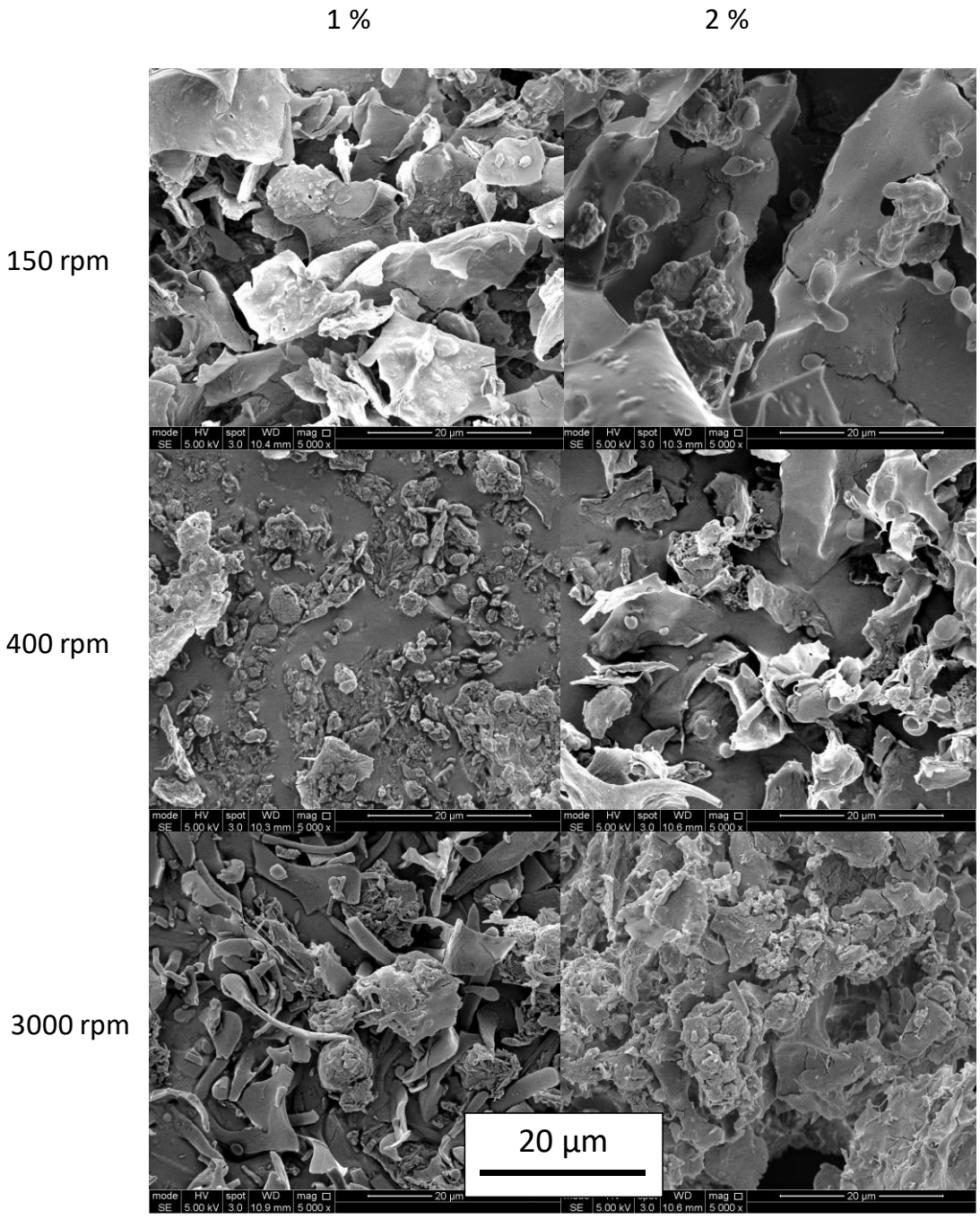


Figure 3-11. SEM images of P(BMA-*stat*-MAA) stabilised ALA-HEX PEU particles at stabiliser concentrations of 1 and 2 w/w% and at 150 and 400 rpm stirring or 3000 rpm homogenisation.

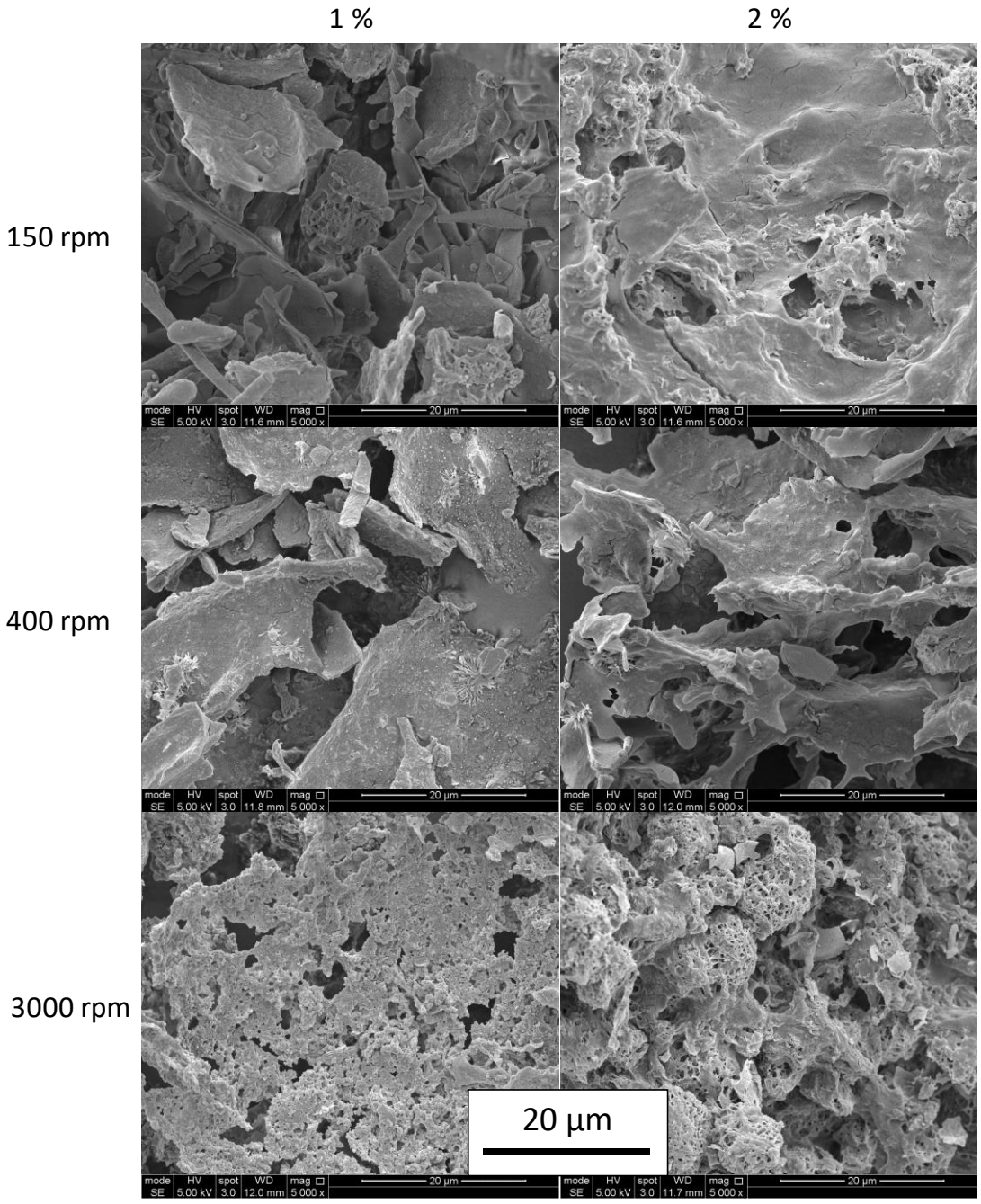


Figure 3-12. SEM images of P(BMA-*stat*-MAA) stabilised GLY-HEX PEU particles at stabiliser concentrations of 1 and 2 w/w% and at 150 and 400 rpm stirring or 3000 rpm homogenisation.

Further magnification of both the 3000 rpm samples of ALA-HEX and GLY-HEX particles by SEM showed what appeared to be nano-sized particles (figure 3-13). This is interesting to note as it implies that the stabiliser has been more efficient as an emulsion stabiliser than as a suspension stabiliser. For this to have occurred it means that the stabiliser has formed micelles, likely surrounding the triphosgene-in-chloroform droplets, and the dissolved salt has fed into these

micelles. These small particles were not seen in samples that were stirred with the anchor stirrer and they are therefore probably caused by the homogenisation process, which has allowed the formation of smaller, more uniform droplets of the oil phase and hence triggered the emulsion polymerisation.

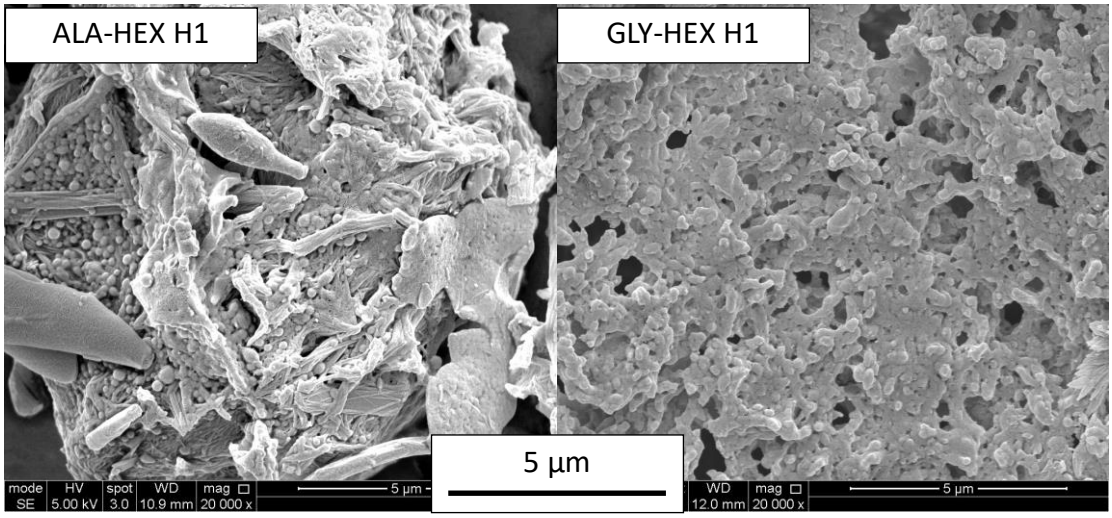


Figure 3-13. SEM images of ALA-HEX and GLY-HEX particles synthesised by stabilised with 1 w/w % P(BMA-*stat*-MAA) under homogenisation at 3000 rpm. In both cases, the polymer fragments are made up of nanosized spherical particles linked together.

3.2.5.Modification of PEUs to improve solubility for analysis

So far, most of the polymer analysis has been by microscopy where particles are dispersed in water and no dissolution is required. NMR analysis has been achieved by using a mixed solvent system of d^6-DMSO and d^4-Methanol. This mixed solvent system was required as the particles that are stabilised with the copolymeric stabiliser have a complex solubility due to having a more complex structure. Where DMF has been suitable for GPC analysis of the unstabilised polymer and the Span 85® stabilised particles, it causes these copolymer stabilised particles to swell, even when heating to boiling point. Therefore, two different approaches have been taken in order to improve the solubility of the particles for GPC analysis. The first is an alkylation reaction (described in the previous chapter) to introduce benzyl functionality onto any methacrylic acid groups of the P(BMA-*stat*-MAA) stabiliser. This should ultimately make the particles less polar and hopefully improve their solubility non-polar solvents. Because the polyester urea is soluble

in DMF for GPC when not synthesised in the form of particles, it was predicted that the stabiliser was the cause of the solubility issues. However, unlike in the alkylation reactions of the stabiliser alone (see page 22), these particle reactions did not become homogeneous in DMF and hence no alkylated polymer was retrieved after 24 h of reaction.

Another method was attempted, derived from work on improving the solubility of polyamides and polyester amides where modification of these polymers by trifluoroacetylation of the NH group and has been used to enable analysis by GPC.[165] Further to this, polyurethanes have been modified in the same way but are found to require longer reaction times and higher temperatures for the trifluoroacetylation to reach full conversion.[166] It was, therefore, predicted that this method would be appropriate for these polyester ureas. Initially, the non-stabilised GLY-HEX PEU was chosen as this was insoluble in DMF for GPC despite the ALA-HEX PEU being soluble. After 24 h at room temperature this mixture was homogeneous and, on removal of solvent and excess trifluoroacetic anhydride, the sample dissolved in DMF but not sufficiently to pass through the PTFE syringe filter to allow the analysis. A sample of GLY-HEX PEU which was stabilised with the P(BMA-*stat*-MAA) stabiliser was also trifluoroacetylated in the same way but its solubility in THF or DMF did not improve. This is further evidence to suggest that it is perhaps the stabiliser that is causing the insolubility of the polymers.

Overall, GPC analysis of the P(BMA-*stat*-MAA)-stabilised particles has therefore not been carried out as they would not sufficiently dissolve in any common GPC eluent. A wide range of mixed solvent systems have been tested with all failing to be soluble enough to pass through a syringe filter. As the Span®85 stabilised particles were able to dissolve in DMF, this insolubility is therefore indication that the copolymer stabiliser has chemically reacted with the PEU and is preventing it from dissolving. This could be in the form of cross-links by the formation of an anhydride group between the methacrylic acid of the stabiliser and the ester groups of the PEU.

This would also explain why the individual spherical nanoparticles seem to be joined together when dried and imaged by SEM.

Evidence for the grafting or potential cross-linking of the polymeric stabiliser onto the PEU can be seen from ^{1}H NMR spectroscopy of washed and dried samples (figure 3-14). A mixed solvent system of 1:3 ratio of d^{4}-Methanol and d^{6}-DMSO was required in order to fully solubilise the polymer particles, whereas for the Span® stabilised particles and the PEU without stabilisation, DMSO alone was sufficient to solubilise the polymer. This was the case for all ALA-HEX and GLY-HEX particles stabilised with P(BMA-*stat*-MAA).

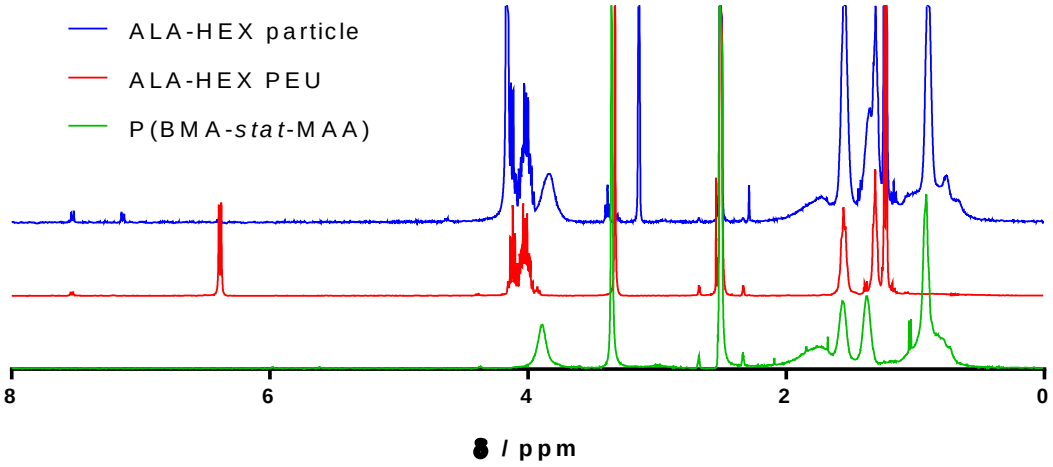


Figure 3-14. ^{1}H-NMR spectra in d^{4}-Methanol and d^{6}-DMSO 1:3 for the particles synthesised using the P(BMA-*stat*-MAA) stabiliser.

3.2.6.Synthesis of mixed PEU from alanine and glycine diester salts

The main reason for choosing these particular two amino acids, glycine and alanine, stems from PLGA studies where the composition, specifically the proportion of each of the lactide (L) and glycolide (G) units, determines the overall polymer degradability (see figure 3-15).[38] This is because the polymer degradation rate in water or DPBS can be attributed to the hydrophilicity, and hence, higher hydrophilic content (or higher glycolide content in the case of PLGA) means more favourable interactions with water and therefore faster degradation. To compare this to PEU degradation, mixed PEUs have been synthesised by mixing different proportions of the

diester salts before polymerisation. From the PLGA work, it was predicted that the larger proportion of glycine would cause a faster degradation in an aqueous environment due to it being less hydrophobic than alanine. Three mixed compositions have been targeted at ratios of 1:3, 1:1 and 3:1 alanine to glycine by mass. Reactions were carried out using the homogenisation protocol and at a total of 2 w/w% P(BMA-stat-MAA) stabiliser. SEM of each of the dried polymers showed similar results to the wholly alanine and glycine based PEU particles, where a defined microparticle structure is not seen (figure 3-16). From NMR spectroscopy it can be concluded that the proportion of the ALA-HEX salt included in each mixed polymer corresponds to the initial reaction concentrations, however this is difficult to analyse quantitively due to overlap of peaks. The key information from NMR spectra is the increase in intensity of the peak at 1.5 ppm which corresponds to the extra methyl group on the ALA-HEX salt only (figure 3-17).

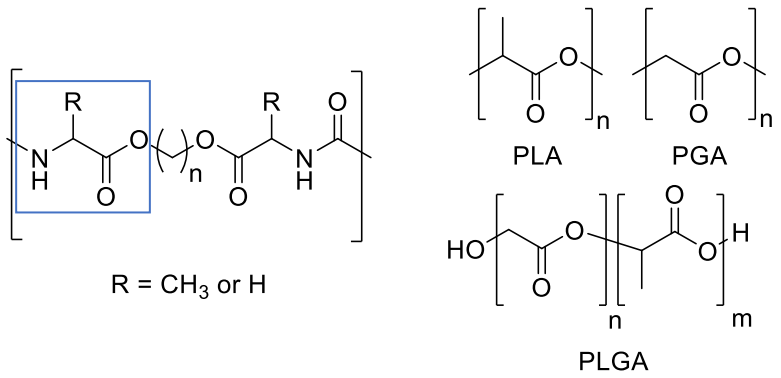


Figure 3-15. Mixed PEUs from both alanine and glycine can be synthesised analogous to the synthesis of PLGA from lactide and glycolide units.

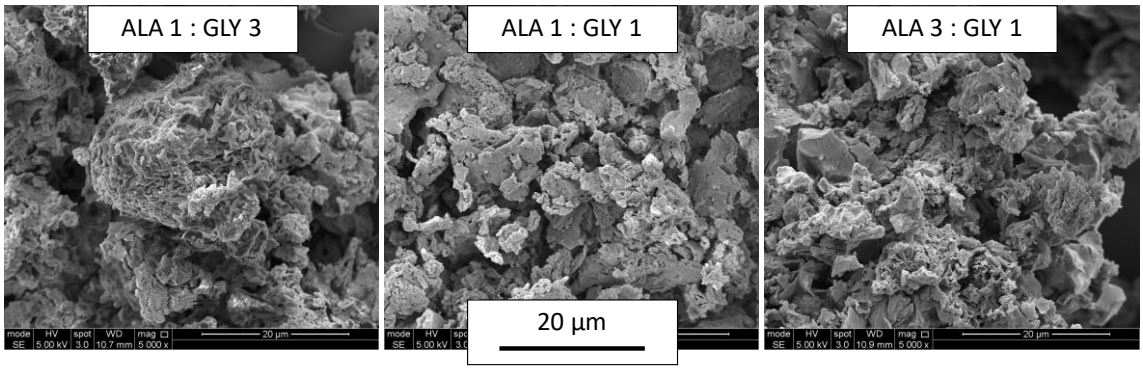


Figure 3-16. SEM images of the mixed PEUs.

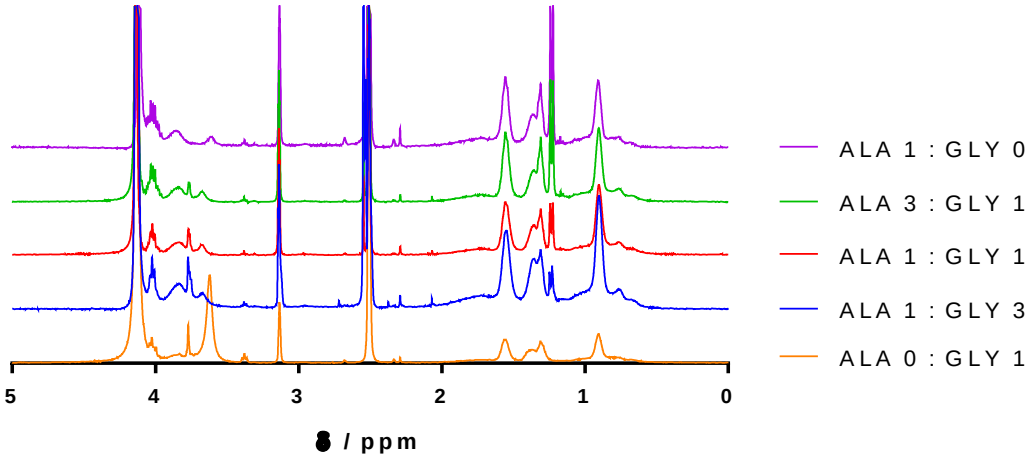


Figure 3-17. ^{1}H NMR spectra in d^{4}-Methanol and d^{6}-DMSO 1:3 for the particles synthesised using the P(BMA-*stat*-MAA) stabiliser and different ratios of the ALA-HEX and GLY-HEX salts. Stacking is in order of increasing ALA-HEX and decreasing GLY-HEX.

3.2.7.Degradation of PEU particles in lipase/DPBS solution

Degradation studies were carried out on 5 samples of P(BMA-*stat*-MAA) PEU particles. A pure ALA-HEX and a pure GLY-HEX sample as well as samples of the three mixed PEUs were tested by suspending in a 0.1% Lipase CalB solution in DPBS and incubated at 37 °C for up to 8 weeks. This specific enzyme was selected as it has previously been shown to be an effective catalyst for the degradation of polyesters.[167] After 2, 4- and 8-weeks samples were washed and dried by centrifugation before analysis by SEM and NMR spectroscopy. Ideally, GPC would have been used to follow any degradation of the polymer by molecular weight analysis, but due to the solubility problems this was not achievable. After 8 weeks, there was no change in the NMR spectra for the particles and SEM showed very little change to the particle structure in all cases. On further magnification of the ALA-HEX PEU sample, there was some visible etching of the polymer surface but no actual break down of the particles (figure 3-18). This further supports the previous PVAc work where even a known biodegradable polymer can be stable to degradation when in particulate form. It is likely in this case that due to the hydrophobic nature of the polymer particles, the water and therefore lipase was unable to reach the core of the particle and was only able to interact very slowly with the surface. A much longer study could be carried out to investigate the total degradation of the particles, however for this application

it is required that degradation occurs over 28 days and therefore the particles here have failed this particular test.

