Functional polyesters for drug delivery and 3D printing

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Metadata Record: [https://dspace.lboro.ac.uk/2134/20177](https://dspace.lboro.ac.uk/2134/20177)

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Functional Polyesters for Drug Delivery and 3D Printing

Samuel Kilsby, PhD Candidate

Thesis Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy at Loughborough University

Supervisor: Dr S. D. R. Christie
Dr S. Edmondson
Dr R. Goodridge
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Acknowledgements

First of all I would like to thank my supervisors Dr Steve D. R. Christie, Dr Stephen Edmondson and Dr Ruth Goodridge. I would like to thank the two Steve’s for being amazing supervisors and making me into a far better chemist. Without you both none of this would be possible, and I will be forever grateful for all you have done for me. I would like to thank Ruth for being the kind and cheerful person you are, and without even knowing it raising my spirits when things became challenging.

Thanks to Al Daley for the quirky stories, Andy Kowalski for the great chats and getting those all-important chemicals for me, and Mark Edgar for making NMR make sense.

To my parents Martin and Julie Kilsby, thank you for all your support not just over the last few years, but for my entire educational career. I wouldn’t be here if it wasn’t for all your hard work and encouragement over the years. My in-laws, Chris and Phil Standen, thank you for all the meals and drinks and for making such a welcoming and relaxing home whenever I came round. I dread to think how many beers I owe you both.

The friends I have made while at Loughborough have been incredible. Rossi we were there at the beginning of this whole school thing and we finished together, cheers for the journey, it’s been amazing. Loughborough wouldn’t have been the same without: Alex, Bully, Nat, Capel, Shuqi, Yuqi, Yamin, Jade, Noble, Jimi, Mickey, Shahzad and Fatemeh. A huge mention goes to all the Spanish friends I have made: Bea, Carlos, Vanessa, Maria. You guys were both crazy and some of the friendliest and happy people I have met. Thank you for putting up with my English lessons.

I would also like to thank the people who I have met since starting in the world of work. Lisa, Joe, John and Emma, you have all helped me when writing up by providing great dinners and fantastic nights in.

Finally the most important acknowledgement of all goes to my wife Trish. I would be lost without you. You have not only had to deal with my constant grumblings, picking me up from moments of despair and put up with seeing me every day at work (both now and at Uni), but you married me and made every day for the rest of my life worthwhile.
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<tbody>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>ATRP</td>
<td>Atom transfer radical polymerisation</td>
</tr>
<tr>
<td>BAPO</td>
<td>Biacylphosphine oxides</td>
</tr>
<tr>
<td>BIBB</td>
<td>α-Bromoisobutyril bromide</td>
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<td>BMS</td>
<td>Bare metal stent</td>
</tr>
<tr>
<td>BOC</td>
<td>tert-Butyloxycarbonyl</td>
</tr>
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<td>CAD</td>
<td>Coronary artery disease</td>
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<td>CIP</td>
<td>Ciprofloxacin</td>
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<td>CIP.HCl</td>
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<td>CBz</td>
<td>Carboxy benzyl</td>
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<td>DBU</td>
<td>Diazobicyclo[5.4.0]-undec-7-ene</td>
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<td>Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless enhancement by polarisation transfer</td>
</tr>
<tr>
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<td>Drug eluting stent</td>
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<td>Lithium diisopropylamine</td>
</tr>
<tr>
<td>MAAS</td>
<td>Methacrylic acid</td>
</tr>
<tr>
<td>P(MAA)</td>
<td>Poly(methacrylic acid)</td>
</tr>
<tr>
<td>mCPBA</td>
<td>meta-Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>MeCN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>PCC</td>
<td>Pyridinium chlorochromate</td>
</tr>
<tr>
<td>PCL</td>
<td>Poly(caprolactone)</td>
</tr>
<tr>
<td>Pd/C</td>
<td>Palladium on carbon</td>
</tr>
<tr>
<td>PEN</td>
<td>Penicillin G</td>
</tr>
<tr>
<td>PET</td>
<td>Polyethylene terephthalate</td>
</tr>
<tr>
<td>Pet. Ether</td>
<td>Petroleum ether</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly(lactic acid)</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly(lactic-co-glycolic acid)</td>
</tr>
<tr>
<td>PLLA</td>
<td>Poly(L-lactic acid)</td>
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<tr>
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<td>Polymer chain</td>
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<tr>
<td>pTSA</td>
<td>para-Toluene sulfonic acid</td>
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<tr>
<td>RAFT</td>
<td>Reversible addition-fragmentation chain transfer polymerisation</td>
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<td>Arginylglycylaspartic acid</td>
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<td>Azide</td>
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<td>ROP</td>
<td>Ring opening polymerisation</td>
</tr>
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<td>Thiol</td>
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<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
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<td>Stannous octanoate</td>
</tr>
<tr>
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<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>T3P®</td>
<td>Propane phosphoric acid anhydride</td>
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<td>UV</td>
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<td>Water soluble carbodiimide</td>
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<td>X-ray photoelectron spectroscopy</td>
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<td>εCL</td>
<td>ε-Caprolactone</td>
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<td>Three dimensional</td>
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5. Abstract