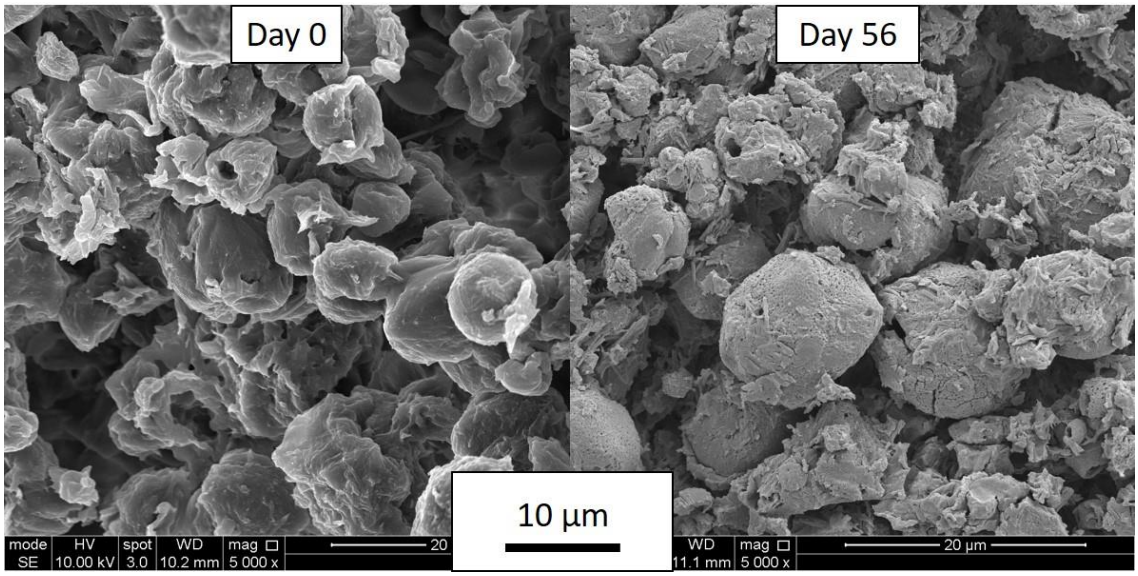


Figure 3-18. SEM comparison of an ALA-HEX PEU sample before and after degradation in 0.1% lipase solution.

3.2.8.Cosmetic application testing of PEU particles

In order for formulation testing of these particles at CRODA where the smallest scale formulation is typically 100 g and actives are usually included at 5 or 10 w/w%, a scale up was required. Due to the simple synthesis method this was easily achieved and from 15 g targeted of solid particles approximately 13 g was produced with some wasted as coagulum due to splashing. The reaction was carried out for 2 hours longer than when carried out on a smaller scale to ensure that all reagents were thoroughly mixed and had chance to interact. Once centrifuged and freeze-dried, SEM was used to compare the particle structure from the small- and large-scale reactions (figure 3-19). No obvious change in particle structure is observed on scaling up the polymerisation which would imply that this process would be potentially suitable for carrying out on even larger scales if required.

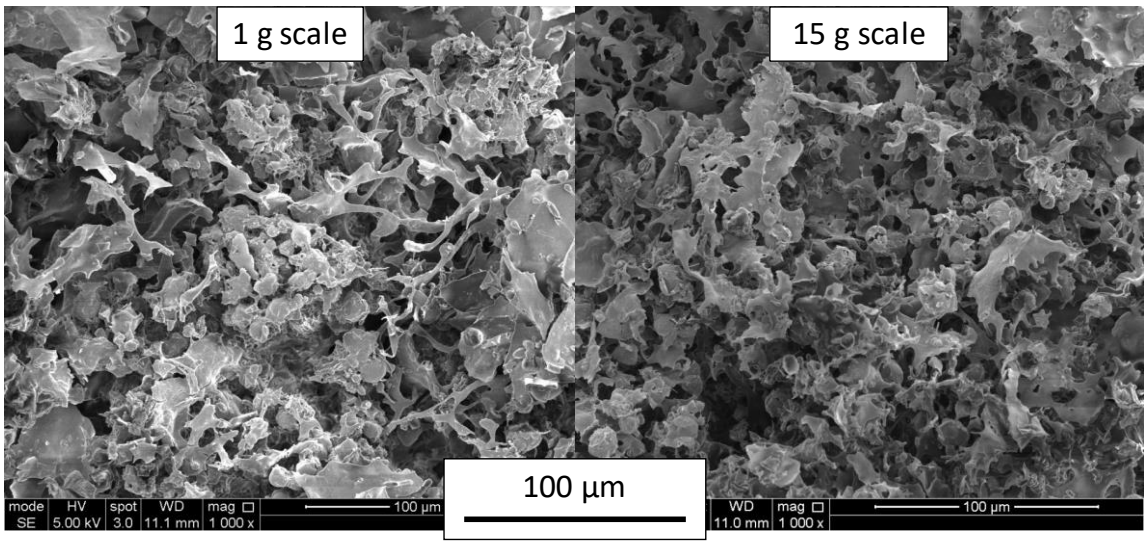


Figure 3-19. SEM images of ALA-HEX PBMMA 2% produced on a 1 g scale and on a 15 g scale.

The dried particles were added into a simple CRODA formulation at 5 and 10 w/w%. The formulation consisted of an oil in water emulsion using Crodafos™ CES, a mixture of cetearyl alcohol, dicetyl phosphate and ceteth-10 phosphate as the emulsifier and mineral oil as the oil phase. Due to the water immiscibility of the particles, they were added in after the emulsion had been produced. The light and fluffy nature of the particle powder meant that addition needed to be slow with vigorous stirring and the resulting formulation was a viscous, opaque paste. This paste thickened further on addition of sodium hydroxide to increase the pH to an appropriate level for a topical cosmetic, any value between 5 and 7. This viscosity increase could be explained by increasing particle distances caused by deprotonation of the methacrylic acid groups of the stabilising copolymer.

The paste was then applied to two test methods designed by CRODA and commonly used to test their own materials as well as commercial benchmarks. An analogous formulation without any PEU material added was tested alongside as a control. The first test is to qualitatively assess the blurring ability of the particles. An even layer is applied onto a textured acetate sheet and allowed to dry. This is then placed over a page of printed text consisting of evenly spaced ‘C’ and ‘O’ at different sizes. A positive result from this test is a sample which causes the letters to appear the same, therefore blurring the ‘C’s enough to make them appear as ‘O’s. In this case,

the 10% formulation showed an increase in blurring in comparison to the control and this was determined to be a positive result showing that the particles do appear to have a blurring effect (figure 3-20a).

The second test method is the pore-filling test. A flexible silicone casting material is applied to sand paper and allowed to dry. On removal of the cast, a reverse surface is produced consisting of a random array of pores of a range of shapes and sizes. Different gauge sandpaper can be used to produce different sized pores. The gauge selected for this test best represents the size of the facial pores and wrinkles which are visible to the naked eye. The formulation paste is then spread evenly over the silicone cast and allowed to dry. The control sample shows no filling of the pores and simply forms a light film across the surface. The sample including the PEU particles filled the pores effectively again giving a strong positive result (figure 3-20b). Overall, the PEU material performed well in the formulation testing and would be useful as a potential cosmetic material.

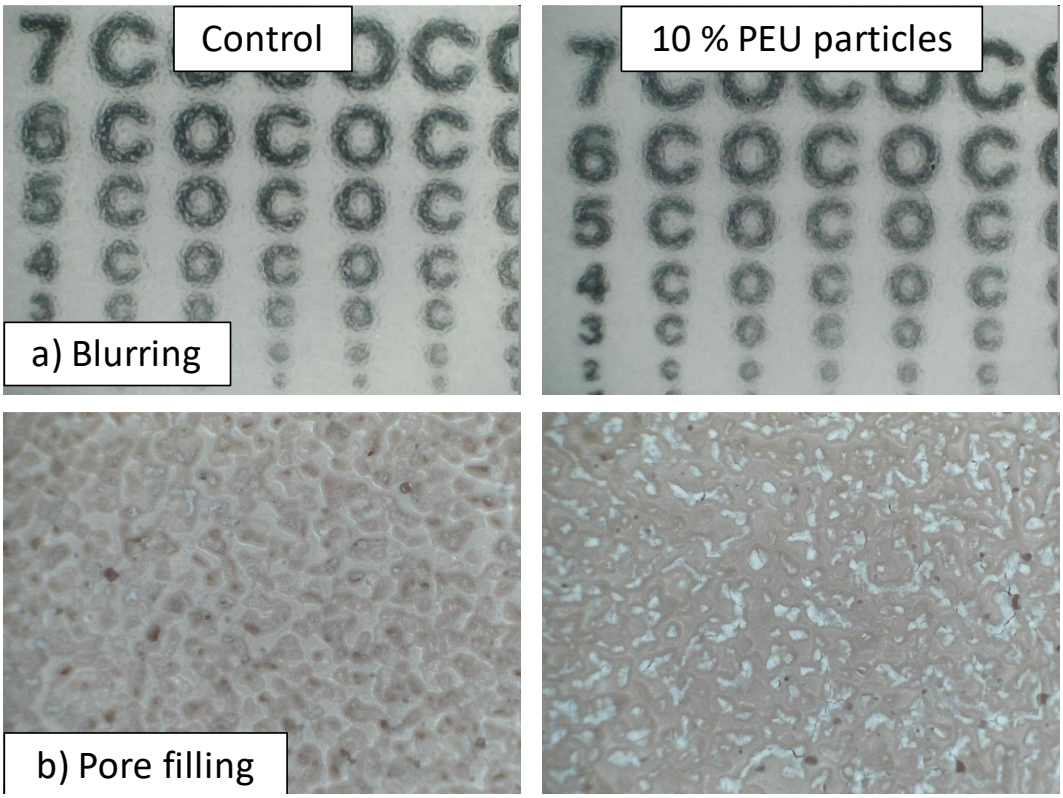


Figure 3-20. Two simple tests have been carried out on a 2% P(BMA-*stat*-MAA) stabilised ALA-HEX PEU sample synthesised on a large-scale using homogenisation.

3.3.Conclusions

Previously, PEUs from a range of amino acids have been designed as biodegradable materials for use *in vivo* where they have shown faster degradation rates than aliphatic polyesters and don't cause localised inflammation.[153–155,168] In this work, the amino acids alanine and glycine have been used successfully in the synthesis of PEUs for *in vitro* uses. Interfacial polymerisation with triphosgene successfully produced polymers with the alanine-based salt reacting more readily and producing higher molecular weight materials than the glycine-based salt. NMR and IR spectroscopies have shown that the polymerisation is successful due to removal of the toluenesulfonic acid and introduction of the urea functionality respectively.

The polymerisation can be manipulated to make irregularly shaped particles by introduction of a stabilising species such as Span® 85 or P(BMA-*stat*-MAA) copolymer as shown by microscopy. The molecular weight distributions of these polymers have not been determined due to poor solubility. Use of the water-soluble stabiliser produces less coagulum and was investigated further using homogenisation. SEM showed that all samples consisted of irregularly shaped particles with rough surfaces, however, those synthesised using homogenisation have an interesting bimodal particle size distribution implying that both emulsion and suspension polymerisation have taken place. NMR spectroscopy shows evidence of grafting of the copolymer stabiliser to the PEU and the poor solubility of the stabilised PEUs also implies this with a possibility that the stabiliser has crosslinked between PEU chains on reaction with triphosgene. This cross-linking between particles is also evident from SEM as material can be seen coating and linking the particles together. The cross-linking polymers would not be expected to be degradable giving a possible explanation for why degradation of these particles is slow in comparison to PEUs from the literature. In order to test this further, the interfacial polymerisation conditions could be changed to use a reagent that would be less reactive with the methacrylic acid group of the stabilising polymer. Alternatively, a different stabilising

polymer could be synthesised which contains a different water-soluble group instead of the methacrylic acid such as an methacrylamide or ethylene glycol units.

Although the most appropriately sized particles synthesised in this work were not biodegradable by the lipase degradation test, they did perform well in the simple applications tests. The two particle size distributions might be the reason for this, as the larger particles will successfully fill in large pores with the nanoparticles adding to the smoothing effect by filling in smaller gaps. From this work, further study into the polymer structure to increase its hydrophilicity and hence its interaction with water should be carried out by, for example, use of a more water-soluble stabilising polymer. If synthesised by a similar method using the homogenisation, this should lead to particles which perform similarly as a cosmetic ingredient but also have enhanced degradation rates in comparison to these PEUs assuming no cross-linking occurs between particles as has been shown here. Ultimately, this chapter has shown that PEUs from amino acids can be manipulated into particle-like structures but that their biodegradability rates are not sufficient to pass the strict testing required for use in rinse-off cosmetics. Due to legislation prohibiting the use of polymer microbeads in cosmetics, a different method of hiding pores and wrinkles must be explored.

Chapter 4: Synthesis and degradability of polyethyl glyoxylate, a self-immolative polymer and the improvement of its film properties by copolymerisation

4.1 Introduction

4.1.1.Biodegradability legislation

As previously described, there are a wide range of polymers that are classed as biodegradable. However, the recent government legislation which came into force in January 2018 has banned completely the manufacture of rinse-off products containing any form of polymer microbeads. This extends to biodegradable polymer particles as well as conventional polyethylene-based microbeads.[169] Therefore, there is currently a rising demand that the cosmetics industry focuses on the production of alternative materials which will have less environmental impact. The use of polymer films in cosmetics has not been condemned in the same way as microbeads so there is scope for the development of biodegradable materials with film-forming properties. The hydrolytically biodegradable polymers synthesised in this project up to now have failed in the biodegradability tests that have been performed. It was concluded that, although the polymers have the chemical capability to degrade, the dense particle structure prevents this from occurring at a practical rate. There is potential that a polymer film of the same material will also be resistant to degradation, however there is currently little evidence in the literature of direct comparisons between microparticle and film degradation rates.

4.1.2.Self-immolative polymers

An alternative to the well-known biodegradable polymers which have degradable backbones are polymers which can spontaneously fall apart on exposure to an external stimulus (figure 4-1). These fall into two main categories, depolymerisable polymers and self-immolative polymers.[170] The former refers to polymers which will respond to a trigger to break down

monomer by monomer into its constituent units, whereas the latter follows a similar process but is specific to polymers which afford molecules that are different to the monomer.[171,172] These molecules are usually analogous to the thermal degradation products of the monomer itself. In terms of ultimate biodegradability (as in the OECD 301 b 'Modified Sturm Test') self-immolative polymers are most appropriate as they yield small molecular fragments which can then easily metabolised to CO_2 and water. Therefore, with intelligent design and tailoring of properties these materials may be appropriate for the cosmetic application.

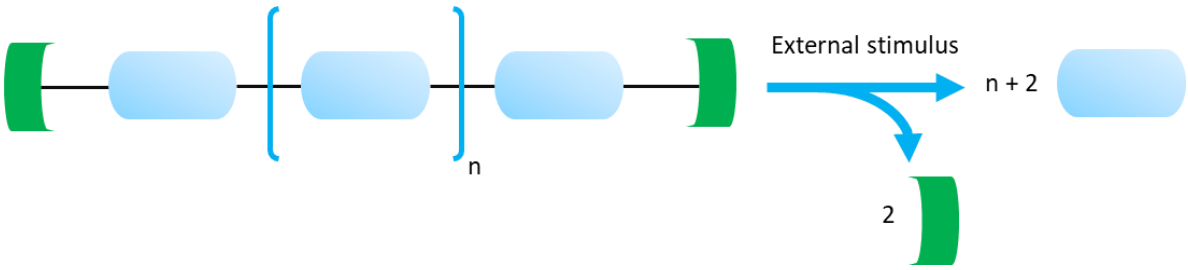


Figure 4-1. Representation of the breaking down of a depolymerisable polymer. An external stimulus triggers the depolymerisation by cleavage of an end-group

The backbone 'unzipping' process is the reversal of polymer propagation and this is only possible due to a low polymer ceiling temperature (T_c). The ceiling temperature is the temperature at which the monomer and polymer are in dynamic equilibrium (figure 4-2). In terms of thermodynamics, at this temperature the overall free energy of propagation is zero. Hence, above this temperature, the reverse 'depropagation' reaction is favoured due to increased entropy. For the majority of polymers, spontaneous depolymerisation is not observed because their T_c is greater than the degradation temperature required to permanently cleave the polymer backbone. This implies that a low ceiling temperature polymer will be susceptible to spontaneous depolymerisation in standard atmospheric conditions without the need for chemical degradation.[173]

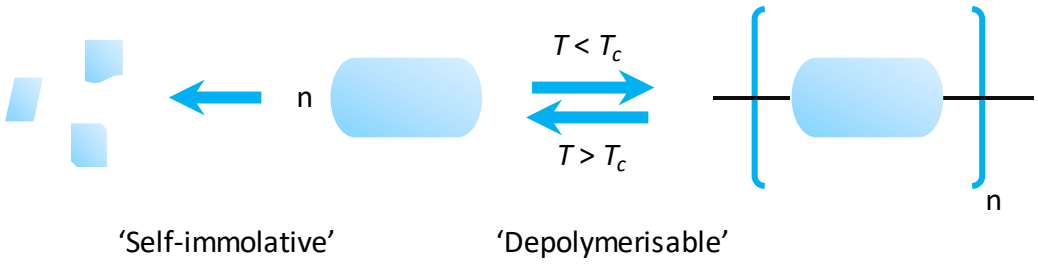
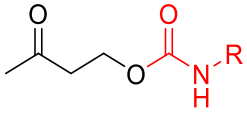
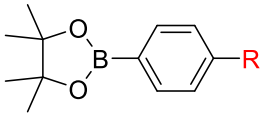
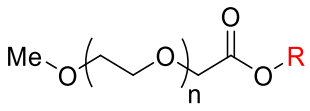
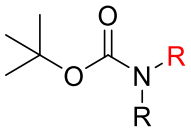
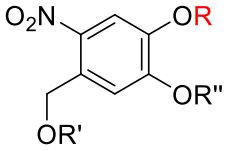


Figure 4-2. Depolymerisation occurs when the temperature of a system is higher than the polymer ceiling temperature. Self-immolative behaviour describes the scenario when the individual monomer units break down further into smaller fragments.

In most reported cases of depolymerisation, an end-capping species is added into the polymerisation to kinetically stabilise the polymer and prevent its spontaneous depolymerisation. Without this end-capper, a chain reaction readily occurs where the monomer units are removed in succession eventually resulting in total depolymerisation. It is possible to design and attach end cappers which respond to a specific external stimulus allowing selective depolymerisation (table 4-1). For example, a polymer end-capped with 4-hydroxy-2-butanone produced a stable carbamate linkage that was removed by a β-elimination reaction with bovine serum albumin (BSA). Once removed, the polymer chain broke down to release a fluorescent amine, as observed by an increase in fluorescence emission using a spectrophotometer. Hence, low T_c polymers can be exploited for a wide range of applications such as targeted drug delivery and smart coatings by choosing an appropriate end capper.[174]

Table 4-1. An example of end-capping molecules (black) used in the synthesis of self-immolative polymers, designed to respond to specific stimuli.

End capper	External stimulus	Reference
	Enzymatic (BSA)	175
	Redox (peroxide)	176

	Nucleophile (water)	[177]
	Acid/base (piperidine)	[178]
	Photo (UV radiation)	[179]

4.1.3.Polyglyoxylates

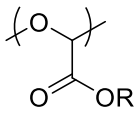
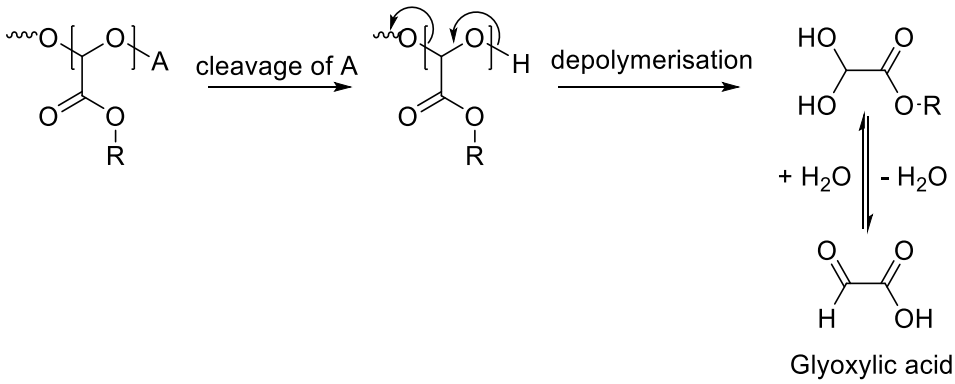


Figure 4-3. The general structure of a polyglyoxylate

Self-immolative linear polymers are a fairly recent technology first patented in 2007.[175] Nevertheless, the low ceiling temperature phenomenon of a polymer has previously been exploited long before this with glyoxylate-based polymers simply for their 'biodegradability'. Polyglyoxylates are a group of polyacetals which can be synthesised from readily available starting materials and degrade to generally benign products. For instance, one intermediate product is glyoxylic acid,[180,181] a naturally occurring molecule which plays an important role in many biological processes. Because of this, they have been manipulated into materials suitable for agrochemical and pharmaceutical industries where such safe degradation products are vital.[182] However, the complete mineralisation of sodium polyglyoxylate is shown to be fastest in mildly acidic conditions,[183] which when considering most cosmetic products are at a pH between 4 and 5.5 means that any glyoxylate-based material would only be suitable in formulation if sufficiently stabilised.

On the other hand, a polyglyoxylate which is stable in formulation and on the skin's surface but will decompose on interaction with enzymes in an aquatic environment offers a simple solution to the current problem. There are multiple routes which could be exploited to enable this to occur in a natural environment from simple functional groups such as readily hydrolysable esters to more complex carbohydrate molecules which bacteria will metabolise. Once rinsed-off, these polymers will be broken down further by the cleavage of the end group and then unzipping of the backbone and finally complete mineralisation of the monomer units to CO_2 and H_2O (scheme 4-1).



Scheme 4-1. The degradation of a polyglyoxylate to glyoxylic acid occurs via the depolymerisation followed by hydrolysis in mildly acidic conditions.[181]

Previously, Gillies and co-workers have synthesised glyoxylate polymers end-capped with phenyl isocyanate. Their work focussed on the simplicity of the syntheses of these materials in comparison to the multi-step syntheses and the potentially toxic degradation products of other self-immolative polymers and dendrimers.[184] Ethyl glyoxylate is the most readily available of the glyoxylate-based monomers and is usually supplied at a low cost in the form of a 50% toluene solution. Methyl, butyl and benzyl derivatives have been synthesised and polymerised as homopolymers and copolymers with glass transition temperatures (T_g) ranging from -30 °C to 25 °C (Table 4-2).[184] When capped with a UV-responsive trigger and exposed to UV radiation, all of the glyoxylate polymers were all able to degrade fully within 10 days. Non-irradiated samples did not undergo any degradation but remained stable. It's useful to know that the degradation

characteristics of the glyoxylate family are similar because it gives more scope to change the polymer structure to target specific film properties, in particular to target a T_g which will produce the best film on human skin.

Table 4-2. The glass transition temperatures of common polyglyoxylates.

Polyglyoxylate	T_g
Poly(ethyl glyoxylate)	-32 °C
Poly(butyl glyoxylate)	-30 °C
Poly(benzyl glyoxylate)	12 °C
Poly(methyl glyoxylate)	25 °C

For an ideal cosmetic film former, the T_g needs to be above a standard storage temperature but slightly lower than the temperature of the skin's surface (figure 4-4). Therefore, these polyglyoxylate homopolymers will not be appropriate for use in cosmetic formulation without modification to increase their T_g. Poly(methyl glyoxylate) would potentially be the easiest T_g to manipulate as it is closest to physiological temperatures, however the monomer itself requires many steps to synthesise as opposed to commercially available ethyl glyoxylate. Another reason for the use of poly(ethyl glyoxylate) (PEtG) rather than its methyl analogue is that ethanol as an intermediate degradation product is safer than methanol, especially if the polymer has any potential to degrade when in contact with the skin. Instead, an increase in T_g could be achieved by introduction of more rigid functional groups within the polymer chain. However, due to the biodegradation aspect of the polymer specifications, any comonomer introduced must either be introduced in small quantities so that the overall polymer does not fail the regulatory testing, i.e. at less than 30% non-degradable component, or it should also be biodegradable. For example, PLGAs of a range of compositions have T_g between 30 and 50 °C[185] and therefore offer potential for inclusion into a PEtG polymer backbone to achieve an overall T_g at an appropriate skin temperature.

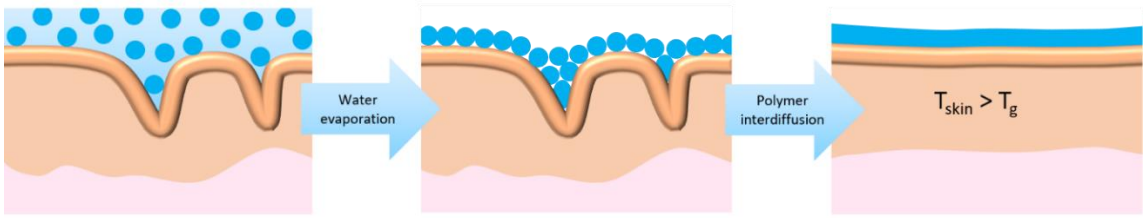


Figure 4-4. A water-based polymer dispersion is applied to the skin. On evaporation of the water the particles come into contact and at a temperature higher than the MFFT (thus, the polymer T_g), for example T_{skin}, the polymer particles coalesce to form a mechanically strong film.

4.1.4.Polyglyoxylate copolymers

Another method of improving the film-forming properties of a polyglyoxylate is its copolymerisation. Statistical copolymerisation with a compatible polymer with a higher T_g should create a polymer with a T_g intermediary between the two, however, the statistical nature would likely prevent any end-to-end depolymerisation from occurring. Block copolymerisation of PEtG with PEG has been shown to produce amphiphilic ABA triblock copolymers which self-assemble into micelles. The copolymerisation is carried out by a copper-assisted azide-alkyne 'click' cycloaddition with an end-capped PEtG and an azide-terminated PEG.[184]

Polystyrene has a T_g of approximately 100 °C and is used in the production of many thermoplastic elastomeric materials such as S-B-S and S-I-S where it is copolymerised with butadiene and isoprene respectively. The 'hard' polystyrene blocks form separate domains dispersed in the continuous elastomer phase and the polymer exhibits an amount of flow when heated before returning to a rigid network when cooled.[186] The block copolymerisation of PEtG with polystyrene therefore would potentially produce a similar effect and lead to the production of flexible films that would be flexible on application but tighten on cooling.

4.1.5.Chapter aims

The aims of this chapter are to synthesise a range of PEtG homopolymers and copolymers and analyse their T_g and determine their biodegradability. The end-capper type and concentration will be studied in order to find the optimal polymerisation conditions. The inclusion of a linking

molecule and the effects of increasing concentration will also be optimised to increase the T_g of the polymer in order to reach T_g values as close to skin temperature as possible. A further attempt to increase the T_g of the polymer will be copolymerising with a higher T_g polymer such as styrene by first synthesis of an ATRP macroinitiator of a halide-terminated PEtG. Finally, the biodegradation of the synthesised homopolymers and copolymers will be analysed by their dispersal in enzyme solutions over a period of 28 days. Ideally, a balance will be found so that both overall polymer degradation and overall polymer T_g will both be improved sufficiently to be appropriate for the cosmetic application.

4.2.Results and discussion

4.2.1.Distillation of monomer solution

The first step in these syntheses of PEtG was the purification of the ethyl glyoxylate/toluene solution. Ethyl glyoxylate (EtG) is usually supplied as a mixture of oligomers due to its spontaneous polymerisation at room temperature. It is also most often in solution at 50% in toluene, presumably to lower viscosity. The purification steps required have been described in the work by Gillies as a process of multiple distillations.[184] First, the toluene was removed under vacuum at approximately 50 °C. Although ethyl glyoxylate monomer has a very similar boiling point to toluene (approximately 120 °C), this distillation is successful due to the majority of the ethyl glyoxylate being present as oligomers at this temperature and therefore have lower volatility. As well as the monomer/toluene solution, phosphorous pentoxide was added as a drying agent to remove any water from the solution and prevent it from contaminating the purified monomer. Once the toluene was removed, the distillation was conducted under an inert gas atmosphere at 150–160 °C. The P_2O_5 reacts with any water that is liberated as the oligomers are cracked at the high temperature. In the literature, it is common for a second distillation under the same conditions to be carried out.[180,187,188] However, here it was found that using the monomer immediately after the first distillation was not detrimental to the end

product and has therefore not only reduced the purification time but also reduced the amount of wasted starting material. The main drawback in this purification process is that any contamination from toluene in the monomer cannot easily be accounted for in the polymerisation concentrations. For all polymerisations described, it was assumed that the distillated contained 100% EtG. To ensure the purity, the initial mixture was distilled until no further toluene was collected and a forerun of the second distillate was always removed before collection of the monomer. As, for this work, it has not been vital to target specific degrees of polymerisation by varying the amount of initiator used in comparison to the amount of monomer, this approximation has not caused any difficulties. With multiple attempts it was found that the faster the distillation was carried out the purer the distillate, therefore a heat gun was used to increase the rate of distillation.

4.2.2. Initial polymerisation attempt

Once the pale-yellow monomer was obtained it was vital for the polymerisation to be carried out immediately to prevent any contamination by water vapour from the air which would lead to the gradual oligomerisation. The initial polymerisation was carried out in the sealed collection vessel without any exposure to air by use of a Schlenk flask. Excessive drying of glassware was not carried out due to a catalytic amount of water being required to allow polymerisation to occur. Dry dichloromethane was added at approximately 50 v/v% followed by addition of 10 µl of triethylamine to catalyse the reaction. This catalytic amount of Et_3N is all that is required to deprotonate any residual water molecules to form hydroxyl anions which then initiate the polymerisation by attack at the carbonyl groups.[189] On polymerisation, a colour change from pale yellow to colourless was observed 10-20 seconds after addition of Et_3N indicating successful initiation. This colour change was also observed when monomer was left for more than a few hours at room temperature and exposed to air. The reaction was then stirred for 1 hour at -20 °C in an ice/NaCl bath before an end capper was added. Acetyl chloride was first selected and added as an end capper and allowed to react for 24 hours at room temperature without addition

of any extra catalyst to produce a stable polymer. Regarding isolation of the polymer product, work by Gillies and others have described precipitation from petroleum ether and/or methanol, however, neither solvent was able to precipitate the polymer in this case. This was later attributed to the lower molecular weights synthesised here. Instead, the best isolation method was found to be precipitation into hexane to give a viscous liquid which settled on the bottom of the vessel. Decantation of the hexane, a second hexane wash and then removal of solvent was then carried out to ensure clean product. The remaining sticky fluid was then dissolved in THF at approximately 10 w/w% before a final precipitation step into 50 cm^3 centrifuge tubes filled to 40 cm^3 with deionised water. Centrifugation twice with refreshing of the supernatant resulted in a white viscous liquid at the bottom of the tube which was then freeze-dried to remove water. The final product was a colourless, transparent, viscous liquid - characteristic of a low T_g polymer.

4.2.3. Initial polymer analysis

Where successful end-capping had occurred, ^{1}H NMR spectroscopy was used to estimate the DP of the polymer by comparison of the CH_3 signal of the acetyl chloride to the polyethylglyoxylate pendant CH_3. As every polymer chain is end-capped at both ends, there are two acetyl CH_3 units per chain. The integrals from the NMR spectra were then used to calculate the approximate average DP. For the initial polymerisation, ^{1}H NMR spectroscopy showed an approximate DP of 250 (figure 4-5) which corresponds to a molecular weight of approximately 25500 g mol^{-1}. The syntheses of PEtG by Gillies *et al*. produced higher molecular weight polymers but were not end-capped by acetyl chloride and therefore this could potentially be due to the kinetics of the end-capping reaction being faster in our work.[184,188,190] GPC was also used to analyse the molecular weight showing a M_n of 10400 g mol^{-1} against PMMA standards. The large difference in molecular weights between the two techniques can be attributed to two things. Firstly, the comparison to PMMA standards might not translate well to these polymers. Furthermore, the end-capping creates an ester linkage which may not be stable to acid hydrolysis and therefore

may be cleaved by the GPC THF/acetic acid eluent system. If such hydrolysis has occurred, the reported polymer molecular weight would be expected to be lower due to breaking down of the polymer chain from the self-immolative nature of the polymer. From this, it has been assumed that actual molecular weights are significantly higher than those reported by GPC analysis and that although it is useful to qualitatively compare polymers, it does not give accurate values for M_w.

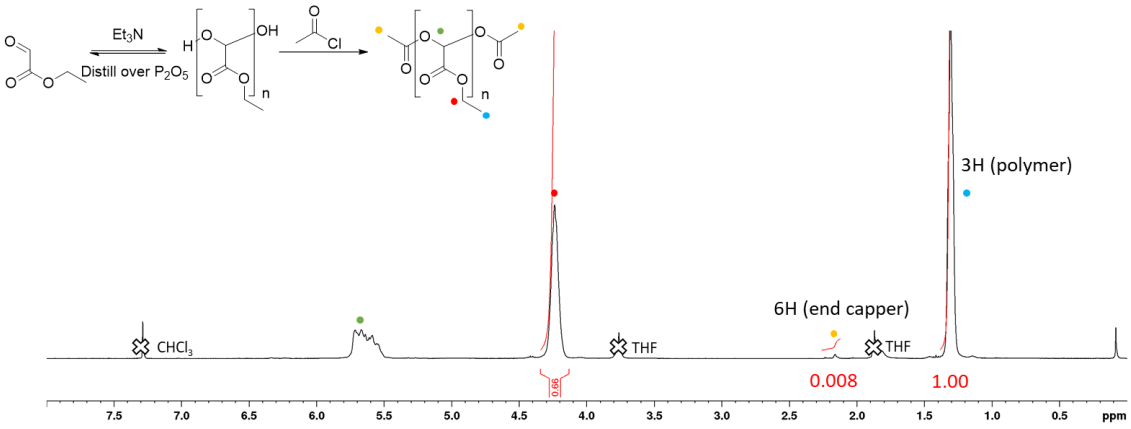


Figure 4-5. ^{1}H-NMR spectrum of PEtG end-capped with acetyl chloride in $CDCl_3$. The peaks at 1.3 ppm (ethyl glyoxylate pendant CH_3) and 2.2 ppm (acetyl CH_3) can be used to determine the approximate DP of the polymer.

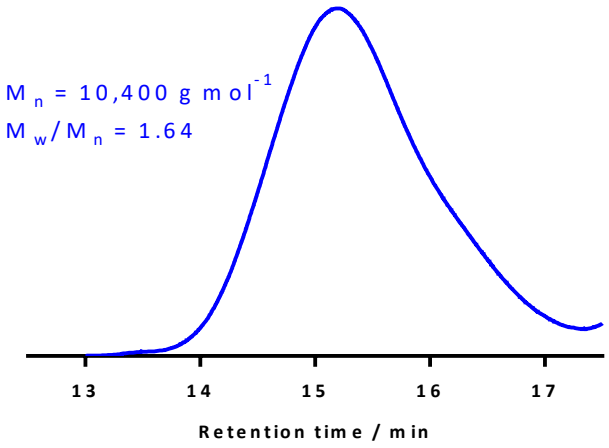


Figure 4-6. GPC trace of PEtG end-capped with acetyl chloride at 10 mol%. THF/acetic acid system, PMMA standards.

In contrast to this attempt, a second polymerisation without the addition of any end-capper was attempted and gave a similar colourless viscous liquid product. However, once isolated, this

polymer became less viscous over time as it spontaneously fell apart. NMR spectroscopy of this polymer showed low conversion of monomer and therefore it was confirmed that end-capping is vital for polymer stability.

4.2.4.Optimisation of polymerisation conditions

Small changes in the polymerisation apparatus also had a large effect on the amount of polymer that could be isolated. A smaller distillation set-up, which prevented loss of monomer as far as possible, allowed a better yield from the distillation. For all further reactions, the distillation was carried out on this smaller equipment which included a short Vigreux column to ensure the best separation of the monomer/toluene mixture. Further to this, it was found that excessive heating should not be applied directly to the distillation mixture as this caused the thermal degradation of the P_2O_5, shown by evolution of white smoke and discolouration of the distillate. In cases where the mixture was heated too intensely, a dark yellow/orange liquid was collected instead of the pale-yellow monomer and when used in polymerisation this produced inferior polymers with lowest molecular weights. This discovery has previously been described in the work by Gillies and the inferior products are due to the reaction which occurs between P_2O_5 and water to produce H_3PO_4. This acid boils at 155 °C and it is therefore important to keep the mixture below this temperature and remove the heat source if it begins to react violently.[188]

The next step in the optimisation of the polymerisation was to enable multiple polymerisations to be carried out at once from the same distillate. This would then eliminate any variation between polymerisations in a series that may be caused by the purity or quality of the starting monomer. A pair of identical polymerisations were set up and carried out over the same duration in salt/ice baths. For both of these polymerisations, only a small amount of polymer was isolated by precipitation. End group analysis by ^{1}H NMR spectroscopy showed an average of only 14 EtG units per chain, a DP of approximately 1500, indicating low monomer conversion.

Nonetheless, both polymers were of the same approximate DP showing that when all reactions conditions are under close control, similar outcomes are achieved.

From this it was necessary to try and produce polymers with larger molecular weights. Initially this was attempted by varying the concentration of end capper used. Thus far, acetyl chloride had been added at 10 mol% with respect to monomer concentration and produced low molecular weight polymers. By decreasing this to 5 mol% an increase in molecular weight should have been achieved, however, this was not the case and the resulting polymer had a molecular weight of approximately 2,900 g mol^{-1} by GPC. Until this point, polymers synthesised had a wide range of molecular weights despite reaction conditions being similar and there did not appear to be any dependence on end-capper concentration. Due to the low ceiling temperature, it was predicted that the success of the polymerisation depends mostly on the reaction temperature. The ice bath temperature was the most difficult parameter to control and required refreshing the mixture multiple times over the course of a polymerisation to ensure the temperatures remained at approximately -20 °C. On warmer days this was refreshed more frequently to accommodate the faster rise in temperature. To overcome the temperature changes, all further reactions were carried out on stirrer plates placed in a -20 °C freezer. This led to much better control over the polymerisations and sets of 4 or 5 polymerisations were also then readily carried out at the same time.

4.2.5.Preliminary degradation test of PEtG

Using the acetyl chloride end-capped polymers of molecular weight 25,500 g mol^{-1} by NMR spectroscopy, a crude degradation test was carried out in order to scope for future development of the polymer. A 10% w/w PEtG solution in THF was dropped onto glass coverslips and placed in a 6-well plate. The solvent was then removed under vacuum to leave transparent, sticky film droplets on the cover slip surfaces. The dried samples were submerged in a 0.00, 0.05 or 0.10% w/w solution of lipase CalB from *Candida antarctica* in DPBS and placed in a 37 °C oven for 24

hours. Coverslips were then washed gently to remove DPBS without disrupting the film and dried under vacuum. Even before drying it was apparent that the samples that had been in contact with lipase had reacted differently to the control samples as the transparent film was now a white flaky residue and this remained the case after drying (figure 4-7). Analysis of this solid could not be carried out as there was an insufficient mass of remaining material. However, mass spectroscopy analysis of freeze-dried DPBS solutions was carried out to determine the identity of any dissolved degradation products. Ignoring any peaks due to the DPBS salts, the two highest intensity signals at m/z of 72.9 and 90.9 are contributions from glyoxylic acid and dihydroxyacetic acid, both of which are formed on hydrolysis of ethyl glyoxylate (figure 4-8).

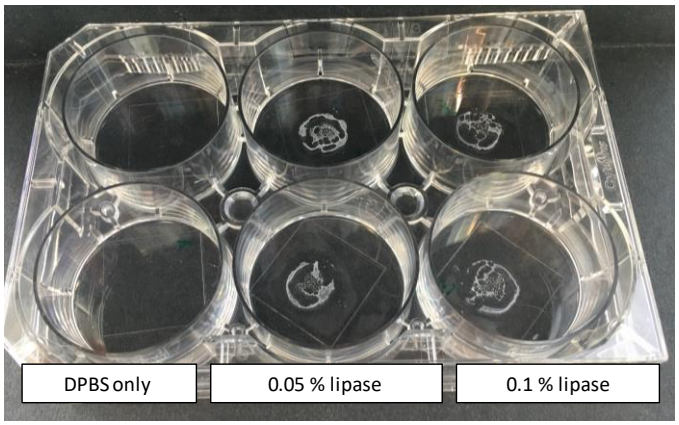


Figure 4-7. Photograph showing the end result of 24 h degradation test carried out on PEtG films after removal of DPBS/lipase solutions and drying of cover-slips. Those inoculated with lipase resulted in a white, water-insoluble residue as opposed to a transparent film.

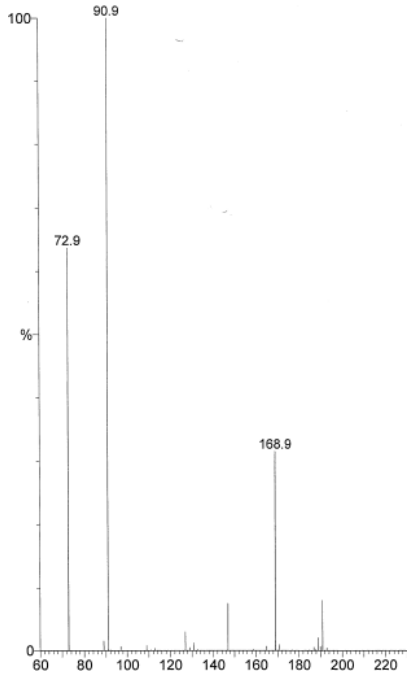


Figure 4-8. ES mass spectrum (negative mode) of PEtG which was dispersed for 7 days in DPBS and 0.1 w/w% lipase resulting in the degradation of the polymer chain.

4.2.6.End-capping strategies

As the enzymatic degradation of PEtG was confirmed, the next aim was to determine whether the molecular weight and stability by using different end cappers. Acetyl chloride was sufficient to end-cap and hence stabilise the polymer but was also quickly removed by lipase hence triggering the self-immolation of the polymer. Previously reported phenyl isocyanate and phenyl chloroformate[184,187] were trialled as alternatives with appropriate catalysts to yield more stable polymers, however after many attempts end-capping was unsuccessful and these polymers could not be successfully isolated (figure 4-9).

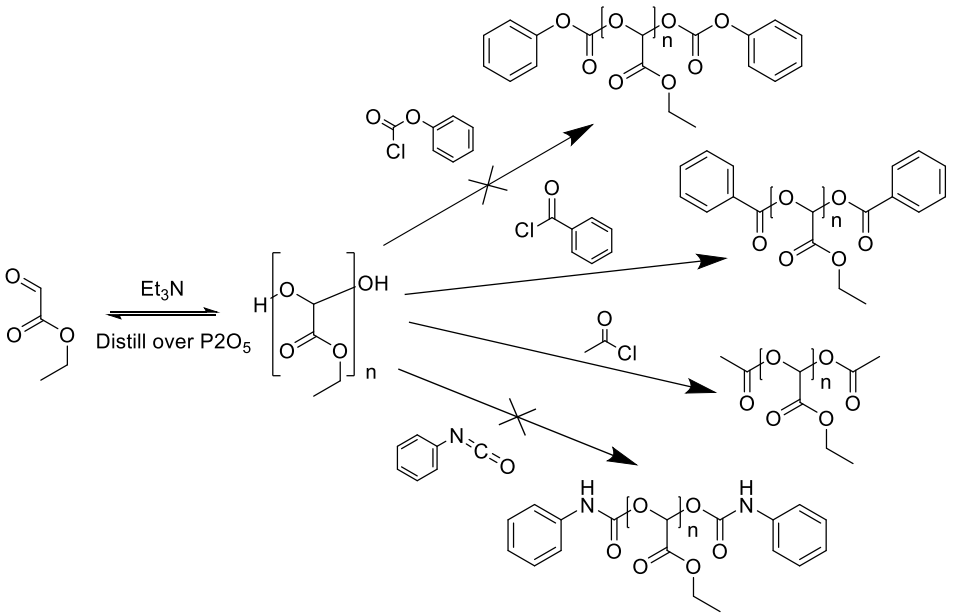


Figure 4-9.End-capping routes of PEtG with phenyl chloroformate, benzoyl chloride, acetyl chloride and phenyl isocyanate.

Benzoyl chloride was predicted to end-cap in a similar reaction to acetyl chloride and produce a higher stability polymer as the benzoate functionality produced in the end-capping reaction should have enhanced stability in comparison to acetate.[191] This should mean the PEtGs would be able to withstand slightly harsher conditions and thus be less susceptible to cleavage and triggering of the self-immolation. Reactions with benzoyl chloride did produce stable polymers under the same polymerisation conditions as the acetyl chloride capped polymers. However, on using 2 wt % of benzoyl chloride, the molecular weight by GPC was still low at 4,870 g mol^{-1} (figure 4-10).