Functional εCL monomers have been successfully synthesised utilising a primary amine (protected as a CBZ or BOC carbamate) and also a protected ketone (using an acetal protecting group). Amine derivatives were synthesised from the commercially available \textit{trans}-4-aminocyclohexanol. The acetal protected ketone was synthesised from the commercially available 1,4-cyclohexanedione monoethylene acetal.

The subsequent exposure of these monomers to ring opening polymerisation conditions (using stannous octanoate) in the presence of εCL, yielded random copolymers with a functionalised polyester backbone. The acetal copolymer was successfully deprotected and reduced to yield an alcohol pendant. A polymer – drug composite was successfully synthesised through the use of this alcohol polymer reacting with ciprofloxacin.

A polymer – drug conjugate was also successfully synthesised using surface chemistry on PCL and PLA films. PCL and PLA films were cast as films and subsequently aminolysed using 1,6-hexamethylene diamine in aqueous media at pH = 11.5. These aminolysed films were then treated with both ciprofloxacin and penicillin G, together with amide coupling chemistry to synthesise biologically active surfaces. Ciprofloxacin and penicillin G were successfully attached to the PCL surface with ciprofloxacin being attached to the PLA variant.

A functional PCL monomer was also successfully synthesised from the commercially available PCL diol. Both methacrylate and acrylate variants were synthesised through acid chloride chemistry. The acrylate and methacrylate analogue were UV cured using both type I (Irgacure 2959) and II (DETX/EDB) photoinitiators. The relative ratios of these initiators were changed along with the composition of the curing material to investigate curing parameters, to aid in 3D printing conditions. To achieve viscosity criteria for 3D printing a ratio of 70:30 (PCL DMA:PEG DA) was used, utilising PEG DA as a reactive diluent. A ratio of 3 wt% DETX and 3 wt% EDB was found to give the best UV curing results and were successfully applied on a jetting machine to yield 3D printed constructs.
6. Thesis overview

This thesis will consist of three chapters each utilising a different feature or route of pendant functionalised polyesters. The first of these sections will concentrate on what coronary artery disease is and how medical stents are being employed to tackle the needs of patients. This will be followed by a look at a potential material to solve this problem, the degradable polymer polycaprolactone (PCL). Synthesis of this polymer with a detailed look at how the polymerisation is catalysed from a literature standpoint will be discussed. This will be focused around ring opening polymerisation, as it will be the method of choice in this line of research. This will then move onto synthetic efforts to synthesise pendant functional PCL and then their attachment reactions, to yield a drug eluting biodegradable polyester.