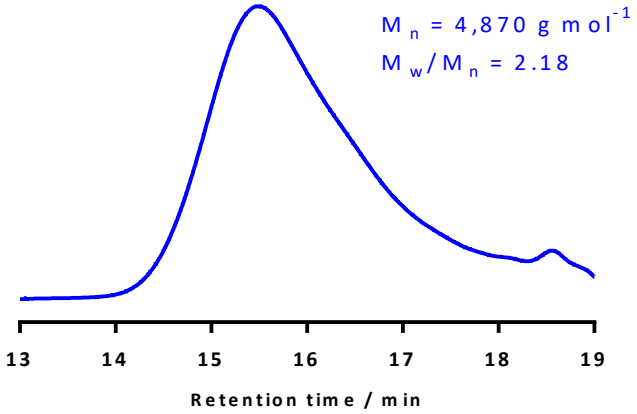
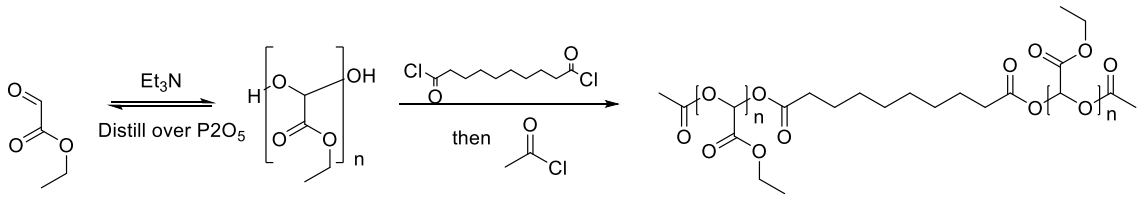


Figure 4-10. GPC trace (RI detection) of PEtGs produced using benzoyl chloride as an end capper at 2 mol%. THF/acetic acid system, PMMA standards.

4.2.7.PEtG syntheses with sebacoyl chloride 'linker'

A different approach to increasing molecular weight was to include a 'difunctional end-capper' which would hypothetically link together PEtG oligomers and hence build up a blocky copolymer. Sebacoyl chloride was selected due to its similarity to acetyl chloride and was assumed to react readily without the need for extra catalysts. Also, the product formed upon the polymer degradation would be sebacic acid, a naturally occurring molecule which is often used in cosmetics as an antimicrobial.[192] This was added after 1 hour of the polymerisation reaction with a final dose of acetyl chloride at 5 mol% added after a further hour to fully end-cap the polymer (scheme 4-2). On work-up, a solid, tacky polymer was precipitated from hexane, a contrast to the viscous liquids that were isolated previously. NMR spectroscopy of this precipitate showed successful polymerisation with evidence of acetyl chloride and of extra CH_2 environments from the sebacoyl chloride linkers.



Scheme 4-2. PEtG synthesis with addition of sebacoyl chloride followed by acetyl chloride to end cap the resulting linked polymers.

Further reactions were then carried out with concentrations of sebacoyl chloride of 0, 2, 5 and 10 mol% with respect to monomer concentration to determine the effect that linker concentration had on polymer molecular weight. The four reactions were carried out simultaneously using the same monomer distillate and under the same conditions. GPC analysis of the precipitated polymers showed multimodal traces and accurate molecular weights could not be obtained, however no visible trend in sebacoyl chloride concentration and retention time was observed (figure 4-11).

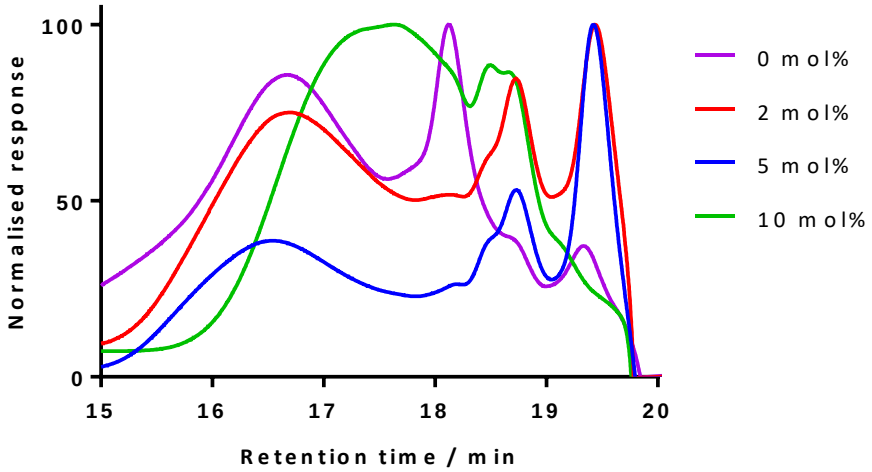


Figure 4-11. GPC traces (RI detection) of PEtGs produced using sebacoyl chloride concentrations of 0-10 mol%. M_n values are not quoted due to overlapping of peaks. THF/acetic acid system, PMMA standards.

After leaving all the purified sebacoyl-chloride-linked polymers overnight, a white solid began to precipitate and continued over three days. The solid was insoluble in THF and was easily removed by gravity filtration to give white crystals. On analysis by NMR spectroscopy in D_2O it was determined that this solid was sebacic acid (figure 4-12). This is most likely formed from

degradation of the polymer chain at the newly formed ester groups. From this it was concluded that sebacoyl chloride was not suitable to link the PEtG units due to the instability of the products it formed. Polymers which include sebacate units have previously been shown to degrade under mild degradation conditions giving further evidence that it is probably too unstable to use in a potential cosmetic material which will require a significant shelf-life at a pH between 5 and 6.[193]

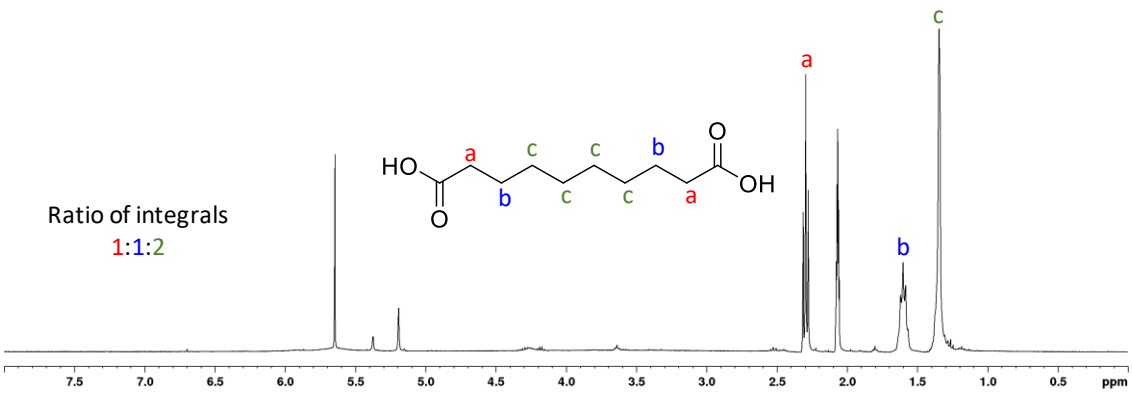


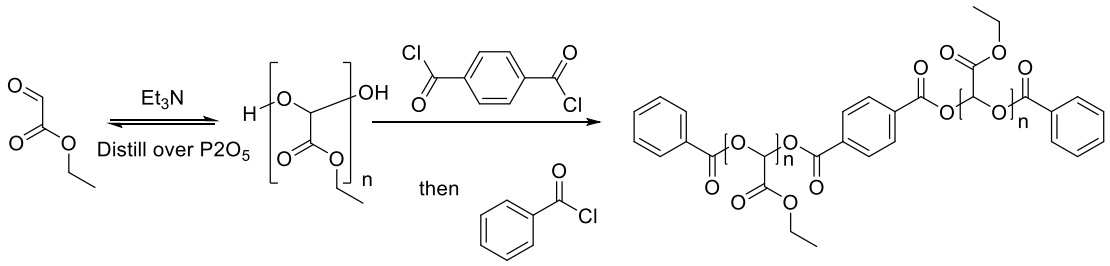
Figure 4-12. ^{1}H-NMR in D_2O of the crystalline material which spontaneously crashed out from the polymer samples over a few days at room temperature.

4.2.8.PEtG syntheses with terephthaloyl chloride 'linker'

Terephthaloyl chloride (TereCl) is another difunctional acid chloride that is commonly used in polyamide synthesis; specifically in the synthesis of Kevlar, a material which is known for its thermal stability.[194] Therefore if successful as a linker it should also produce more stable polymers. The increased stability in comparison to sebacoyl chloride is likely to do with the increase in steric hinderance of the aromatic ring in comparison to the alkyl chain.

To begin with, TereCl was substituted for sebacoyl chloride at the same concentrations (0-10 w/w%) and the polymerisations were carried out as described previously being finally end-capped with benzoyl chloride an hour after the addition of linker (scheme 4-3). Benzoyl chloride

was selected as end-capper for its improved stability over acetyl chloride. As with the sebacoyl chloride reactions, the molecular weight by GPC was not increased by the addition of linker but the washed polymer product showed extra aromatic peaks in the ^{1}H NMR spectrum implying that the TereCl had successfully been incorporated (figure 4-13).



Scheme 4-3. PEtG synthesis with addition of TereCl followed by benzoyl chloride to end cap the resulting linked polymers.

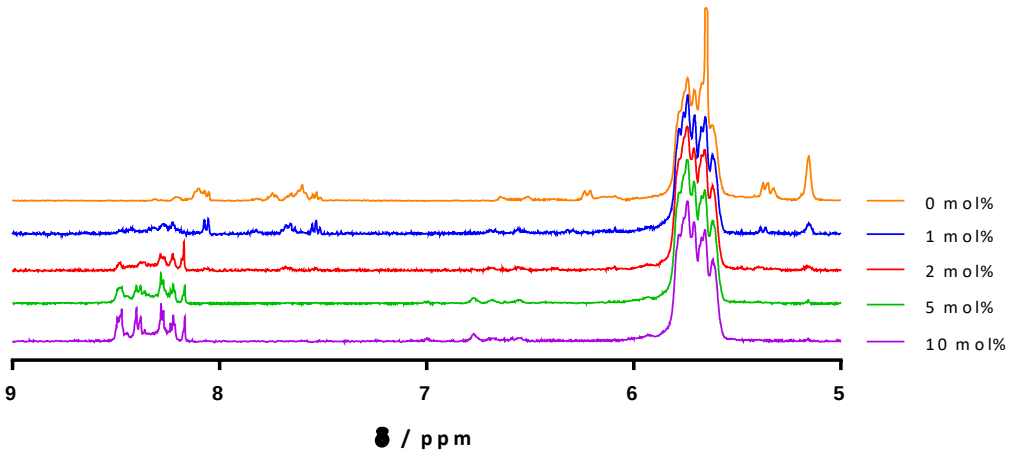


Figure 4-13.Stacked ^{1}H-NMR spectrum in d^{6}-DMSO of PEtG synthesised with increasing concentration of TereCl linker from 0-10% and end-capped with benzoyl bromide. Magnified to show increase in intensity of aromatic signals (8.1-8.5 ppm) with respect to TereCl concentration with PEtG backbone signal intensity (5.7 ppm) remaining constant.

As well as the increased stability with respect to sebacoyl chloride, an advantage of using TereCl was the increase in polymer T_g it imparted by increasing the rigidity of the PEtG backbone. Poly(ethylene terephthalate) (PET) has a T_g of 67 °C when amorphous and 81 °C when crystalline,[195] therefore by incorporating its stiffer structure randomly into the PEtG backbone, an overall increase of T_g would be expected. This was visually observed by the isolation of a

white solid when TereCl linker was used, instead of the free-flowing viscous liquid when no linker was added or the tacky substance when sebacoyl chloride linker was used. DSC was later used to confirm this.

4.2.9.Kinetics of TereCl linking reaction

In order to optimise the linking reaction, analysis of the kinetics of polymerisation was carried out over 1 week. Four monomer preparations were made and terephthaloyl chloride was added simultaneously and the vials were then placed in the freezer to react. Benzoyl chloride end-capper was added at 2 w/w% after 1 h. After time intervals of 24, 48, 72 and 168 h, the vials were warmed to room temperature and the polymers were collected by precipitation. Differential scanning calorimetry (DSC) was used to determine the T_g of the polymers and their molecular weight were analysed by GPC in THF/acetic acid. An increase in T_g was seen over the second 24 h with the highest T_g polymer being isolated at 48 h. The M_n values obtained from GPC analysis show that there is no increase in molecular weight after 24 h and they are also further evidence that the polymerisations are reliable and reproducible when conditions are controlled. From these findings, all reactions were carried out for 48 h to ensure maximum TereCl incorporation into the polymer backbone.

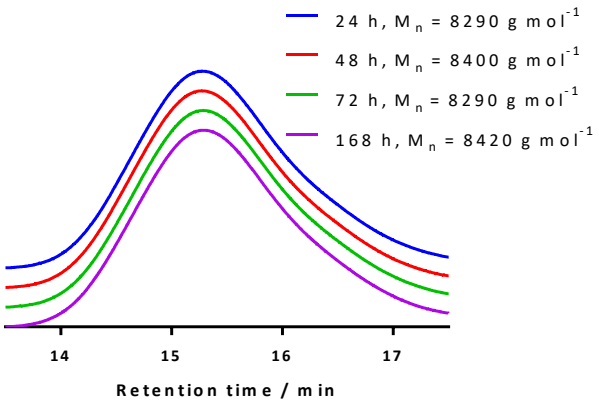


Figure 4-14. Stacked GPC chromatograms (RI detection) used to obtain the M_n of the PEtG synthesised with TereCl linker over 168 h (7 days). THF/acetic acid system, PMMA standards.

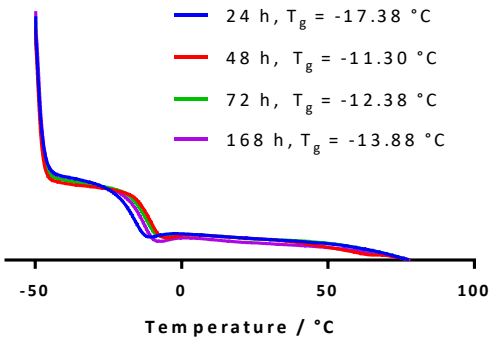


Figure 4-15. Stacked and normalised DSC traces (plotted 'exo up') of the second heating cycle to obtain the T_g values of the PEtG synthesised with TereCl linker over 168 h (7 days)

4.2.10.Effect of TereCl and benzoyl chloride concentration on PEtG molecular weight and glass transition temperature

After optimisation of the polymerisation process, T_g and M_n were examined alongside concentration increases of TereCl and benzoyl chloride. For this set of experiments, the concentrations of both benzoyl chloride and TereCl were increased to find the optimal conditions for high molecular weight polymers with increased T_g. Concentrations of benzoyl chloride were 1, 2 and 5 mol% and concentrations of TereCl were 0, 1, 2, 5 and 10 mol%. The polymers synthesised are listed below in table 4-3. For all polymers, DSC was used to obtained T_g values and GPC was used to analyse the effect of concentration on molecular weight. The second heating cycle was used to obtain T_g values as this represents this transition more accurately as the first heating cycle can often involve other thermal transitions such as residual solvent loss and therefore results can vary from the true T_g values. There is also argument to suggest that on the second heating there is less thermal lag due to better contact between the polymer and the aluminium pan caused from increased mobility and lowered viscosity after the first cycle.[185] T_g values were obtained by taking the temperature value at half of the height of the incline of the transition.

Table 4-3. Concentrations of terephthaloyl chloride (TereCl) and benzoyl chloride that have been studied as linker and end-capper respectively.

	0 mol% TereCl	1 mol% TereCl	2 mol% TereCl	5 mol% TereCl	10 mol% TereCl
1 mol% benzoyl chloride	B1T0	B1T1	B1T2	B1T5	B1T10
2 mol% benzoyl chloride	B2T0	B2T1	B2T2	B2T5	B2T10
5 mol% benzoyl chloride	B5T0	B5T1	B5T2	B5T5	B5T10

For comparison, polymerisations without any TereCl were carried out. These polymers have T_g values from DSC that are closest to the reported values of -32 °C for PEtG homopolymers[184] and were far lower than the values obtained when linkers were used (figure 4-16). Each set of five polymerisations were performed under controlled conditions from the same distillation of monomer and this is likely to explain why trends occur within sets but no trend is seen between them. With exception of the B5 series of polymerisations, the T_g increased with increasing TereCl concentration, implying that more TereCl is incorporated into the backbone as would be expected. Overall, the T_g was only increased to 0 °C which is not sufficient for this polymer to have prospects to be used in the cosmetic application.

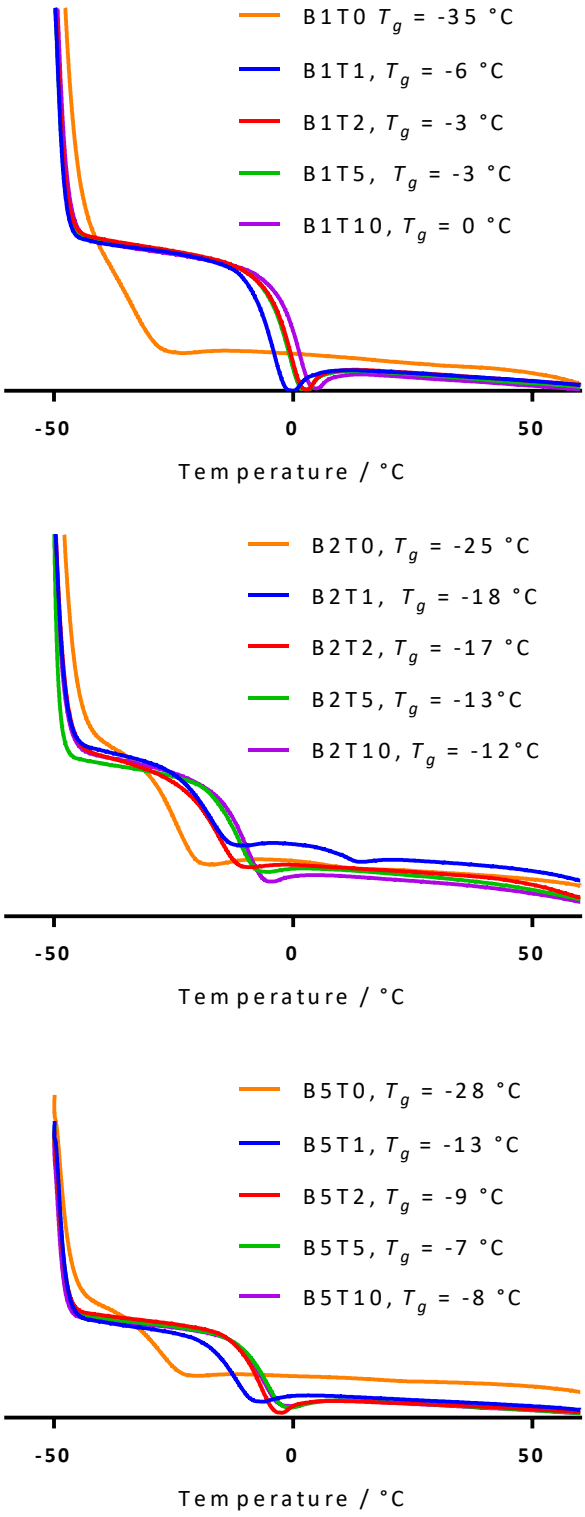


Figure 4-16. Stacked and normalised DSC traces (plotted 'exo up') of the second heating cycle to obtain the T_g values of the PEtG synthesised with increasing benzoyl chloride and TereCl concentrations.

GPC analysis shows that the increase of TereCl has little effect on overall polymer molecular weight (figure 4-17). In the B1 and B5 series there is a small increase but ultimately the molecular weight is more likely to be dictated by the end-capper concentration as it is the end-capping that terminates the polymerisation. As discussed previously, molecular weights of PEtG synthesised in this work also have a dependence on temperature due to the low ceiling temperature of the polymerisation.

Overall, the incorporation of increasing concentrations of TereCl in the polymerisation has a strong influence on the T_g of the product but not much influence on the molecular weight. This is intuitive because the individual molecules of TereCl will be included into the polymer chain at a higher ratio. Taking the B1 series as an example, the molecular weight difference by GPC between B1T1 and B1T10 is approximately 4200 g mol^{-1}, which equates to almost a 20% increase. This difference can be accounted for by the 9% increase in TereCl added. If PEtG calibration standards had been used it would be more likely that the increase seen in the GPC analysis would correlate with the increase that can be accounted for by the TereCl.

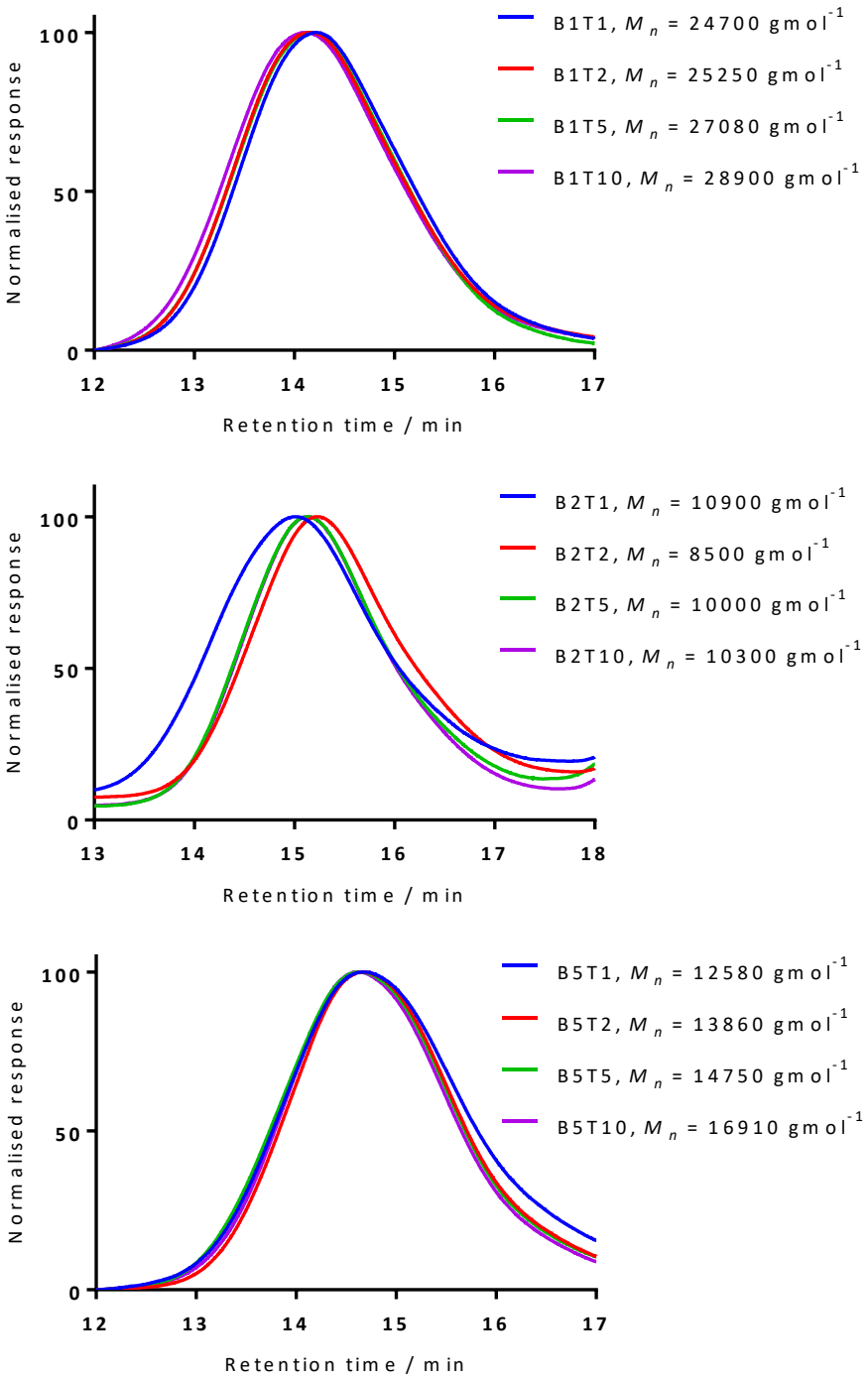


Figure 4-17. Stacked GPC chromatograms (RI detection) used to obtain the M_n of the PEtG synthesised with increasing benzoyl chloride and TereCl concentrations. THF/acetic acid system, PMMA standards.