The focus on functionalising polyesters is then further explored through surface modification via aminolysis. Aminolysis of a polyester surface is extremely advantageous as it requires only a single step to generate a functional surface. Various types of surface chemistry will be explored as an introduction to the field, along with analysis techniques and finally the field of aminolysis will be introduced and how the technique is utilised in surface modification. This modification will be employed synthetically to functionalise both PCL and poly(lactic acid) (PLA) surfaces. X-ray photoelectron spectroscopy will be utilised a a key method in analysing aminolysed surfaces, with these substrates being further reacted to yield biologically active surfaces.

The final section of the thesis will discuss the use of 3D printing with particular attention to the use of radical polymerisation, as a tool for curing materials being the primary focus. The types of 3D printing will briefly be explored, with the radical curing being the main area of focus. Radical chemistry will also be discussed highlighting it’s versatility in the synthesis of polymers. The synthesis and curing of an acrylated semi-biodegradable polymer will be explored, with the results leading a jettable ink from which to generate 3D constructs.
7. Aims

The aim of this research project is to utilise various types of functional polyesters in the fields of drug delivery and 3D printing. The first area of research will concentrate on the field of drug delivery for use in cardiovascular stents. The purpose of this line of research is to synthesise a polymer that can be used not only as the structural scaffold of a stent to help support the artery, but also to act as a drug delivery system to aid recovery.

![Scheme 1: Functional polyester retrosynthetic route](image)

The polymer could be generated from the co-polymerisation of commercially available $\varepsilon$-caprolactone and a synthesised functional analogue. Once polymerised, a biologically active molecule could be reacted using the functionality present on the polymer backbone (Scheme 1). The biologically active molecule could be attached in such a way so that it is released using the same route as the degradable polyester.
As a further project surface chemistry could be investigated as a route towards a drug delivery system using polyesters. This route would explore the use of aminolysis as a one step method in generating functional polyesters from which to attach biologically active molecules (Scheme 2).

![Scheme 2: Aminolysis retrosynthetic route](image)

Using pure polycaprolactone films could be cast and then treated with a diamine to yield an amine functionalised surface. This in turn could be reacted with a drug compound to yield a biologically active surface.

The final theme of research aims to look at the use of a functional polyester as a jettable ink for 3D printing. Linking back to the drug delivery theme, if a drug molecule was dispersed in the ink they could act as drug eluting scaffolds once printed.

![Scheme 3: UV curing ink retrosynthetic route](image)

Polycaprolactone (PCL) again could be used with a commercially available diol being a potential starting point. The diol could be reacted to form the dimethacrylate using methacryloyl chloride. This could then be used in conjunction with UV initiators as a precursor for use in a 3D printer. Another feature would be that the 3D printed scaffold could be semi-biodegradable through the use of PCL as the core backbone of the material.
8. Use of functional poly(caprolactone) as a drug delivery system

8.1. Introduction

In this introduction there will be an overview of what coronary artery disease is and how medical stents are being employed to tackle the needs of patients. Several types of stents will be discussed in an attempt to introduce the need for a fully biodegrading drug eluting alternative. This is because current technology has the stent implanted in the body for life, whereas having a biodegradable stent allows the implant to be removed after the required treatment period.

This will then lead onto a detailed look at the polymerisation of lactones, as they are key materials in the field of biodegradability. These polyesters are commonly produced through a process known as ring opening polymerisation therefore the types of this polymerisation along with the wide variety of catalysts will be discussed. This then introduces the field of functional polyesters from which the idea of a fully biodegradable drug eluting stent can take form. This will be via the attachment of a drug to one of these functional polymers and is a key idea within this research.