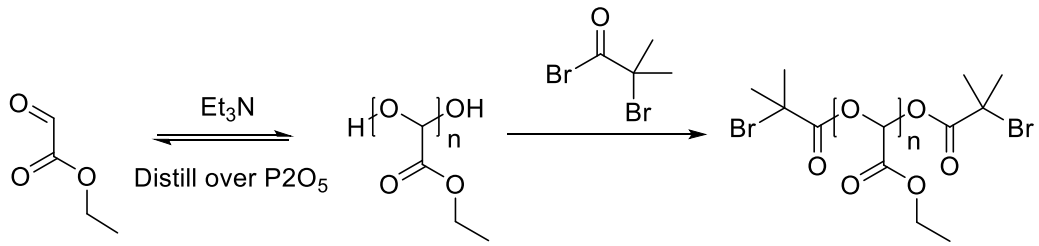
4.2.11.Synthesis of PEtG with α-bromoisobutyryl bromide (BIB) end-capper

Due to the increase in T_g not being sufficient when using a comonomer, the synthesis of block copolymers with a higher T_g polymer was attempted. Due to the double-ended nature of the

PEtG polymers synthesised, ABA triblock copolymers rather than AB diblock copolymers were synthesised in the chain-extension.

The living character of atom-transfer radical polymerisation (ATRP) enables it to be used to produce block copolymers. However, it can also be used to synthesise block copolymers from pre-formed polymeric macro-initiators, such as a PEtG chain, end-capped with a suitable initiator species. This would replace the 'click' reaction outlined previously in the synthesis of the ABA triblock copolymer micelles of PEG-*b*-PEtG-*b*-PEG.[184]

Firstly, in order to synthesise a suitable ATRP macroinitiator, the PEtG was synthesised with α-bromoisobutyryl bromide (BIB) as an end capper to give it a terminal halide which could be used to initiate the copolymerisation with another monomer. The BIB end-capper reacted readily under the same conditions as previously and the resulting polymer had a similar viscous liquid form to the polymers synthesised without a chain extender. The end capper was added at 1 and 5 mol% to determine the difference on molecular weight. Some dependence on concentration was observed with M_n by GPC of 19,640 g mol^{-1} and 10,140 g mol^{-1} respectively. Following this, the reactions were carried out on a larger scale using approximately 15 g of EtG monomer to produce batches of polymer for use in multiple copolymerisations. The molecular weight by GPC of the scaled-up 1% and 5 % end-capped polymers were 32,000 g mol^{-1} and 9,910 g mol^{-1} respectively, further confirming that the reaction conditions rather than specifically the concentration of end-capper control the polymerisation reproducibility.



Scheme 4-4. PEtG synthesis with α-bromoisobutyryl bromide to end cap the resulting linked polymers

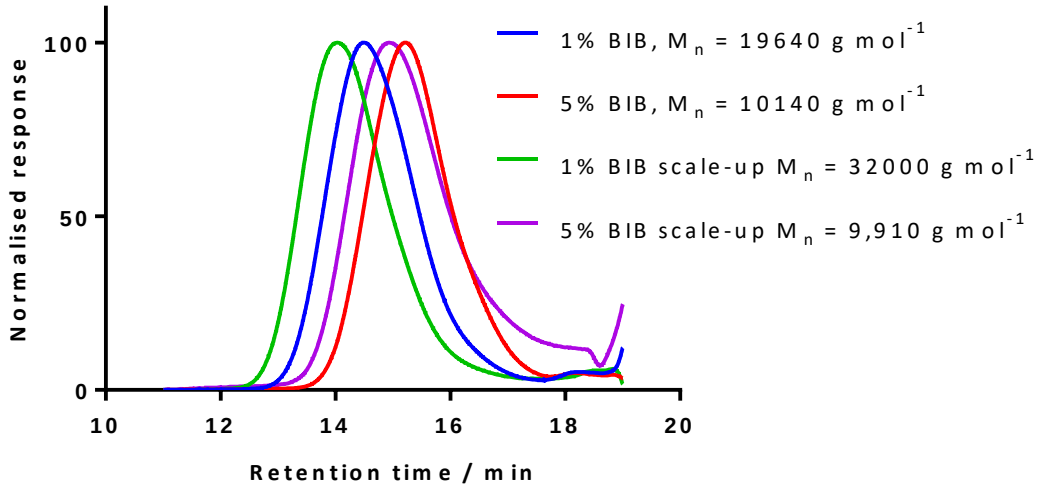
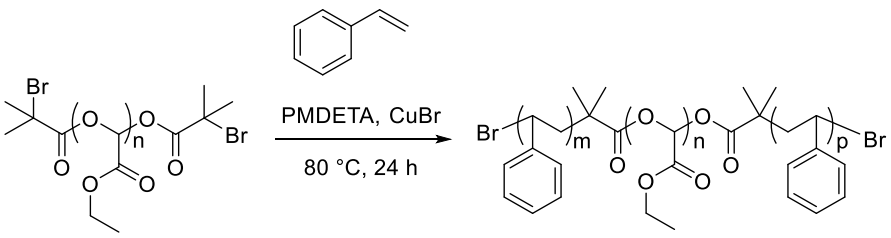


Figure 4-18. GPC chromatograms used to obtain the M_n of the PEtG synthesised with BIB at 1 or 5 mol%. Conditions were then repeated at a larger scale to obtain largers batches of PEtG.

4.2.12.Synthesis of polystyrene-block-poly(ethyl glyoxylate) copolymers by ATRP

Once isolated, the PEtG macroinitiator of M_n = 32,000 gmol^{-1} was used in the copolymerisation with styrene by ATRP. Monomer to macroinitiator mole ratios of 100:1, 500:1 and 1000:1 were attempted in order to target polymers with styrene blocks of approximately 100, 500 and 1000. All three of these polymerisations were unsuccessful resulting in blue/green sludge-like liquids. The lower molecular weight PEtG was then used under the same conditions, though due to the lower molecular weight they were carried out on a larger scale to enable better stirring and for the production of a large enough amount of copolymer for the analysis that was carried out. All reagents were deoxygenated for longer than the previous attempts to ensure that oxygen contamination did not affect the polymerisation.



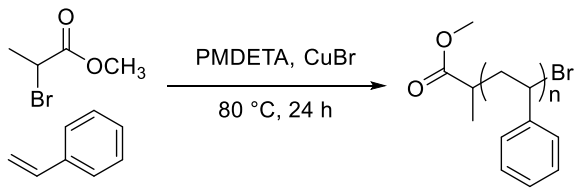
Scheme 4-5.The copolymerisation of styrene by ATRP from a PEtG macroinitiator.

The PEtG macroinitiator is bifunctional and therefore the polystyrene reacted at both ends to produce an ABA triblock copolymer (as seen in scheme 4-5). Due to these polymerisations being carried out in bulk, the macroinitiators were heated to 80 °C before addition of monomer, catalyst or ligand as this made it less viscous and enabled better mixing. Polymerisations were carried out for 24 h to ensure high conversions of monomer as final chain functionality was not important as no further copolymerisation was to be carried out. The synthesised polymers ranged in colour from dark blue solution to brown/green solid, characteristic of copper in different oxidation states but were homogenous in comparison to the previous failed attempts. The blue colours formed indicate that the copper catalyst is trapped in the +2-oxidation state and therefore no longer able to catalyse the polymerisation, hence the polymerisation product being liquid. This could be due to the occurrence of some chain termination or due to the presence of oxygen in the system. This polymer (the targeted 100:1) was discarded as it contained mainly unreacted styrene. The remaining green/brown polymers (the targeted 500:1 and 1000:1) were isolated by dissolving in DMF and precipitating from water. The two successfully synthesised block copolymers precipitated as white solids with the colourful copper salts remaining in solution. The reason for the success of these polymers could be simply due to the increased amount of styrene monomer providing a larger volume and hence improving the stirring of the reagents.

4.1.1 *Synthesis of polystyrene homopolymer by ATRP*

In order to have a control for future T_g and degradation studies, a polystyrene homopolymer was first synthesised by ATRP (scheme 4-6) based on a literature procedure.[196] An initiator to monomer ratio of 1:100 was used and was stopped at approximately 50% conversion to target a polymer of approximately 6,300 $gmol^{-1}$. The polymerisation was carried out in bulk and on cooling from the reaction temperature of 80 °C to room temperature, a green/brown glassy solid remained. To remove the colour and precipitate the polymer it was first dissolved in THF and precipitated from deionised water which turned bright blue as the copper (II) ions remained

in solution. The precipitate was a white solid which was hard and brittle once dried. GPC analysis of the polystyrene gave M_n of 6,680 g mol^{-1} and a dispersity of 1.29 against PMMA standards. Both values are higher than expected due to the polymerisation being allowed to go to higher than 50% conversion. The conversion was estimated to be >99% by ^{1}H NMR spectroscopy of a crude sample due to the absence of any vinyl peaks at around 5 ppm (figure 4-19). Usually, at conversions > 60% in ATRP, polymers begin to terminate by combination and thus lead to higher molecular weights and dispersities which reflect the polymers which have and haven't terminated. Therefore, where block copolymers are to be synthesised, the initial homopolymerisation is often only allowed to get to approximately 50% to ensure that end-group functionality remains and to keep dispersities narrow.[197,198]



Scheme 4-6.Polymerisation of styrene by ATRP with methyl-2-bromopropionate initiator, CuBr catalyst and PMDETA ligand to yield polystyrene homopolymer with low dispersity.

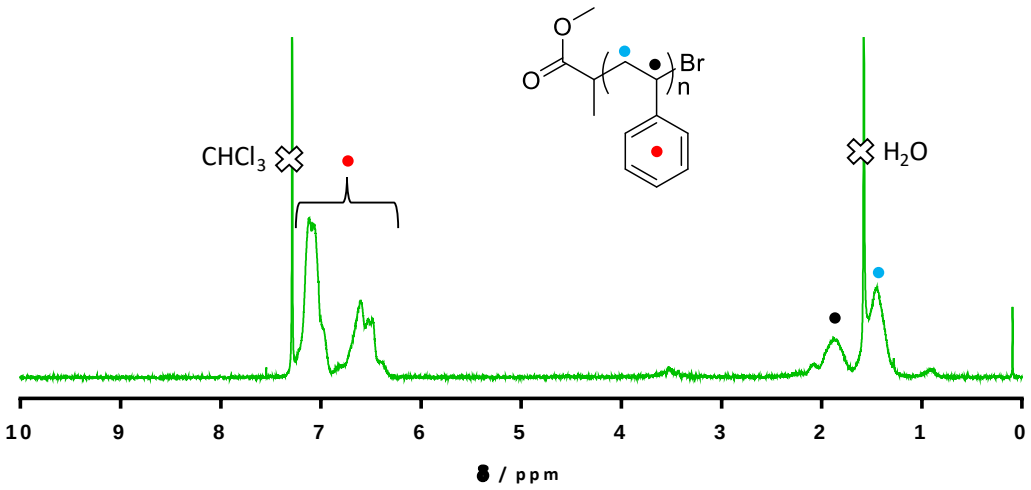


Figure 4-19. ^{1}H-NMR spectrum in $CDCl_3$ of crude polystyrene synthesised by ATRP.

4.1.2 *Analysis of copolymers*

The successful copolymerisations were analysed by GPC, ^{1}H NMR spectroscopy and DSC. GPC analysis showed an increase in molecular weight on copolymerisation as expected (figure 4-20).

The overlaid chromatograms show that the precipitated product is the copolymer rather than two separate homopolymers of PEtG and PS due to the narrow molecular weight distributions and little overlap of molecular weights.

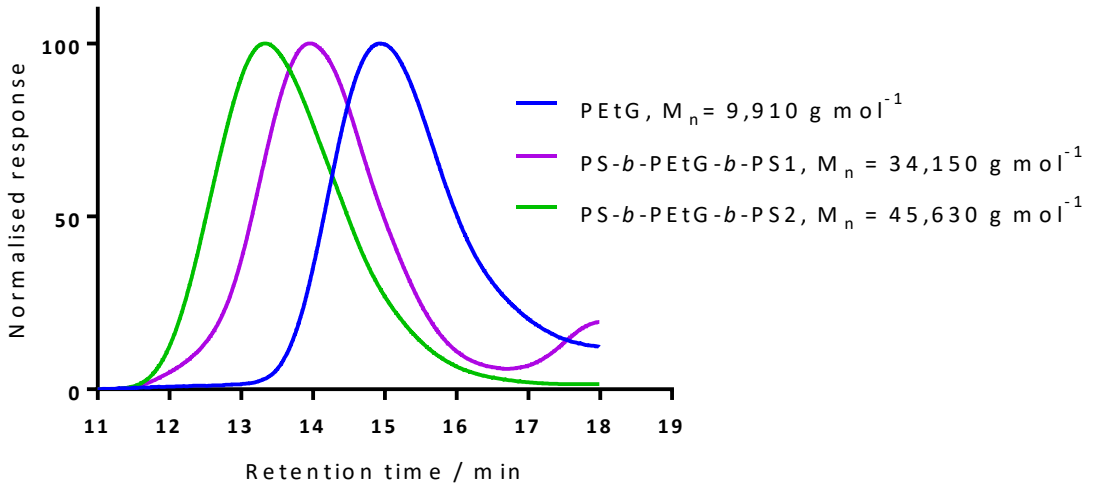


Figure 4-20. Stacked GPC chromatograms (RI detection) used to obtain the M_n of the PEtG homopolymer and PS-b-PEtG-b-PS copolymers synthesised from this homopolymer.

From NMR spectroscopy of the purified copolymers, a higher styrene content is apparent in the 1000:1 targeted copolymer PS-*b*-PEtG-*b*-PS2 than the 500:1 targeted copolymer PS-*b*-PEtG-*b*-PS1 as would be expected. The PEtG peaks have been normalised to show this increase (figure 4-21). Approximate composition of the copolymers has been estimated from NMR integrals as 1:6 and 1:15 EtG/S units for copolymer 1 and 2 respectively.

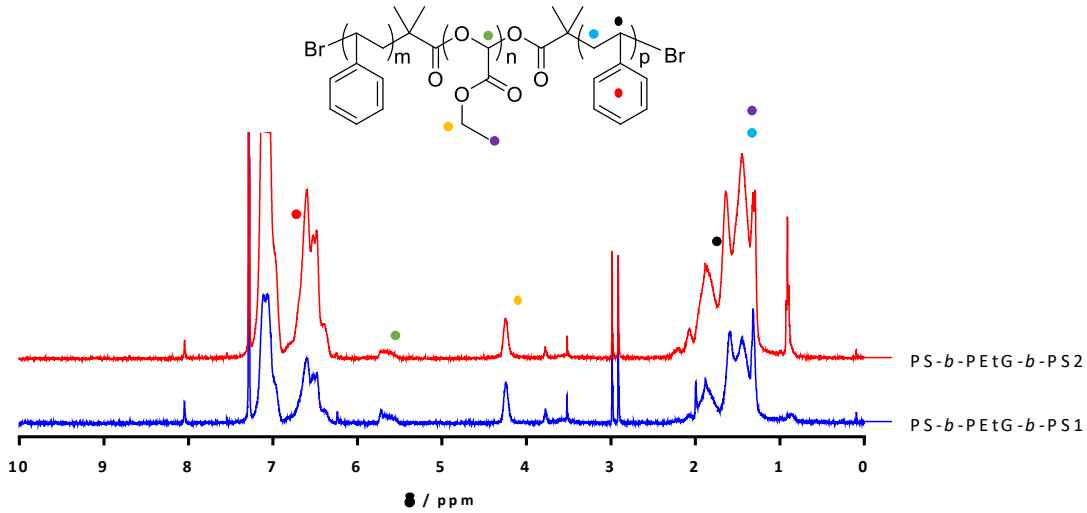


Figure 4-21. Stacked ^{1}H-NMR spectra of PS-b-PEtG-b-PS copolymers.

The T_g of the triblock copolymers were determined by DSC and compared to the previously synthesised homopolymers of PS and PEtG end-capped with BIB. For the PEtG homopolymer, the T_g is -10 °C. This is higher than all of the non-linked PEtGs despite being a similar molecular weight, implying that the end-capper has some effect on the interactions between the individual polymer chains and causing the solid polymer to have a less amorphous structure. The T_g for PS is at the lower end of the T_g range in the literature, but this could be due to the method of obtaining the T_g and its relatively low molecular weight. The method used is by drawing tangents on the heat flow curve at temperatures above and below the transition and therefore are subjective. Although the tangents are carefully selected, individual T_g values from DSC are not perfectly accurate and should be used cautiously.

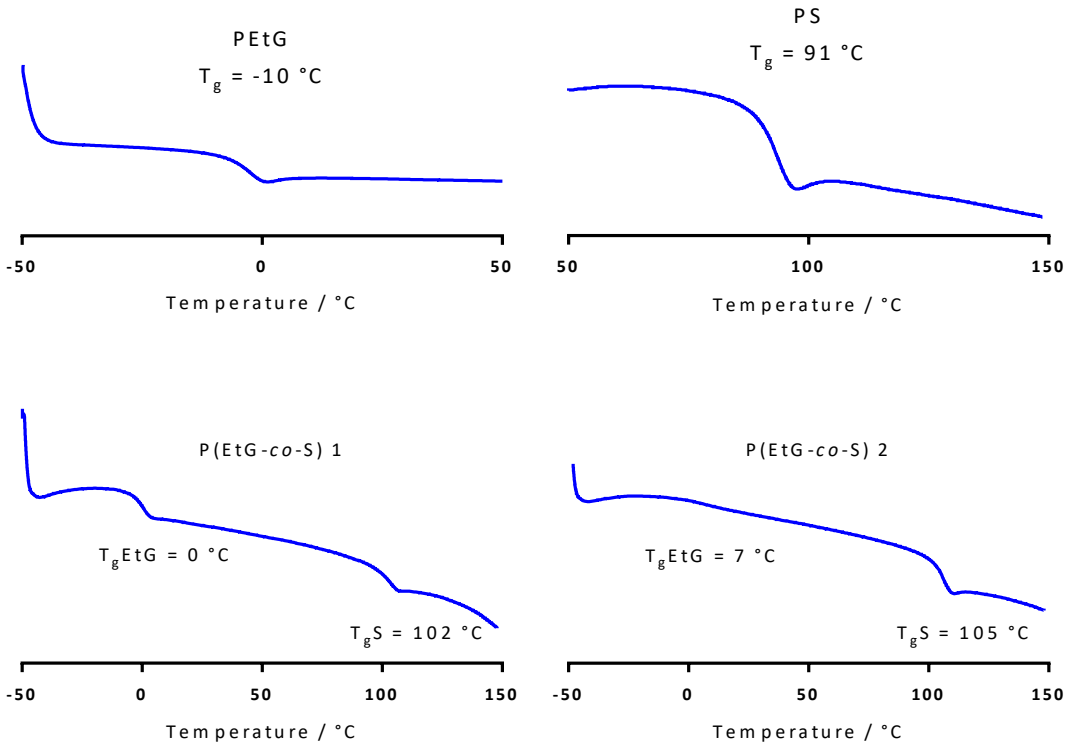


Figure 4-22. T_g data from DSC analysis (plotted 'exo up') of the PEtG homopolymer and copolymers. The PS homopolymer synthesised previously is included for comparison.

The heat flow curves for the copolymers show multiple glass transitions and this is indicative of a copolymer which exhibits phase separation. The transitions themselves occur at temperatures similar to the individual homopolymer temperatures which also strongly suggests that the blocks are phase separated and their glass transitions are not affected by the other polymer. PS chains will favourably interact with each other due to the aromatic stacking intermolecular force, and the PEtG chains will exhibit more polar interactions due to the oxygen atom in their structure. There is a small increase in temperature of the PEtG T_g and this could indicate a small amount of phase interaction but also could be due to the increase of molecular weight causing the end-group effect on T_g to have been diluted. However, the ultimate goal of increasing the T_g of the polymer to physiological temperature has not been achieved by the formation of block copolymers. Analysis of the mechanical properties of the films rather than thermal properties would give a better understanding of the properties and if sufficient improvement has been made for the copolymers to yield appropriate films.

4.1.3 *Biodegradation analysis of copolymers*

Finally, the polymers synthesised in this work were subject to a biodegradation study over 28 days. Five samples of each of the five polymers were prepared as solutions in THF and cast as films into 2 cm^3 centrifuge tubes by removal of the THF under vacuum to give 25 degradation test samples. The masses of the dried polymers were recorded so that the change in mass could be followed should any degradation of the polymers occur. The five different polymers tested were the PEtG labelled B5T5, PEtG end-capped with BIB, PS-*b*-PEtG-*b*-PS 1, PS-*b*-PEtG-*b*-PS 2 and the PS homopolymer synthesised by ATRP. It was expected that all polymers would degrade except for the PS homopolymer due to the previous evidence of degradation of PEtG subjected to a lipase solution. Four of each of the samples were spiked with 1 cm^3 of a 1% lipase CalB from *Candida Antarctica* in deionised water solution, with a final control sample spiked with 1 cm^3 of deionised water only. All samples were then submerged in a water bath at 38 °C for a period of 0, 7, 14 or 28 days. The control was also incubated for 28 days.

After the allocated time frame for each sample, the water was removed by centrifugation and the remaining solid was washed with fresh deionised water. The removed supernatant was freeze-dried to leave any lipase and water-soluble degradation products as a residue. This residue was then dissolved in D_2O for analysis by ^1H NMR spectroscopy. The samples which did undergo degradation gave multiple peaks in their NMR spectra showing the presence of degradation products (example shown in figure 4-23).For example, the peak at 5.1 ppm could correspond to glyoxylic acid.[199] The three samples which contained polystyrene did not result in any dissolved degradation products implying that no degradation had occurred. The control samples gave the same results by NMR spectroscopy as the samples which contained lipase implying that the degradation occurred without the need for the enzyme catalysis.