Following on from the overview of functional polyesters, the synthetic efforts to synthesise a variety of these will be discussed followed by the successful attachment of a biologically active molecule.
8.2. Coronary artery disease

Coronary artery disease (CAD) is the blocking or narrowing of the arteries surrounding the heart (coronary artery). The blockage, called atherosclerosis, is usually caused by the buildup of cholesterol and fatty deposits, commonly linked with unhealthy lifestyles and poor diet. As the deposits thicken and harden, the artery becomes narrower, reducing the amount of blood flow to the heart (Figure 1). This in turn reduces the flow of oxygen and essential nutrients into the heart, which can cause a heart attack. If the deficiency becomes too high cells begin to die causing angina, this can then result in a heart attack.

![Diagram showing the restricted blood flow when atherosclerosis is present](image)

Figure 1: Diagram showing the restricted blood flow when atherosclerosis is present

With a proportion of today’s population not exercising, consuming high fat diets or both, heart disease is becoming more common. This has lead to an increase in the types of treatments available to those suffering of atherosclerosis. Treatments can take the form of medicines or operations with preferred preventative measures being more lifestyle driven than medical intervention. Preventative measures include a lower cholesterol diet, regular exercise and quitting smoking. Medical treatments are varied and include calcium channel blockers, which relax the muscles surrounding the heart causing the arteries to open, therefore increasing blood flow. Aspirin can also be used which thins the blood decreasing the chances of clots, but also allows the blood to bypass blockages easier. The final treatment, which is considered as invasive, is surgery. This is seen as far more specific to the
patient’s needs, for instance for a single blockage an angioplasty may be performed, which is the mechanical widening the artery in question using a balloon catheter. The balloon is inflated and the hardened calcium deposits are broken under the pressure. If there are several blocked arteries, then a coronary bypass may be required. This technique grafts another vessel, such as an artery from the leg in such a way that allows blood to flow around the blockage. This method is often reserved for extreme cases, as open heart surgery is required. The final surgical, less invasive, method is the use of cardiovascular stents.

8.3. Cardiovascular stents

Stents are cylindrical structures that can be inserted into the body to help support weakened blood vessels and were first implanted in 1985 by Palmaz et al.² Cardiovascular stents, or coronary stents, are placed into arteries using the same procedure as angioplasty. A catheter with a balloon attached is inserted into the blocked artery. Once at the site of the blockage, the balloon is inflated forcing the previously narrowed artery to widen. The inflation of the balloon not only widens the artery but also expands the stent, which in turn allows support for the newly widened artery. At this point the structural integrity of the artery is supported by the cardiovascular stent, and the balloon can be deflated and the catheter removed (Figure 2).³
The first generation of stents were made of metal and were known as bare metal stents (BMS). When a narrowed artery is widened by angioplasty, with or without a stent, and the artery begins to narrow post-treatment this is called restenosis. This process can be viewed through coronary angiograms when previously narrowed coronary arteries (Figure 3, parts A white arrow and E) are successfully widened through stent implantation (parts B white arrow and F), followed by late stage restenosis being observed after 20 months (parts C white arrow, D, G and H). Yellow arrows (parts A, B and C) show no signs of restenosis when using a bare metal stent. In the early treatment of CAD when only a balloon catheter was being used (angioplasty), re-narrowing of the artery was a common problem, with ~30% of all patients receiving the treatment for cardiovascular disease developing the symptoms of restenosis. With the introduction of BMS there has been a significant decrease in the chances of restenosis. Although promising, ~20 – 30% of patients still develop restenosis due to scaring of the inima, the inner layer of the artery.
The challenge that scientists face is to create a stent that is radially strong enough to withstand the pressures in an artery with a design that does not require thick struts, therefore reducing the amount of artery coverage. Bare metal stents are excellent for that role, however as previously discussed, carry compatibility problems. This compatibility problem creates a significant challenge in choosing a material which can as strong as the BMS but also that could potentially act as a drug delivery system (but also degrading in such a fashion that does not release inflammatory by-products).

### 8.3.1. Drug Eluting Stents

A drug eluting stent (DES) is a design in which a standard metal stent has a polymer coating on the exterior surface. This polymer coating is designed to contain drug molecules which are released upon degradation of the polymer. These drugs can then be tailored to aid the healing response after the angioplasty operation. The chance of restenosis is lessened as the artery wall inflammation can be reduced by the imbedded drug compounds. This can be observed in the results of the Taxus (Boston Scientific Co.) V clinical trials where restenosis in patients was found to be 13.7% compared to the 31.9% in the bare metal equivalent.⁵

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**Figure 3**: Coronary angiograms showing the treatment of CAD using a sirolimus-eluting stent
8.3.2. Biodegradable stents

The reasoning behind a biodegradable stent is that the degradation is tailored so that once the artery is fully recovered; the stent is removed from the body and is no longer present. This could be advantageous as there would be no chance of any problems occurring with prolonged stent implantation. This tailoring can be achieved through the rate of different polymers to degrade. A fully biodegradable stent was first developed, and implanted into humans, by the Japanese company Igaki Medical Planning Company. The stent, called the Igaki – Tamai stent, was made of poly-L-lactic acid (PLLA) and is comprised of a zig-zag helical coil connected with straight bridges (Figure 4).6 The biodegradability comes from the hydrolysis of the ester linkage liberating lactic acid, water and carbon dioxide. Absorption of the stent occurs at both the surface and inside the structure. This allows the stent to maintain structural function until absorption is in the late stages.

Figure 4: The Igaki-Tamai biosorbable stent, constructed from poly-L-lactic acid

Another example where PLLA is used is the everolimus eluting bioabsorbable stent made by Abbott Vascular.7 This stent has a PLLA core with a poly-D,L-lactide coating which contains the antiproliferative drug. The release of the drug is 80% within 30 days with the stent itself being absorbed by bulk erosion.
Biodegradable stents do not use polymer exclusively, with Biotronik in Germany manufacturing a magnesium stent (Figure 5). The magnesium degrades through surface erosion, with the stent thickness decreasing as absorption increases. The stent is completely degraded within 2 months, but the radial strength was lost much earlier giving rise to issues regarding practical application. Another troubling statistic was the high rate of restenosis, with 50% of patients showing re-narrowing of the artery vessel within 4 months.

A promising example of a stent aiding the patient’s recovery is in the bioabsorbable therapeutic stent made by Bioabsorbable Therapeutics Inc. They have made a stent that upon degradation releases salicylic acid which counteracts the inflammation caused by the angioplasty stretching.

In the previous examples of stents, the materials used act not only as a source of support but also biocompatibility. This leads to the idea that not only is the design of the stent, from clasps lock in place or intricate arching styles, important but also the material used in their construction. This has lead to research into biomedical polymers.

**8.4. Biomedical polymers**

Polymeric materials that are biocompatible, and in some cases are biodegradable, can be used in medical applications. These polymers can therefore be categorised as biomedical polymers. Biocompatibility refers to the body’s ability to integrate the implant without
causing rejection issues. As well as limiting the rejection issues, the implant should be recognised by the body, and also not be toxic in any way. Biodegradability implies that the polymeric material will degrade in a controlled fashion after the implant has performed its desired effect. This makes the polyesters of lactones and lactides very attractive, with their desirable mechanical properties and biodegradability through the hydrolysis of their ester linkages. The importance of the polyester’s biodegradability is twofold, with respect to their use in cardiovascular regeneration. The main advantage over the current metal stents would be that the biodegradable process can be finely tuned to begin after the stent has completed its purpose. An ideal situation would be one in which the stent is implanted, restores function to the blocked artery, and is then removed via a biodegradation pathway when it is no longer required. The second more interesting improvement to existing stent designs would be to exploit the degradation of the polymer, through ester hydrolysis, to allow the release of drug molecules into the body. This would not only allow the specificity of the stent to increase but also may hold potential towards decreasing the treatment time of the patient, i.e. increasing the recovery rate of the blocked artery.