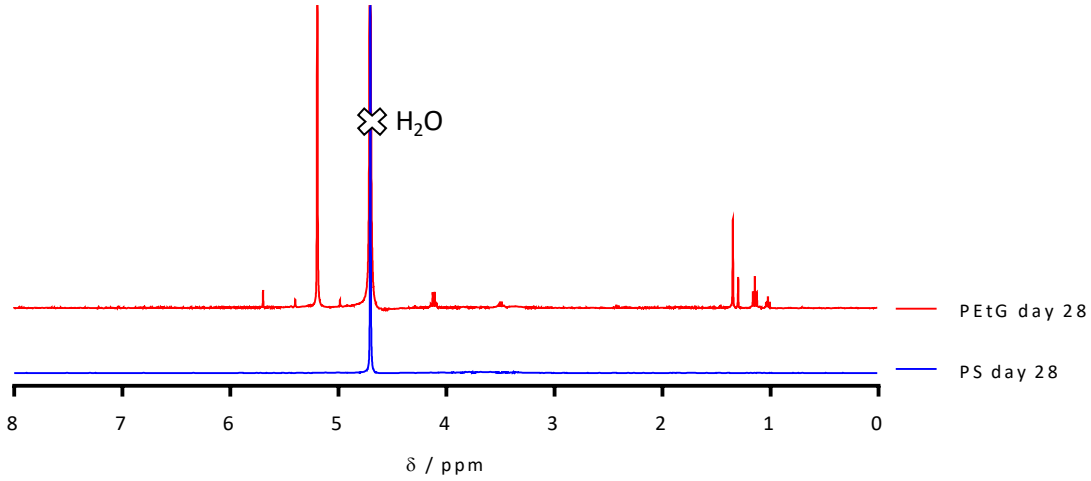


Figure 4-23. Stacked example NMR spectra in D_2O of the freeze-dried supernatants from the 28 day sample of PEtG (BIB-capped) and PS polymers.

The undissolved solid collected by centrifugation was also freeze-dried to remove any residual water and then analysed by GPC to observe any change in molecular weight due to degradation (figure 4-24). For all three polymers which contained styrene, solid polymer remained after 28 days. For the other two polymers, there was no obvious evidence of any remaining polymer in the centrifuge tube, implying total degradation had occurred. GPC analysis of all polymers shows that the polystyrene-based polymers remained generally unchanged and hence did not undergo any degradation over the 28 days. The two PEtG homopolymers which did not contain any styrene groups were successfully fully degraded. In all cases the control sample gave the same results as the sample which had been incubated with lipase, further evidence that deionised water alone was sufficient to cause the degradation to occur.

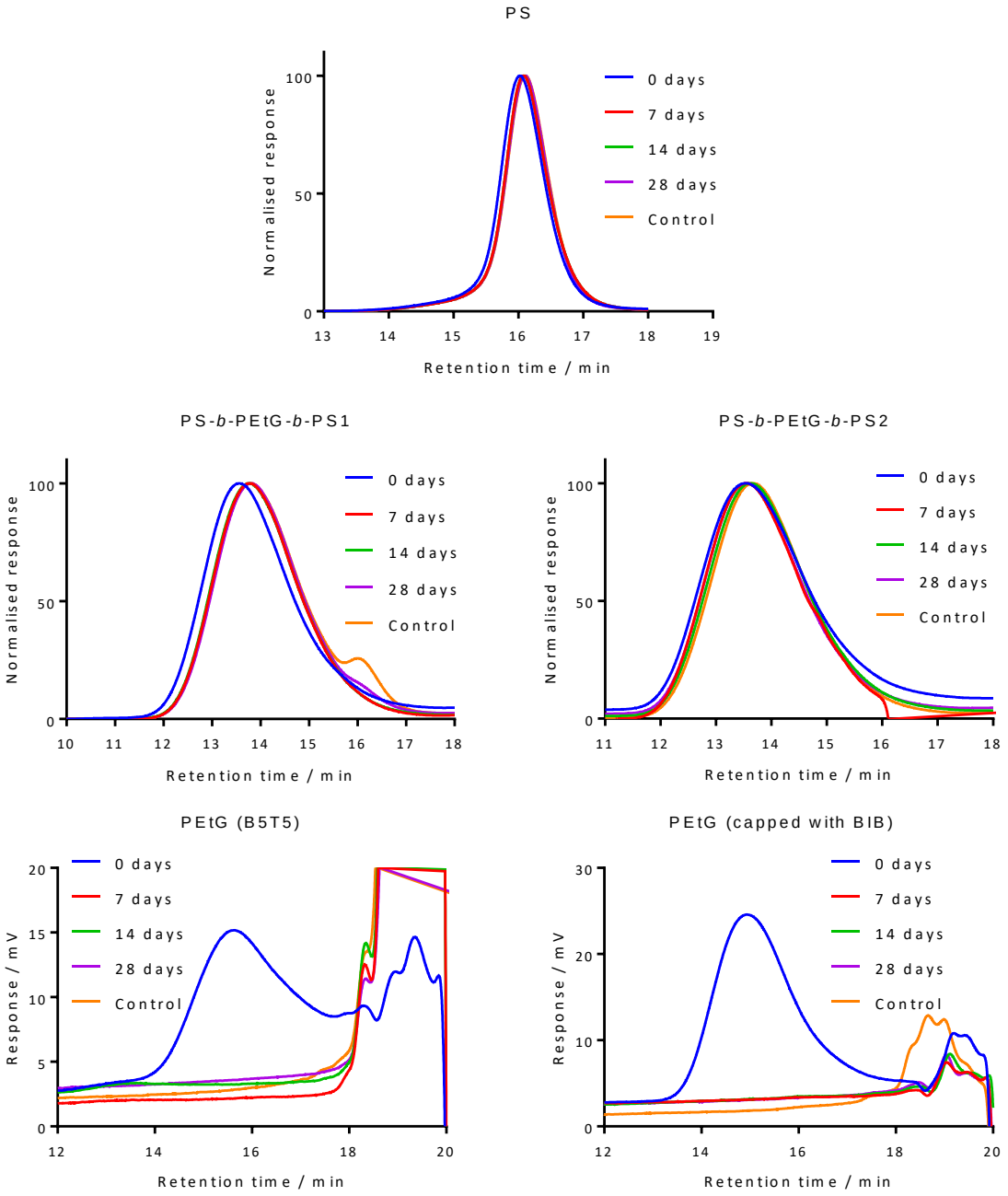


Figure 4-24. Stacked GPC chromatograms (RI detection) of the five test samples. The PEtG samples are not normalised due to the solvent fronts being the predominant peaks.

The final method of degradation analysis was by measuring the solid mass decrease over time. For this, the dried samples which were later dissolved for GPC were first weighed and the mass of solid remaining was determined. The percentage mass lost is shown in figure 4-25. The data corroborates well with the GPC and NMR data and suggests that the polystyrene samples did not lose any mass over time (results within the error of the method). The two PEtG

homopolymers show to decrease in mass by almost 100%, implying that almost 100% of the polymer has degraded to water-soluble molecules or ethyl glyoxylate monomer. This correlates with the self-immolative behaviour of PEtG where degradation would be rapid once the end-group had been removed.

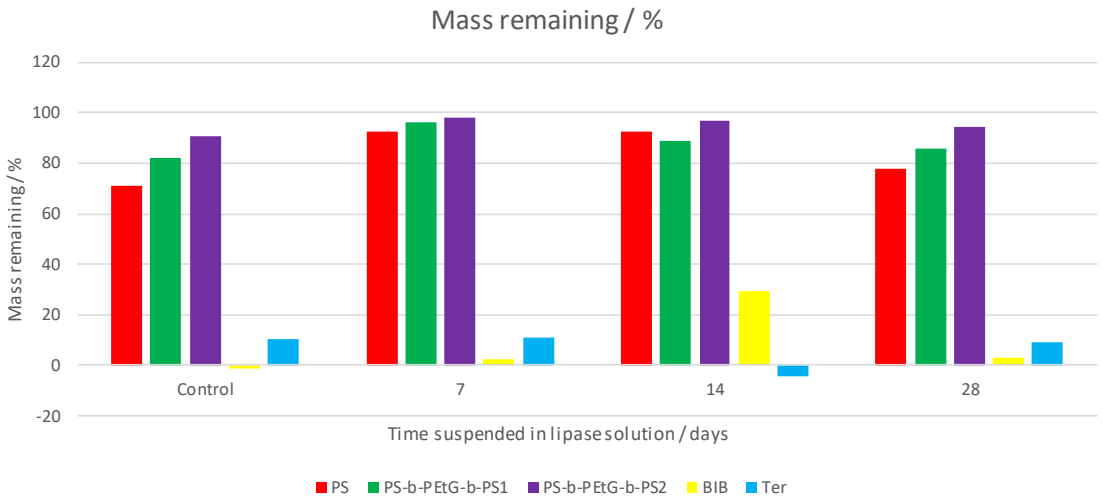


Figure 4-25. Bar chart displaying the results of the mass-lost method of degradation analysis.

4.3.Conclusions

The synthesis of PEtG as outlined in the literature has been carried out successfully with end-capping agents acetyl chloride, benzoyl chloride and α-bromoisobutyryl bromide. The reaction conditions have been optimised to ensure the high conversion of monomer. The key finding regarding the conditions was the importance of a controlled, low, temperature on the reproducibility and yield of polymerisations. Polymerisations that were carried out in a -20 °C freezer from the same batch of distilled monomer were found to reach similar molecular weights. Polymerisations which were carried out in salt/ice baths or from different monomer batches reached entirely different degrees of polymerisation, regardless of the ratio of end-capper to monomer which was used. Overall, the molecular weights did not exceed 19,000 g

mol^{-1} regardless of reaction conditions showing that there is still need for further optimisation if high molecular weight polymers are required.

The linking of the polymers to increase molecular weight was carried out using sebacoyl chloride and terephthaloyl chloride, with the former being largely unsuccessful due to the instability of the polymer product. Terephthaloyl chloride concentrations were varied in order to determine its effect on molecular weight and it was concluded that its use did increase molecular weight but it was not directly dependent on the concentration used, as the series of reactions produced similar molecular weight polymers, further strengthening the argument that the monomer batch and temperature were the most important variables to control.

A change in T_g was observed with the use of terephthaloyl chloride linker and this was then further analysed to determine the effect of linker and end-capper concentration on the T_g increase. For all linked polymerisations, the higher concentrations of terephthaloyl chloride produced a polymer with a larger T_g as determined by DSC and as observed by a decrease in the fluidity and tackiness of the polymer. The maximum T_g increase was from -35 °C to 0 °C by addition of 10 mol% TereCl. Higher concentrations of linker were not attempted due to the 5 mol% and 10 mol% inclusion concentrations having similar effects on T_g. The increase in T_g was required in order to make the PEtG polymers more suitable for the topical cosmetic application.

A final attempt at increasing T_g was carried out by block copolymerisation with the high T_g polymer, polystyrene. The BIB-capped PEtG was used as a macroinitatior in the copolymerisation of styrene by ATRP and was successful where concentrations of styrene were highest due to more efficient mixing. A wider range of molecular weights could be targeted by performing the reactions in solution rather than in the bulk to allow the efficient mixing when only small amounts of styrene monomer are used.

The overall appearance of the polymer was changed significantly by the copolymerisation as the PEtG homopolymers were generally synthesised in the form of viscous liquids whereas the

copolymers were isolated as powders. DSC analysis of the block-copolymers confirmed that they are phase-separated by the presence of two T_gs and therefore an overall increase in T_g had not been achieved. In order to produce block-copolymers with a singular T_g, two compatible polymers must be used. Previous work on copolymer phase separation has shown poly(styrene-*co*-methyl methacrylate) block copolymers to exhibit two T_g values by DSC.[200] However, poly(styrene-*co*-α-methyl styrene) block copolymers exhibit one T_g.[201] Therefore, a singular T_g might be more likely in PEtG copolymers with units such as ethyl methacrylate, methyl methacrylate or methyl glyoxylate due to the similarity in block structures and polarities. There is a large scope for further work in this area as many other copolymers can be synthesised by ATRP, with methyl methacrylate copolymers in particular having already been widely researched.[197] Nonetheless, further analysis to determine their mechanical properties of the copolymers synthesised in this work in comparison to the PEtG homopolymer would give a better insight into their possible application as film-forming materials.

Finally, the degradation studies carried out have shown that PEtG is readily degraded in water and does not require enzyme catalysis. However, once copolymerised with styrene, the self-immolative behaviour does not occur over 28 days. This negative result implies that the use of a biodegradable copolymer would be a more suitable alternative to styrene. One alternative to synthesis of a block copolymer could be the grafting of a synthetic polymer block onto a natural polymer such as cellulose, a technique which has been reported[202] but could be adjusted to find polymers with particular properties such as an appropriate T_g. Generally, ATRP has been used in the synthesis of a wide range of biodegradable block copolymers[203] and there is therefore an abundance of possible copolymer combinations that could be synthesised in order to find a suitable material for the application as a biodegradable cosmetic film-former.

Chapter 5: Overall Conclusions and Outlook

The aim of this work was to synthesise a material which would be appropriate for use in an instant-effect cosmetic product and that would pass an externally performed 'ready biodegradability' test. The initial efforts at producing microbead materials from PVAc afforded spherical particles with some control over particle size and size distribution by use of a copolymer suspension stabiliser. Emulsion polymerisation produced round nano-sized particles whereas suspension polymerisation afforded larger particles of up to 1 cm in length. The design of a P(BMA-*stat*-MAA) copolymeric stabiliser and optimisation of stirring speed and stabiliser concentration enabled the targeting of particles in the size range desired for the application at approximately 5 µm in diameter. However, on external biodegradation testing, these particles degraded too slowly as they reached only 7% degradation over a 28 day timescale. The required degradation for the material to be characterised as biodegradable is >60%. PVAc is known to degrade by hydrolysis to PVA followed by enzymatic degradation, however, in particle form this is slowed likely due to the tight-packing of the polymer preventing water and therefore bacteria from reaching any further than the particle surface.

Nonetheless, attempts were made at making particles from a PEU material which has more recently been shown to degrade reasonably quickly in comparison to PLGA, specifically *in vivo*. Control over the shape and size of these particles was more difficult and SEM showed random shapes with some round nanoparticles and lots of amorphous regions. Simple testing with lipase showed that the enzyme was unable to catalyse any degradation and therefore external testing was not carried out. These particles did however perform well as pore-filling agents, which was somewhat surprising considering their irregular shape and size. It was during this work that legislation was passed which influenced the needs of the industrial sponsor and from this point all work on microparticles was ceased to look at alternative film-forming materials. However, future scope for PEU materials as film-forming materials could be investigated. The blocky

structure could be the key in creating flexible films, for example, by use of a longer and more flexible diol between the hard urea units.[186,204]

The initial attempts at producing a viable film-forming material considered the use of poly(ethyl glyoxylate) as a film-former largely for its self-immolative behaviour. The attempts at increasing its T_g by incorporating a stiffer functional group such as terephthaloyl chloride into the polymer backbone was successful but not to the extent required for the formation of films on skin. A maximum T_g of 0 °C was achieved when using the linking group at 5-10 w/w%. The block copolymerisation with styrene however did alter the material's properties and is a good starting point for the further production of PEtG block copolymers to target specific mechanical properties. Dynamic mechanical analysis (DMA) would be a useful technique used to gather more information about the block copolymers' mechanical properties with respect to temperature to ensure they would behave appropriately when applied to skin.[205]

Ideas for potential future work with respect to these individual areas of study are as follows. For the preparation of monodisperse, round microparticles which pass 'ready biodegradability' testing, a wider range of possible copolymeric stabilisers could be designed, specifically from polymers which themselves are biodegradable. The use of a more hydrophilic stabilising polymer could also improve degradation by better enabling the diffusion of water into the particles hence providing more sufficient contact between the degrading enzymes and the material. In terms of the PEU synthesis in particular, the use of amore hydrophilic amino acid such as serine or asparagine could produce more hydrophilic particles.

The self-immolative behaviour of PEtG shows promise in biodegradable polymers, especially with respect to the wide range of trigger-sensitive end-groups that could be used. The degradation with styrene copolymers was unsuccessful, however, the use of a biodegradable polymer such as an aliphatic polyester in its place could provide the initial biodegradation pathway which could be followed by the self-immolation of the PEtG. The appropriate

copolymer should be selected from those already assessed to be biodegradable to ensure a fast-enough degradation profile whilst ensuring the best elastic properties to be a suitable film-forming material.

Finally, a potentially very useful area to look into would be the design of a simple but accurate degradation testing method to enable more products to be screened for their potential end-use in cosmetics. The modified Sturm test requires large amounts of material and complex apparatus. However, the basic principle of inoculating a sample with a high concentration of bacteria, such as with 'activated sludge', and analysing the amount of CO_2 evolved could potentially be applied on a smaller scale or a shorter timescale by using an "accelerated conditions" method similar to those used in standard cosmetic stability testing.[206]

Overall, this work has shown that properties of polymers which are not traditionally used in topical cosmetics can be optimised towards this application. However, the potential for the use of new and more complex polymers in cosmetics is possibly decreasing due to industry focussing on the use of more natural, sustainable materials. Therefore, I believe that further research in this field should begin to focus on the functionalisation of natural polymers such as polypeptides, as these have already shown promise as elastic film-formers.[105,207] Similarly, for products which give anti-aging effects by mattifying the skin, microparticles synthesised from proteins would be suitable alternatives to the polyethylene and silicone based materials.[122,208] Providing these materials can be made cheaply, they should appeal to the mass market of consumers who are becoming increasingly conscientious of the chemicals they are using, both for their own perceptions about what constitutes a product that is completely safe for use on the skin and for the wider implications on the environment.

Chapter 6: Experimental Methods and Materials

6.1.General methods

Nuclear magnetic resonance (NMR) spectroscopy:

All ^{1}H NMR spectra were recorded in solution state using a Bruker Avance spectrometer at 400 MHz, with 64 scans per spectrum. All spectra were analysed using Topspin 3.2.

Transmission electron microscopy (TEM):

Copper/palladium TEM grids were surface-coated in-house to produce a thin film of amorphous carbon. The grids were then plasma glow-discharged for 30 seconds to create a hydrophilic surface. They were then immersed in 0.1 wt % aqueous dispersions containing the polymer particles for 40 seconds. After blotting to remove excess dispersion, the grids were negatively stained using an aqueous uranyl formate solution for 15 seconds. The grids were blotted and dried with a vacuum hose and imaging was performed using a Philips CM100 instrument operating at 100 kV and equipped with a Gatan 1 k CCD camera.

Scanning electron microscopy (SEM):

The powder samples were applied as a thin layer on carbon-taped aluminium stubs and sputtered with gold at a rate of 5 nm/min for 4 min. SEM was performed on an Inspect F FEG instrument at a voltage of 5 kV.

Dynamic Light Scattering (DLS):

Measurements were conducted at 25 °C using a Malvern Instruments Zetasizer Nano series instrument. Samples were prepared at 0.1 wt % in water in disposable plastic cuvettes and hydrodynamic diameters were determined from measurements averaged over thirty runs using the Stokes-Einstein equation.

Infra-red (IR) Spectroscopy:

IR spectra were recorded in the solid state using a Thermo Scientific Nicolet iS10 ATR spectrometer. The % transmission was recorded over an average of 22 scans between the wavenumbers 4000 and 750 cm^{-1}.

Gel permeation chromatography (GPC):

DMF eluent system: analysis was performed using an Agilent 1260 infinity LC system fitted with two PLgel 5 µm mixed-C columns heated to 50 °C. A series of near-monodisperse PMMA standards were used for calibration in conjunction with a refractive index detector. Samples were prepared at 2 mg/cm^{-3} and filtered through a 45 µm PTFE filter to remove undissolved polymer and contaminants before injection of 100 µL of sample dissolved in DMF with 0.1% toluene as a flow-rate marker. DMF eluent contained 2.5 g lithium bromide per 2.5 L and flow-rate was set at 1 mL min^{-1} for all samples.

THF/acetic acid eluent system: analysis was performed using an Agilent PL-GPC 50 Integrated GPC system fitted with two PLgel 5 µm mixed-C columns heated to 50 °C. A series of near-monodisperse PMMA standards were used for calibration in conjunction with a refractive index detector. Samples were dissolved at 2 mg/cm^{-3} in THF/acetic acid (4 % acid) before injection of 300 µL of sample at a flow-rate of 1 cm^{-3} min^{-1}. Eluent also contained 0.025 % BHT antioxidant.

Optical microscopy:

Images either were recorded using a Motic DMBA300 digital biological microscope equipped with a built-in camera and analyzed using Motic Images Plus 2.0 ML software, or using a Kern Optics OBL 155 digital microscope with a ODC832 Camera and analysed using 'Microscope VIS'. Image J software was used for particle sizing.

Melting point analysis:

Melting points were identified using a Stuart SMP50 automatic melting point instrument. Temperature was increased at 2.0 °C/min and values were calculated from the average of two measurements.

Differential scanning calorimetry:

DSC thermograms were obtained using a TA-DSC25 calorimeter and analysed using Trios software v4.4.0.41651 in 'exotherm up' mode. Liquid samples were packed into Tzero® aluminium pans and solid samples were crimped using a sample press. Thermograms were obtained by heating and samples at 10 °C/min in a N_2 atmosphere. Measurement conditions were as follows:

Terephthaloyl chloride tests	Polystyrene	PEtG-PS copolymers
1. Equilibrate to -50 °C	1. Equilibrate to 30 °C	1. Equilibrate to -50 °C
2. Ramp 10 °C/min to 80 °C	2. Ramp 10 °C/min to 150 °C	2. Ramp 10 °C/min to 150 °C
3. Ramp 10 °C/min to -50 °C	3. Ramp 10 °C/min to 30 °C	3. Ramp 10 °C/min to -50 °C
4. Equilibrate at -50 °C	4. Equilibrate at 30 °C	4. Equilibrate at -50 °C
5. Ramp 10 °C/min to 80 °C	5. Ramp 10 °C/min to 150 °C	5. Ramp 10 °C/min to 150 °C

6.2.Chapter 2 – Experimental procedures

Emulsion polymerisation of vinyl acetate

PVA (Mowiol 8-88, M_n 67 kDa) (1.10 g), sodium dodecylbenzenesulfonate (0.09 g, 2.5 x 10^{-4} moles) and sodium hydrogen carbonate (0.09 g, 1.1 x 10^{-3} moles) were dissolved in deionised water (6.22 cm^3) and purged with N_2 at 66 °C for 30 minutes. An initial portion of vinyl acetate (0.68 g, 7.9 x 10^{-3} moles) was added to the mixture followed by ammonium persulfate (0.091 g, 4.0 x 10^{-4} moles, dissolved in 0.72 cm^3 H_2O). The remaining vinyl acetate (7.0 g, 8.3 x 10^{-2} moles) was added over 135 min via syringe pump (0.05 cm^3 min^{-1}) and after addition the suspension was stirred at 66 °C for a further 2 h. The suspension of was cooled and exposed to air for 1 h. DLS and TEM were performed on 0.1 wt% dispersions.