![Degredation pathways for biodegradable polymers](image)

**Figure 6: Degredation pathways for biodegradable polymers**

**Figure 6** shows the potential routes through which biodegradation of polymer may occur and within these routes two different modes exist, which are through chemical interactions or physical ones. Chemical interactions are concerned with the chemical bonds found within the polymer, whereas the physical types are the attractive forces between polymer chains.
Primary bond degradation is the process of breaking bonds to form smaller or more compatible polymers. This can occur through either side-chain or crosslink scission. The former creates smaller water-soluble fragments whereas the latter turns a previously insoluble crosslinked polymer into a potentially soluble one. Scission can also occur within the bulk of the polymer. These types of scissions can be broken down into two categories, random and zipper-type. Random scission, as the name would suggest, involves the cleaving of bonds in a random fashion. Zipper-type cleavage is so called because it occurs at the end of the polymer chain and continues from one repeat unit to the next, almost unzipping the monomers in sequence.

The use of biodegradable polymers in medical implants was first seen in the 1960s by the company Cyan- amide Co. who used polyglycolide to create absorbable sutures under the trade name Dexon. The use of polyesters were shortly introduced in the form of a copolymer poly[(L-lactide (8 %)-co-glycolide (92 %))] under the trade name Vicryl®. Since these early developments in the field, polyesters have been researched in depth for drug delivery purposes, including antimalarial, contraception and optical drugs for use within the eye.

A polymer which has not received a vast amount of attention in the past is polycaprolactone (PCL). This is due to the fact that poly(lactic acid) (PLA) has better physical characteristics and has inadvertently pushed PCL from the forefront of research. This however is changing as the area of biomedical research in biocompatible polymers, is becoming an increasingly important field. With the increase popularity of PCL the following chapter will introduce the chemistry of the polymer with a focus towards the various polymerisation techniques used to synthesis it.
8.5. ε-Caprolactone and polycaprolactone

ε-Caprolactone (εCL, 3) is the monomer to the biodegradable polymer polycaprolactone, and is an example of a cyclic ester (lactone) (Figure 8). This polymer could be of potential use in both biodegrading stents and also the drug eluting variety, therefore the properties and synthesis will be discussed as this will be the polymer of choice in the following research. εCL is a liquid at room temperature which makes it an attractive possibility for a jetting material (to be discussed later). This lactone is miscible with a variety of solvents including CH₂Cl₂, CHCl₃, toluene, cyclohexanone, with low solubility in acetone, ethyl acetate (EtOAc) and dimethylformamide (DMF). Solvents which εCL is insoluble in include petroleum ether, alcohols, diethyl ether (Et₂O) and water.

![Figure 8: Structures of different lactone](image)

εCL can be polymerised to form polycaprolactone, which exists as hexanoate repeating units and is becoming highly desirable in the biomaterials field of research (Scheme 4). This is due to the favourable properties of biocompatibility, biodegradability and that the polymer itself creates a porous network allowing permability, all of which allow stable incorporation into the body. Another rare property is that the polymer itself is miscible with various other polymers including poly(vinyl chlorides), polycarbonates, and various others. Although the mechanical properties of PCL are not hugely impressive, the polymer is compatible with other, more mechanically strong polymers such as polyethylene and natural rubber.

![Scheme 4: Polymerisation of ε-caprolactone to polycaprolactone](image)
The rate which a polymer degrades depends largely on its molecular weight, crystallinity and the environment it is situated in, with all of these being important factors when wanting to tailor this rate. The glass transition temperature (the temperature at which the polymer exhibits a glass like state) can also impact degradation by altering the physical state of the polymer (PCL has a glass transition temperature of -65 °C and a melting temperature which ranges between 56 – 65 °C), as the morphology of the crystalline material could impact the degradation (surface area change for example). The degradation time can range from months to years and is a key parameter in creating biomedical implants. It has been observed that the amorphous phase of the polymer degrades first, leading to an increase in crystallinity with no loss of molecular weight. The by-product of this degradation are carboxylic acids, which catalyse the degradation process, with the rate being significantly increased with the use of enzymes. Although enzymes can be used to hydrolyse the polymer it has been shown that this process does not occur in the body.