Suspension polymerisation of vinyl acetate with PVA stabiliser

PVA (Mowiol 8-88, M_n 67 kDa) (1.22 g, 2.44 g or 4.88 g) was dissolved in water (60 cm^3) and AIBN (0.2 g, 1.22 x 10^{-3} moles) was dissolved in vinyl acetate (20.0 g, 2.4 x 10^{-1} moles). Both

solutions were purged with N_2 separately for 30 minutes at room temperature. The vinyl acetate solution was added to the PVA solution and the temperature was increased to 64 °C. The temperature was kept constant and the stirring speed was held at 500 rpm for the duration of the reaction (4 hours). At completion, the polymerisation was allowed to cool for 30 minutes. The resulting polymer beads were washed with water and excess liquid was decanted off after centrifugation at 4500 rpm for 5 minutes.

Suspension polymerisation of vinyl acetate using PVP_{90} as a steric stabiliser

PVP_{90} (M_w 10 kDa) (1.22 g, 2.44 g or 4.88 g) was dissolved in water (60 cm^3) at 30 °C and purged with N_2 for 30 minutes. AIBN (0.2 g, 1.22 x 10^{-3} moles) was dissolved in vinyl acetate (15.36, 15.66 or 16.27 g) and purged with N_2 in ice for 30 minutes to prevent evaporation of vinyl acetate. The vinyl acetate solution was added to the PVP solution and the temperature was raised to 64 °C. The stirring rate was held at 300 or 500 rpm for 4 hours. After polymerisation, the reaction vessel was removed from the heat source and exposed to air. The stabilised PVAc particles were separated from the suspension by filtration or by centrifugation at 4500 rpm for 5 minutes and washed in warm water in order to remove excess stabiliser, initiator and unreacted vinyl acetate. For the smaller particles, a 1% dispersion of the polymer beads in water was imaged by optical microscopy. Larger particles were imaged as dry beads. δ_H (400 MHz $CDCl_3$) 1.6 –1.9 (2H, m, PVAc backbone C**H**$_2$) 2.0 (3H, m, PVAc C**H**$_3$) 4.8-5.0 (1H, m, PVAc backbone C**H**).

Polymerisation of vinyl acetate with no stabiliser

AIBN (0.2 g, 1.22 x 10^{-3} moles) was dissolved in vinyl acetate (15.36 g, 1.9 x 10^{-1} moles) and purged with N_2 in ice for 30 minutes to prevent evaporation of vinyl acetate. The vinyl acetate solution was added to water (60 cm^3), the temperature was raised to 64 °C and stirring rate held at 500 rpm for 4 hours. After polymerisation, the reaction vessel was removed from the heat source and exposed to air to terminate the polymerisation. The solid PVAc coagulum was removed from the reaction and dried.

Synthesis of P(BMA-stat-MAA) for use as a steric stabiliser

Isopropanol (IPA) (70 cm^3) and a mixture of butyl methacrylate (17.04 g, 1.2 x 10^{-1} moles), methacrylic acid (6.88 g, 7.9 x 10^{-2} moles) and AIBN (1 g, 6.09 x 10^{-3} moles) were purged with N_2 separately for 30 minutes. The monomer mixture was added to the IPA with stirring and temperature was set at 70 °C for 3 h. The reaction was then exposed to air and allowed to cool for 30 minutes. The polymer was precipitated from water, redissolved in THF and precipitated again into petroleum ether. It was then dried to give the white solid poly(butyl methacrylate-stat-methacrylic acid). ν_{max}/cm^{-1}(solid state) 3200 (O-H) 2900 (CH) 1750 (C=O). δ_H (400 MHz;$(CD_3)_2CO$) 1.0 (9H, m, backbone C**H**$_3$ and BMA C**H**$_3$) 1.4 (2H, s, C**H**$_2$ of BMA) 1.7 (2H, s, C**H**$_2$ of BMA) 1.8-2.1 (4H, m, backbone C**H**$_2$) 4.0 (2H, s, O-C**H**$_2$).

Alkylation of P(BMA-stat-MAA) for composition analysis

P(BMA-stat-MAA) (0.60 g) was dissolved in DMF (6 cm^3). Caesium carbonate (0.710 g, 2.2 x 10^{-3} moles) was added along with benzyl bromide (0.375 g, 2.2 x 10^{-3} moles) and stirred for 22 hours at room temperature. Solid salts were removed by filtration, washed with DMF and the filtrate collected. DMF was removed by rotary evaporation and the crude polymer was redissolved in chloroform and washed with deionised water (3 × 30 cm^3). The chloroform was then removed by rotary evaporation and the solid product was dried under vaccum for 1-2 days to give solid poly(butyl methacrylate-*stat*-benzyl methacrylate). ν_{max}/cm^{-1}(solid state) 2900 (CH) 1750 (C=O). δ_H (400 MHz;$(CD_3)_2CO$) 1.0 (9H, m, backbone C**H**$_3$ and BMA C**H**$_3$) 1.4 (2H, s, C**H**$_2$ of BMA) 1.7 (2H, s, C**H**$_2$ of BMA) 1.8-2.1 (4H, m, backbone C**H**$_2$) 4.0 (2H, s, O-C**H**$_2$) 5.0 (2H, s, C**H**$_2$ of Bz) 7.4 (5H, m, aromatic C**H**).

Suspension polymerisation of vinyl acetate using p(BMA-*stat*-MAA) as a steric stabiliser

P(BMA-*stat*-MAA) (1.30 g, 2.61 g or 5.22 g) was dissolved in water (60 cm^3) with the aid of 2 mol equivalents of $NaHCO_3$ at 30 °C and purged with N_2 for 30 minutes. AIBN (0.2 g, 1.22 x 10^{-3} moles) was dissolved in vinyl acetate (15.43, 15.75 or 16.40 g) and purged with N_2 for 30 minutes in ice to minimise the evaporation of vinyl acetate. The vinyl acetate solution was added to the

P(BMA-*stat*-MAA) solution and the temperature was raised to 64 °C and stirring rate held at 300, 400 or 500 rpm for 4 hours. After polymerisation, the reaction vessel was removed from the heat source and exposed to air. The stabilised PVAc particles were separated from the suspension by centrifugation at 4500 rpm for 5 minutes and washed in warm water twice in order to remove excess stabiliser, initiator and unreacted vinyl acetate. Kinetic analysis by gravimetry showed conversion of the monomer to be approximately 50 %. A 1 wt% dispersion of the polymer beads in water was imaged by optical microscopy. δ_H (400 MHz;$CDCl_3$) 1.6 –1.9 (2H, m, PVAc backbone C**H**$_2$) 2.0 (3H, m, PVAc C**H**$_3$) 4.8-5.0 (1H, m, PVAc backbone C**H**).

6.3.Chapter 3 – Experimental procedures

Synthesis of di-*p*-toluenesulfonic acid salt of bis-L-alanine hexane-1,6-diester (ALA-HEX salt)

Alanine (3.47 g), 1,6-hexanediol (2.00 g) and toluenesulfonic acid monohydrate (7.40 g) were added to toluene (50 cm^3) and stirred under N_2 at 110 °C for 22 hours. A Dean-Stark apparatus was used to remove water during the reaction. A white solid was produced which hardened on cooling and allowed decantation of the toluene. Recrystallisation was performed three times from ethanol to yield the desired product as a white solid. Yield: 10.24 g, 87%; mp: 182 °C; Found: C, 51.6%; H, 6.70%; N, 4.5%; S, 7.4%.Calc. for $C_{26}H_{40}N_2O_{10}S_2$; C, 51.6%; H, 6.7%; N, 4.6%; S, 10.6%; ν_{max}/cm^{-1}(solid state) 2800 (C-H), 1750 (C=O); δ_H (400 MHz; d^6-DMSO) 1.4 (6H, d, C*H*$_3$) 1.7 (4 H, quintet, C*H*$_2$) 2.3 (6H, s, C*H*$_3$) 4.1 (2 H, quartet, C*H*) 4.2 (4H, t, C*H*$_2$) 7.3 (4H, d, aromatic) 7.6 (4H, d, aromatic).

Synthesis of di-*p*-toluenesulfonic acid salt of bis-L-glycine hexane-1,6-diester (GLY-HEX salt)

Glycine (2.92 g), 1,6-hexanediol (2.00 g) and toluenesulfonic acid monohydrate (7.39 g) were added to toluene (50 cm^3) and stirred under N_2 at 110 °C for 22 hours. A Dean-Stark apparatus was used to remove water during the reaction. A white solid was produced which hardened on cooling and allowed decantation of the toluene. Recrystallisation was performed three times from ethanol to yield the desired product as a white solid. Yield: 8.30 g, 74%; mp: 80 °C; Found:

C, 46.7%; H, 6.4%; N, 4.5%; S, 11.1%.Calc. for $C_{24}H_{36}N_2O_{10}S_2$; C, 50.0%; H, 6.3%; N, 4.9%; S, 11.1%; ν_{max}/cm^{-1}(solid state) 2800 (C-H), 1750 (C=O); δ_H (400 MHz; d^6-DMSO) 1.5 (6H, d, C*H*$_3$) 1.7 (4 H, quintet, C*H*$_2$) 2.3 (6H, s, C*H*$_3$) 4.1 (2 H, quartet, C*H*) 4.2 (4H, t, C*H*$_2$) 7.3 (4H, d, aromatic) 7.6 (4H, d, aromatic).

Interfacial polymerisation of the di-*p*-toluenesulfonic acid salt of bis-L-alanine hexane-1,6-diester (ALA-HEX PEU)

ALA-HEX salt (1.00 g) and Na_2CO_3 (0.37 g) were dissolved in water (30 cm ^{3}) at 30 °C with overhead stirring at 250 rpm and under N_2. The aqueous mixture was cooled in ice and a further portion of Na_2CO_3 was added (0.175 g in 6 cm^3 water). Triphosgene (0.17 g in 5 cm^3 $CHCl_3$) was added quickly and the reaction was allowed to stir for 30 minutes in ice. A further portion of triphosgene (0.05 g in 1 cm^3 $CHCl_3$) was added dropwise over 5 minutes. The polymerisation was then allowed to continue for a further 2 hours at room temperature. The reaction mixture was washed with 2 × 10 cm^3 portions of cold deionised water. The organic phase with the PEU product suspended in it was precipitated into hot water (90 °C) to remove chloroform and give solid PEU polymer which was dried under vacuum. ν_{max}/cm^{-1}(solid state) 3300 (N-H), 1650 (C=O); δ_H (400 MHz; d^6-DMSO) 1.2 (3H, C*H*$_3$), 1.3 (C*H*$_2$), 1.6 (C*H*$_2$), 4.0 (C*H*$_2$-O), 4.1 (C*H*), 6.4 (N*H*).

Interfacial polymerisation of the di-*p*-toluenesulfonic acid salt of bis-L-glycine hexane-1,6-diester (GLY-HEX PEU)

GLY-HEX salt (1.00 g) and Na_2CO_3 (0.39 g) were dissolved in water (30 cm ^{3}) at 30 °C with overhead stirring at 250 rpm and under N_2. The aqueous mixture was cooled in ice and a further portion of Na_2CO_3 was added (0.19 g in 6 cm^3 water). Triphosgene (0.18 g in 5 cm^3 $CHCl_3$) was added quickly and the reaction was allowed to stir for 30 minutes in ice. A further portion of triphosgene (0.05 g in 1 cm^3 $CHCl_3$) was added dropwise over 5 minutes. The polymerisation was then allowed to continue for a further 2 hours at room temperature. The reaction mixture was washed with 2 × 10 cm^3 portions of cold deionised water. The organic phase with the PEU product suspended in it was precipitated into hot water (90 °C) to remove chloroform and give

solid PEU polymer which was dried under vacuum. ν_{max}/cm^{-1}(solid state) 3300 (N-H), 1650 (C=O); δ_H (400 MHz; d^6-DMSO) 1.4 (C*H*$_2$), 1.5 (C*H*$_2$), 3.7 (C*H*$_2$-O), 4.1 (C*H*$_2$-NH), 6.5 (N*H*).

Direct synthesis of polymer particles in the interfacial polymerisation of the di-p-toluenesulfonic acid salt of bis-L-alanine hexane-1,6-diester or bis-L-glycine hexane-1,6-diester using Span®85 stabiliser (ALA-HEX SPAN and GLY-HEX SPAN)

ALA-HEX salt or GLY-HEX salt (1.00 g) and Na_2CO_3 (0.37 g) were dissolved in water (30 cm^3) at 30 °C with overhead stirring at 250 rpm and under N_2. The aqueous mixture was cooled in ice and a further portion of Na_2CO_3 was added (0.175 g in 6 cm^3 water). SPAN®85 (0.15, 0.25 or 0.35 wt%) and triphosgene (0.17 g in 5 cm^3 $CHCl_3$) was added quickly and the reaction was allowed to stir for 30 minutes in ice. A further portion of triphosgene (0.05 g in 1 cm^3 $CHCl_3$) was added dropwise over 5 minutes. The polymerisation was then allowed to continue for a further 2 hours at room temperature. PEU particles were washed by centrifugation at 4500 rpm for 5 minutes and were isolated from water and dried under vacuum to give a fine white powder. δ_H (400 MHz; 1:3 d^4-Methanol and d^6-DMSO) ALA-HEX 1.2 (3H, C*H*$_3$), 1.3 (C*H*$_2$), 1.6 (C*H*$_2$), 4.0 (C*H*$_2$-O), 4.1 (C*H*), 6.4 (N*H*); GLY-HEX 1.4 (C*H*$_2$), 1.5 (C*H*$_2$), 3.7 (C*H*$_2$-O), 4.1 (C*H*$_2$-NH), 6.5 (N*H*).

Direct synthesis of polymer particles in the interfacial polymerisation of the di-p-toluenesulfonic acid salt of bis-L-alanine hexane-1,6-diester or bis-L-glycine hexane-1,6-diester using P(BMA-stat-MAA) as a stabiliser

ALA-HEX salt (1.00 g) and Na_2CO_3 (0.39 g) were dissolved in water (30 cm^3) at 30 °C with overhead stirring at 250 rpm and under N_2. The aqueous mixture was cooled in ice and a further portion of Na_2CO_3 was added (0.19 g in 6 cm^3 water). A portion of 4% solution of P(BMA-stat-MAA) was added in order to give an overall reaction concentration with 2% stabiliser. Triphosgene (0.18 g in 5 cm^3 $CHCl_3$) was added quickly and the reaction was allowed to stir for 30 minutes in ice. A further portion of triphosgene (0.05 g in 1 cm^3 $CHCl_3$) was added dropwise over 5 minutes. The polymerisation was then allowed to continue for a further 2 hours at room temperature. PEU particles were washed by centrifugation at 4500 rpm for 5 minutes and were isolated from water and dried under vacuum to give a fine white powder. δ_H (400 MHz; 1:3 d^4-

Methanol and d^6-DMSO) ALA-HEX 1.2 (3H, CH_3), 1.3 (CH_2), 1.6 (CH_2), 4.0 (CH_2-O), 4.1 (CH), 6.4 (NH); GLY-HEX 1.4 (CH_2), 1.5 (CH_2), 3.7 (CH_2-O), 4.1 (CH_2-NH), 6.5 (NH).

Direct synthesis of polymer particles in the interfacial polymerisation of the di-p-toluenesulfonic acid salt of bis-L-alanine hexane-1,6-diester, bis-L-glycine hexane-1,6-diester or a mixture of both using P(BMA-stat-MAA) as a stabiliser with homogenisation

ALA-HEX salt, GLY-HEX salt or both salts (total 1.00 g) and Na_2CO_3 (0.39 g) were dissolved in water (30 cm^3) at 30 °C with magnetic stirring for 30 minutes. The aqueous mixture was cooled in ice and a further portion of Na_2CO_3 was added (0.19 g in 6 cm^3 water). A portion of 4% solution of P(BMA-stat-MAA) was added in order to give an overall reaction concentration with 2% stabiliser. Triphosgene (0.18 g in 5 cm^3 $CHCl_3$) was added quickly and the reaction was homogenised at 3000 rpm for 30 minutes in ice. A further portion of triphosgene (0.05 g in 1 cm^3 $CHCl_3$) was added dropwise. The polymerisation was then allowed to continue for a further 30 minutes with homogenisation at room temperature. PEU particles were washed by centrifugation at 4500 rpm for 5 minutes and were isolated from water and dried under vacuum to give a fine white powder. δ_H (400 MHz; 1:3 d^4-Methanol and d^6-DMSO) ALA-HEX 1.2 (3H, CH_3), 1.3 (CH_2), 1.6 (CH_2), 4.0 (CH_2-O), 4.1 (CH), 6.4 (NH); GLY-HEX 1.4 (CH_2), 1.5 (CH_2), 3.7 (CH_2-O), 4.1 (CH_2-NH), 6.5 (NH).

PEU particle degradation studies

ALA-HEX, GLY-HEX and mixed ALA-GLY PEU particles stabilised with P(BMA-*stat*-MAA) (0.1 g) were suspended in a Lipase CalB/DPBS solution (1 cm^3, 0.1% lipase) and incubated at 37 °C for 2,4 and 8 weeks. After the allocated time, samples were centrifuged to remove supernatant and freeze-dried before analysis by SEM.

6.4.Chapter 4 – Experimental procedures

Synthesis of poly(ethyl glyoxylate)

Ethyl glyoxylate (10 cm^3, 50% solution in toluene) was first distilled over P_2O_5 (1.5 g) under vacuum at 60 °C to remove solvent and residual water to leave a mixture of ethyl glyoxylate

oligomers as a colourless liquid. This mixture was then cracked over P_2O_5 at 150 °C and distilled under N_2 to give pure ethyl glyoxylate monomer as a yellow liquid. After collection of approximately 2 cm^3 of monomer and without exposing to air, dry DCM (2 cm^3) was added and the reaction vessel was submerged in an insulated ice/salt bath to maintain a temperature of approximately -20 °C. Triethylamine (20 µL) was added to initiate the polymerisation which was then stirred for 1 h before removal of the ice bath. The reaction was stirred at room temperature for a further 24 h and then heated to 40 °C for another 16 h overnight. The vessel was then removed from the heat and exposed to air and the colourless, viscous liquid diluted with a further 2 cm^3 of DCM before precipitating from hexane. A translucent white residue of impure polymer settled on the bottom and was dissolved into THF before precipitating directly into centrifuge tubes filled with deionised water. After 10 minutes at 4,500 rpm, followed by lyophilisation, polyethylglyoxylate was collected as a colourless, viscous liquid. δ_H (400 MHz; d^6-DMSO) 1.3 (C*H*$_3$ of pendant ethyl), 4.2 (C*H*$_2$ of pendant ethyl), 5.5-5.7 (C*H* of polymer backbone).

Synthesis of poly(ethyl glyoxylate) with acetyl chloride, benzoyl chloride and α-bromoisobutryl-bromide end cappers

Distillation of ethyl glyoxylate solution was performed as previously described. Without exposure to air, 2 cm^3 portions were added into sealed scintillation vials and stirred in an ice/salt bath or freezer at approximately -20 °C. Dry DCM (1.6 cm^3) and triethylamine (20 µL) were added to begin polymerisation. The solution changed from yellow to colourless on addition of initiator. After 1 h, acetyl chloride, benzoyl chloride or α-bromoisobutryl bromide at concentrations of 1, 2, 5 or 10 mol% were added as an end capper and stirred at room temperature. After 24h, the reactions were heated to 40 °C for 16 h overnight. Vials were opened to air and the reaction mixture diluted with 2 cm^3 DCM before precipitation and isolation as previously described. All end-capped polymers were isolated as colourless viscoelastic solids. (400 MHz; d^6-DMSO) 1.3 (C*H*$_3$ of pendant ethyl), 1.7 (C*H*$_3$ of acetyl group), 4.2 (C*H*$_2$ of pendant ethyl), 5.5-5.7 (C*H* of polymer backbone), 8.0-8.4 (benzyl protons).

Revised polymerisation procedure

Distillation of ethyl glyoxylate solution was performed as previously described. Without exposure to air, 2 cm^3 portions were added into sealed scintillation vials with anhydrous DCM (1.6 cm^3) and stirred for 5 minutes at room temperature. Triethylamine (20 µL) was added to begin polymerisation and the vials were stirred for 20 min in the freezer (-20 °C). Benzoyl chloride (1 mol%) was added and reactions were stirred for a further 3 h. Polymerisations were then stirred at room temperature for 16 hours overnight. Vials were opened to air and the reaction mixture diluted with 2 cm^3 DCM before precipitation and isolation as colourless viscoelastic solids. (400 MHz; d^6-DMSO) 1.3 (CH_3 of pendant ethyl), 1.7 (CH_3 of acetyl group), 4.2 (CH_2 of pendant ethyl), 5.5-5.7 (C*H* of polymer backbone), 8.0-8.4 (benzyl protons).

Synthesis of poly(ethyl glyoxylate) with sebacoyl chloride linker

Distillation of ethyl glyoxylate solution was performed as previously described and 2 cm^3 portions of monomer with DCM (2 cm^3) and triethylamine (20 µL) were added into sealed scintillation vials immersed in an ice/salt bath and stirred for 1 h. Sebacoyl chloride (1, 2 , 5 and 10 mol%) was added at this stage and the reactions were stirred for a further hour at -20 °C. Acetyl chloride was then added (5 mol%) and the reactions were stirred for 24 h at room temperature followed by 16 h at 40 °C. A white residue formed in reactions with high sebacoyl chloride concentration which dissolved at the higher temperature. The polymers were then isolated by precipitation twice from hexane followed by dissolving in THF and precipitation into water. (400 MHz; d^6-DMSO) 1.2 (CH_2 of sebacoyl), 1.3 (CH_3 of pendant ethyl), 1.7 (CH_3 of acetyl group), 1.8 (CH_2 of sebacoyl), 2.4 (CH_2C=O of sebacoyl), 4.2 (CH_2 of pendant ethyl), 5.5-5.7 (C*H* of polymer backbone).

Synthesis of poly(ethyl glyoxylate) with terephthaloyl chloride linker

Distillation of ethyl glyoxylate solution was performed as previously described and 2 cm^3 portions of monomer with DCM (1 cm^3) and triethylamine (20 µL) were added into sealed scintillation vials and shaken before placing in a -20 °C freezer for 1 h. TereCl (1, 2 , 5 and 10

mol% dissolved in 1 cm^3 dry DCM) was added at this stage and the reactions were left for a further hour at -20 °C. Acetyl chloride or benzyl chloride was then added (5 mol%) and the reactions were stirred for 24 h at room temperature followed by 16 h at 40 °C. The polymers were then isolated by precipitation twice into hexane followed by dissolving in THF and precipitation into water to give colourless, viscoelastic solids. (400 MHz; d^6-DMSO) 1.3 (CH_3 of pendant ethyl), 1.7 (CH_3 of acetyl group), 4.2 (CH_2 of pendant ethyl), 5.5-5.7 (C*H* of polymer backbone), 8.0-8.4 (aromatic protons from terephthaloyl and benzyl).

Synthesis of polystyrene by ATRP

Styrene (5.01 g, 0.05 mol), CuBr (0.07 g, 4.8×10^{-4} mol) and PMDETA (0.08 g, 4.8×10^{-4} mol) were deoxygenated individually 3 times by evacuating the flask and refilling with N_2 using a Schlenk line. The styrene and PMDETA were injected into the vessel containing the CuBr and stirred for 20 min at room temperature under N_2 to form the catalyst complex. The mixture was then heated to 80 °C and methyl-2-bromopropionate was added (0.08 g, 4.8×10^{-4} mol). The polymerisation was carried out for 24 h before removal from the heat source and exposure to oxygen to give a green solid. The polymer was dissolved in DMF and precipitated from water twice and freeze-dried to give solid white flakes of polystyrene. δ_H (400 MHz; $CDCl_3$) 1.4 (2H, backbone CH_2), 1.8 (1H, backbone C*H*), 6.3-7.3 (5H, aromatic C*H*).

Synthesis of polystyrene-*b*-poly(ethyl glyoxylate)-*b*-polystyrene

Styrene ((100:1 target, 0.55 g, 5.3×10^{-3} mol), (500:1 target, 2.74 g, 2.6×10^{-2} mol), (1000:1 target, 5.48 g, 5.2×10^{-2} mol), CuBr (7.6×10^{-3} g, 5.3×10^{-5} mol), PMDETA (9.1×10^{-3} g, 5.3×10^{-5} mol) and PEtG macroinitiator (1.00 g, 5.3×10^{-5} mol) were deoxygenated individually 5 times by evacuating the flask and refilling with N_2 using a Schlenk line. BIB-terminated PEtG was then stirred at 80 °C in the reaction vessel to melt the polymer. Styrene, PMDETA and CuBr were mixed and stirred for 20 min at room temperature under N_2 to form the catalyst complex. The complex was injected into the PEtG charged vessel and temperature was increased to 80 °C for 24 h. The polymerisation was terminated by exposure to air to give brown/green solids.

Polymers were dissolved in acetone and precipitated from water twice and freeze-dried to give solid polystyrene-*b*-poly(ethyl glyoxylate)-*b*-polystyrene as an off-white powder. δ_H (400 MHz; $CDCl_3$) 1.4 (2H, PS backbone CH_2), 1.5 (3H, PEtG CH_3), 1.8 (1H, PS backbone C*H*), 4.3 (2H, PEtG CH_2), 5.5 (1H, PEtG backbone C*H*), 6.3-7.3 (5H, PS aromatic C*H*).

Polymer degradation studies

Polystyrene, polyethylglyoxylate and the triblock copolymers were dissolved in THF at 10 w/w% and pipetted into 1 cm^3 centrifuge tubes and dried under vacuum to coat the inside in polymer film. Once dried, the solid polymer mass was recorded. Lipase CalB from *Candida antarctica* in distilled water (0.5 w/w%, 1 cm^3) was added and the tubes were closed. For each polymer, 3 tubes were prepared with lipase alongside a fourth control which contained only deionised water (1 cm^3). All tubes were submerged in a water bath at 37 °C for 7, 14 and 28 days. After each time interval, tubes were centrifuged and supernatant was pipetted into clean vials and freeze dried. Fresh deionised water (1 cm^3) was added to the degradation vials to freeze-dry any residual solid. Supernatants were analysed by ^{1}H NMR spectroscopy. Polymers were analysed by GPC (THF/acetic acid eluent). Final solid mass measurements were recorded to give degradation profiles.

6.5.Materials used

Chemical	Purity	Supplier
Chapter 2		
vinyl acetate	>99%, 3-20 ppm hydroquinone	Sigma Aldrich
butyl methacrylate	99%, stabilised	Acros organics
methacrylic acid	99.5% 'extra pure'	Acros organics
ammonium persulfate	>99%	Sigma Aldrich
azobisisobutyronitrile	98%	Sigma Aldrich
Mowiol® 8-88	67 kDa	Sigma Aldrich
polyvinylpyrrolidone	10 kDa	Sigma Aldrich
sodium bicarbonate	99%	Fisher
sodium dodecyl benzyl sulfate	-	Fisher

isopropanol	99.9%, HPLC grade	Sigma Aldrich
petroleum ether	75% bp 40-60 °C	Sigma Aldrich
tetrahydrofuran	HPLC grade	VWR
N,N-dimethylformamide	HPLC grade	VWR
chloroform	HPLC grade	VWR
deuterated chloroform	99.8% D	VWR
deuterated acetone	99.9% D	Sigma Aldrich

Chapter 3

glycine	≤ 99%	Sigma Aldrich
l-alanine	≤ 99%	Sigma Aldrich
1,4-butanediol	99%	Alfa Aesar
1,6-hexanediol	97%	Alfa Aesar
p-toluene sulfonic acid monohydrate	97%	Alfa Aesar
triphosgene	reagent grade, 99%	Sigma Aldrich
sodium carbonate	anhydrous	VWR
sodium hydrogen carbonate	reagent grade	Fisher
Span 85 (sorbitan trioleate)	-	Sigma Aldrich
P(BMA-stat-MAA)	-	as synthesised
Lipase CalB from *Candida Antarctica*	recombinant from Aspergillus oryzae	Sigma Aldrich
toluene	HPLC grade	Fisher
chloroform	HPLC grade	Fisher
deuterated dimethylsulfoxide	99.9% D	Sigma Aldrich
deuterated chloroform	99.8% D	VWR

Chapter 4

ethyl glyoxylate	50 % mixture of oligomers in toluene	Fluorochem
styrene	ReagentPlus ≥99%	Sigma Aldrich
phosphorous pentoxide	98%	Alfa Aesar
triethylamine	≥99%	Sigma Aldrich
acetyl chloride	≥99.0%	Sigma Aldrich
benzoyl chloride	99+%	Alfa Aesar
α-bromoisobutyryl bromide	98%	Sigma Aldrich
sebacoyl chloride	≥95%	Sigma Aldrich
terephthaloyl chloride	≥99%, flakes	Sigma Aldrich
Lipase CalB from *Candida Antarctica*	recombinant from Aspergillus oryzae	Sigma Aldrich

copper (I) bromide	unknown	unknown
N,N,N′,N′′,N′′-Pentamethyldiethylenetriamine	99%	Sigma Aldrich
methyl-2-bromopropionate	98%	Sigma Aldrich
dichloromethane	99.9%	Sigma Aldrich
hexane	≥97.0%	Sigma Aldrich
tetrahydrofuran	HPLC grade	VWR
acetic acid (glacial)	Analytical reagent grade	Fisher
N,N-dimethylformamide	HPLC grade	VWR
deuterated dimethylsulfoxide	99.9% D	Sigma Aldrich
deuterated chloroform	99.8% D	VWR

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Chapter 8: Appendices

1. Full biodegradation report of PVaC particles – issued by Chemex Environmental International Limited

Commercial-in-Confidence

Chemex reference: ENV11379/170201

An evaluation of the ready biodegradability of PVAc particles / 2% P(BMA-stat-MAA) using the OECD 301B CO_2 evolution test.

Report for University of Sheffield

Report issued by:

Chemex Environmental International Limited
Unit J
Broad Lane Industrial Estate
Cottenham
Cambridge CB24 8SW
UK

Sponsor:

University of Sheffield
Department of Chemistry
Brook Hill
Sheffield
S3 7HF

November 2017

Contents

Page

Figures

Compliance with Good Laboratory Practice standards

I, the undersigned, hereby declare that the study described in this report was performed under my supervision, and that the final report fully and accurately reflects the raw data generated during the conduct of the study, in compliance with international codes of Good Laboratory Practice including:

- Section II of Annex 1 to the European Parliament and council Directive 2004/10/EC and Annex 1 to the European Parliament and council Directive 2004/9/EC (Official Journal No. L 50) and embodied within:
- *The UK Good Laboratory Practice Regulations 1999 (The United Kingdom GLP Regulations 1999, Statutory Instrument 3106)* as amended by:
- *The UK Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004 (Statutory Instrument No 994)*

These principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

J. Crane ..... 30 November 17 .....

Jimi Crane BSc (Hons)
Study Director

Date

Key personnel

Other key personnel at Chemex involved in this study were:

Monika Zuba-Sosnowska

Quality Assurance Statement

The Quality Assurance unit inspects the final report to confirm that the methods, procedures and observations are accurately and completely described, and that the reported results accurately and completely reflect the raw data of a regulatory study.

This is achieved by conducting routine annual facility and system inspections at approximately 12 monthly intervals. In addition, an internal process-based audit programme is also adhered to at 3 monthly intervals. Where required, study specific inspections are also conducted. All study plans and amendments are verified by the QA unit to confirm compliance with GLP.

The inspections applicable to this study are detailed below. The dates are given as dd/mm/yy.

Study Number: ENV 11379
Study Title: An evaluation of the ready biodegradability of PVAc particles / 2% P(BMA-stat-MAA) using the OECD 301B CO_2 evolution test.

Procedures and Processes	Type	Date of inspection	Date reported to Study Director / Management
Biodegradation test set up	P	21/03/17	03/05/17
Weighing out test or reference materials	P	22/03/17	03/05/17
Preparation of growth medium / nutrients / standards	P	21/03/17	03/05/17
Changing solutions	P	10/04/17	03/05/17
Determination of carbon content	P	22-23/03/17	03/05/17
Preparation of sludge	P	21/03/17	03/05/17
Taking samples for chemical analysis	P	10/04/17	03/05/17
Taking and recording readings	P	27/02/17	02/03/17
Equipment calibration	P	21/03/17	03/05/17
Labelling and paperwork	P	21/03/17	03/05/17
Preparation of solution/WAF	P	27/02/17	02/03/17

Key: P- Process-based, S- Study specific, O- other inspection type.

This report has been inspected by the undersigned and, as far as can be reasonably established, the methods, procedures and observations are accurately and completely described and the results incorporated into this report accurately and completely reflect the raw data generated during this study.

Final report and data inspection started: 20 June 2017

Final report and data inspection completed: 30 November 2017

Signed: .....
Jane Hawkins MRQA
Quality Assurance

Date: 30 November 2017

Summary

This section summarises the ready biodegradability results obtained by Chemex Environmental International Limited on a sample as detailed below:

Substance under test:	PVAc particles / 2% P(BMA-stat-MAA)
Chemex reference:	Sample: ECO170201 Study: ENV11379
Test commissioned by:	University of Sheffield
Test type:	Ready Biodegradability – CO_2 Evolution Test
Test conditions:	Degradation of test compound according to OECD Guidelines for Testing of Chemicals CO_2 Evolution Test 301B[(1)]
Test temperature range:	20.9°C to 21.8°C
Test period:	22/03/17 to 20/04/17 (Biodegradation phase)
Test carried out at:	Chemex Environmental International Limited Unit J, Broad Lane Industrial Estate Cottenham, Cambridge CB24 8SW, UK

Results:

Time (days)	Biodegradation (%)	
	Reference material Sodium acetate	Test material PVAc particles / 2% P(BMA-stat-MAA)
0	0	0
2	32	-1
5	51	-1
7	59	0
9	62	1
14	70	4
19	74	6
23	76	4
28	76	5
29	82	5
29	90	7

The carbon content of PVAc particles / 2% P(BMA-stat-MAA) was calculated to be 55.81%.

1. Introduction

This report contains a description of the methods used and the results obtained during a study to evaluate the ready biodegradability of PVAc particles / 2% P(BMA-stat-MAA). The object of the study was to measure the ready biodegradability of the sample in a freshwater environment. The biodegradation is defined as the ratio of the carbon dioxide (determined as dissolved inorganic carbon) evolved within 28 days to the theoretical carbon dioxide ($ThCO_2$).

2. Materials and Methods

Unless otherwise specified all methods mentioned in this report are according to Chemex Environmental International Limited standard procedures.

All records of measurements and observations made during this test will be collated and held in the Chemex Environmental International Limited archives at Unit J, Broad Lane Industrial Estate, Cottenham, Cambridge CB24 8SW, UK.

2.1 Test substance

Identification:	PVAc particles / 2% P(BMA-stat-MAA)
Lot/batch number:	1
Supplied by:	University of Sheffield
Homogeneity (supplied):	Homogeneous
Homogeneity (observed):	Appears Homogeneous
Date of receipt:	02 February 2017
Expiry date:	July 2017
Composition/Purity:	Polyvinyl acetate particles which have been formed in suspension polymerisation stabilised by poly(butylmethacrylate-stat-methacrylic acid) copolymer. Trace amounts of stabiliser remains grafted on to particles. (TSDS) CAS No. 9003-20-7 (SDS). 100%.
Chemex reference:	ECO170201
Required storage conditions:	Ambient (15±10°C), no protection from light
Actual storage conditions:	Ambient (15±10°C)
Stability in container after opening:	Stable
Stability in water:	Stable
Quoted appearance:	White powder, some crystalline lumps due to aggregation of the particles.
Observed appearance:	White powder
Quoted solubility in water:	Insoluble

Unless otherwise stated as Study Data Sheet (SDS) all the test substance data is taken from the Test Substance Data Sheet (TSDS).

2.2 Inoculum

Type:	Activated Sludge
Source:	Cambridge Sewage Treatment Works, Cowley Road
Date of collection:	21/03/17

2.3 Sludge pre-treatment

Sludge pre-treatment:	Sieved to 850µm to remove coarse particulates, settled and centrifuged at ~ 4000rpm for ~ 5-10 minutes. The supernatant discarded, sludge re-suspended in mineral media and centrifuged at ~ 4000rpm for ~ 5-10 minutes. This stage repeated once more then the supernatant discarded again and homogenised thoroughly by mechanical stirring (spoon). Dry sludge solids determined on the pellet produced.
Dry sludge solids:	7.31%
Volume of mineral medium:	1.5l per bioreactor
Dry sludge solids in test:	0.0300g/l

2.4 Test methods - Theory

Prerequisites

The test material should be non-volatile and its carbon content known. It is also preferable that the purity or relative proportion of the major components is known.

Principle of the test method

A measured volume of inoculated mineral medium, containing a known concentration of the test substance (10 to 20 mg/litre total organic carbon) as the nominal source of organic carbon, is aerated by the passage of carbon dioxide free air at a controlled rate in the dark at 22 ± 2°C. Degradation is followed over 28 days by determining the carbon dioxide produced. The carbon dioxide is trapped in sodium hydroxide and is measured as Dissolved Inorganic Carbon (DIC) using a Tekmar-Dohrmann Phoenix 8000 (the UV-Persulfate Analyser). The amount of carbon dioxide produced is usually expressed as a percentage of the theoretical carbon dioxide ($ThCO_2$). In practice, carbon dioxide (determined as DIC) is expressed as a percentage of the organic carbon in the test material. Test and reference values are corrected for inoculum blank or surfactant control as appropriate.

On day 28, 2ml of 50% (v/v) hydrochloric acid is added to each bioreactor, which are then aerated overnight, to drive off the remaining carbon dioxide. One last analysis of evolved carbon dioxide is made on day 29.

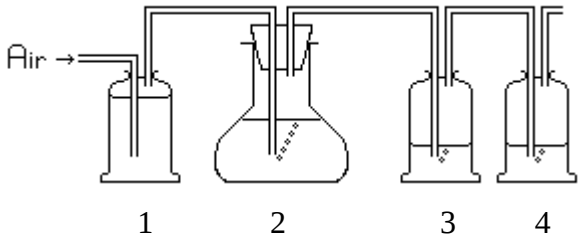
Toxicity control

A single toxicity control is run to indicate whether the material is inhibitory to the microorganism population. This is prepared by adding to the flask the same quantities of carbon as used in each of the respective test and reference material flasks (20mg/l of each). The degradation of the reference material in the toxicity control is determined by using the average test sample value as the 'blank' at each time point. Inhibition is indicated by a marked reduction in reference material degradation in the toxicity control compared to the reference material. The toxicity control data is only useful if the test material is not degradable.

2.5 Test methods - Conditions

The 28 day degradation was determined by a procedure following the OECD Guidelines for Testing of Chemicals reference 301B [(1)].

Conical flasks of nominal volume 2000ml were filled with 1500ml of inoculated mineral medium. The blanks, reference and test bottles were set up in duplicate, with a single replicate for the toxicity control. Test and reference materials were added to appropriate bottles to a final concentration of 20mg Carbon/l. Atmospheric air was pumped into the test system and scrubbed clean of carbon dioxide by passing over soda lime. The air continued into the test vessel where it collects any evolved carbon dioxide before moving into the carbon dioxide traps, each containing 200ml of 0.05M sodium hydroxide solution. The test solutions were stirred for the duration of the study.



Key:

1 Soda lime
2 Test vessel
3 Carbon dioxide trap
4 Carbon dioxide trap

Test material

Quantity of PVAc particles / 2% P(BMA-stat-MAA) in Test 1	53.7mg
Quantity of PVAc particles / 2% P(BMA-stat-MAA) in Test 2	53.7mg
Percentage carbon in PVAc particles / 2% P(BMA-stat-MAA)	55.81%
Total organic carbon (TOC) in Test 1	29.970mg
Total organic carbon (TOC) in Test 2	29.858mg

Reference material

Quantity of sodium acetate in reference 1	102.42mg
Quantity of sodium acetate in reference 2	102.42mg
Percentage carbon in sodium acetate	29.3%
Total organic carbon (TOC) in reference 1	30.009mg
Total organic carbon (TOC) in reference 2	30.009mg

Toxicity control

Quantity of PVAc particles / 2% P(BMA-stat-MAA) in toxicity control	54.0mg
Quantity of sodium acetate in toxicity control	102.42mg
Total organic carbon (TOC) in toxicity control	59.867mg

2.6 Deviations from the study protocol

The study plan states that in order to strip atmospheric CO_2 from the test aeration system, “air is pumped over self-indicating soda lime before entry into the test vessels”.

Due to a defective batch of soda-lime, the standard test configuration (see 2.5) was changed from 2 soda-lime traps to 2 soda-lime traps followed by 2 500ml dreschels of 3M NaOH and 1 dreschel of Maxima H_2O to act as a caustic vapour trap on 30/03/17 (day 8).

The increased number of filtration stages led to a significant rise in air pressure and flow rate through the trap vessels. As a result some CO_2 is believed to have made it through the filters and contaminated the system, which can be seen in the high blank CO_2 results. Blank degradation is accounted for in result calculations and test results are adjusted accordingly. Therefore, in the opinion of the study director this contamination would not have had an impact on the overall result of the study.

3. Results

3.1 Inorganic carbon (mg) in 200ml NaOH

Time (days)	Blank		Reference		Test chemical		Toxicity control
	1	2	1	2	1	2	
2	2.28	1.72	12.10	10.86	2.01	1.66	9.33
5	2.52	2.31	8.34	8.24	2.30	2.25	9.47
7	1.28	1.21	3.96	3.55	1.78	1.60	3.48
9	0.91	0.80	1.90	1.58	1.02	0.99	2.69
14	2.14	1.98	3.92	4.64	3.09	2.98	6.07
19	3.15	3.33	4.89	4.19	4.22	3.50	4.91
23	3.04	3.44	3.28	4.21	2.91	2.47	3.07
28	2.83	2.45	2.12	3.23	2.93	2.87	3.78
29	0.90	1.14	3.12	2.45	0.86	0.75	2.52
29	3.47	2.44	4.01	6.68	3.64	3.66	6.17

3.2 Cumulative inorganic carbon (mg) from test

Time (days)	Reference		Test chemical		Toxicity control
	1	2	1	2	
0	0.00	0.00	0.00	0.00	0.00
2	10.10	8.86	0.01	-0.34	7.49
5	16.02	14.68	-0.11	-0.51	14.68
7	18.73	16.98	0.42	-0.16	16.47
9	19.77	17.70	0.58	-0.03	18.15
14	21.63	20.28	1.61	0.89	21.18
19	23.28	21.23	2.59	1.15	22.23
23	23.32	22.20	2.26	0.38	22.61
28	22.80	22.79	2.55	0.61	23.49
29	24.90	24.22	2.39	0.34	25.20
29	25.95	27.94	3.07	1.04	27.72

3.3 Percentage degradation

Time (days)	Reference			Test chemical			Toxicity control
	1	2	Mean	1	2	Mean	
0	0	0	0	0	0	0	0
2	34	30	32	0	-1	-1	25
5	53	49	51	0	-2	-1	49
7	62	57	59	1	-1	0	55
9	66	59	62	2	0	1	60
14	72	68	70	5	3	4	71
19	78	71	74	9	4	6	74
23	78	74	76	8	1	4	75
28	76	76	76	9	2	5	78
29	83	81	82	8	1	5	84
29	86	93	90	10	3	7	92

4. Discussion

PVAc particles / 2% P(BMA-stat-MAA) failed to meet the requirements for a pass in this test (≥60% degradation relative to the $ThCO_2$ value) with a maximum of 7% recorded on day 29. Because of the stringency of the test, this does not necessarily mean that the test substance is not biodegradable under environmental conditions, but indicates that more work would be necessary to establish biodegradability.

The test protocol also requires that a 10 day window is applied to the degradation results (60% degradation to be reached within 10 days of 10% of the theoretical carbon dioxide produced). As PVAc particles / 2% P(BMA-stat-MAA) failed to meet ≥60% degradation relative to the $ThCO_2$ value the 10-day window is not applicable to this test.

The inoculum blank should not normally produce more than 40mg/l CO_2 (10.9mg/l C). However, a value of 53.0mg/l CO_2 (14.4mg/l C) was recorded. High values observed in the blank flasks are believed to be the result of CO_2 contamination from the air system caused by a defective batch of soda-lime. The blank degradation is taken into account when the test degradation is calculated so the additional CO_2 in the test set-up is accounted for in the results. As such, it is the opinion of the study director that the high blank CO_2 levels will have had no impact on the overall result of the study.

The percentage degradation of the reference material should be ≥60% $ThCO_2$ within 14 days, the reference material reached 62% degradation at approximately day 9.

It can be determined that given the considerable degradation of reference material in the toxicity control (92% at day 29), PVAc particles / 2% P(BMA-stat-MAA) was non-inhibitory to the sludge inoculum.

The final conclusion of this study is therefore that PVAc particles / 2% P(BMA-stat-MAA) is not readily biodegradable in an aerobic aqueous environment.

5. References

(1) OECD Guidelines for Testing of Chemicals 301B Ready Biodegradability: CO_2 Evolution test 1992.

Standard operation procedures:

SOP E231 – Ready biodegradability - CO_2 Evolution Test (Modified Sturm).

SOP E523 – Laboratory Total Organic Carbon analyser and auto-sampler.

Figure 1

Ready Biodegradation of PVAc particles / 2% P(BMA-stat-MAA) - CO_2 Evolution test