The monomer εCL is industrially produced by the Baeyer-Villiger oxidation of cyclohexanone using peracetic acid. Thomas et al. found that it can also be synthesised using bacteria, where εCL (3) and 6-hydroxyhexanoic acid (19) are intermediates in the conversion of cyclohexanol (17) into adipic acid (21) (Scheme 5).
Polycaprolactone is one of the polyester family of polymers and is mainly formed through ring opening or condensation reactions. Braud et al.\textsuperscript{20} carried out the condensation of 6-hydroxyhexanoic acid to produce PCL. The reaction was catalyst free and required a gradual increase of temperature from 80 – 150 °C, and it took 6 h. One practical drawback was that it had to be carried out under a vacuum to remove the water produced from the condensation. The removal of water acts as the driving force, shifting the equilibrium towards the formation of polymer. Ring opening polymerisation has however been implemented far more successfully leading to PCL being commercially available in large quantities.

Using enzymes as catalysts during ester formation is another method towards polyester synthesis. An example of an enzymatic catalyst is \textit{Candida antartica} Lipase B (Scheme 6).
Scheme 6: Enzymatic synthesis of polyesters using *Candida antarctica* Lipase B

The lactone (red) enters the active site of the enzyme (22) to create the acyl enzyme (24) via the first transition state (23). An initiator (blue) enters the active site attacking the acyl enzyme via the second transition state (25). This regenerates free enzyme and the opened lactone. Propagation is achieved through the attack of the acyl enzyme by the propagating chain (green) to regenerate the free enzyme and longer polyester chain. The advantage of
using enzymes is the generally mild and non-toxic reaction conditions required for the polymerisation, with the structure of the enzyme also giving rise to control of the stereochemistry (when using lactides). The only limitation of enzymatic polymerisation is the lack of high molecular weight polymers. This is shown when using the lipase enzyme from *Candida antarctica* as this gives rise to PCL polymers with molecular weights of 9,000 g/mol, with a polydispersity of under 1.5 in 2 days.

The most common method to polymerise a cyclic lactone however is ring opening polymerisation (ROP), where the lactide or lactone’s internal ester is cleaved and the polymer grown from there using a ‘living’ process (where a living process is one in which the polymer is constantly growing rather than in any equilibrium with reagents for example). This technique has been shown to give high molecular weight polymers with the main drawback being racemisation of the repeating ester linkages (in the case of lactides). The following section will highlight the ways in which ROP is achieved, and the catalytic systems that are used to initiate the polymerisation.
8.6. Ring opening polymerisation

As previously mentioned the main polymerisation method for ε-caprolactone is ring-opening polymerisation, and will be the method employed during this research. This type of polymerisation is characterised by the chemical cleavage of the ester linkage which acts to propagate the polymerisation cycle, with the general mechanism for ROP is shown in Scheme 7. ROP of lactones is generally carried out in either bulk or solution (tetrahydrofuran, toluene, etc) but can also be undertaken in more specialised methods e.g. emulsions or dispersions. Bulk polymerisation conditions can range from 100 – 150 °C, depending on the required kinetics, but undesired side reactions can be reduced at lower temperatures, often requiring more specialised initiators and/or catalysts. Although the polymerisation occurs through the cleavage of the ester linkage, how the reactions are initiated, and therefore catalysed, can be divided into three sub-categories, which are cationic, anionic and coordination insertion. Which of these methods is responsible for the polymerisation depends on which catalyst and initiators are used.

Scheme 7: General mechanism for ROP using lactide as an example
8.6.1. Cationic polymerisation

As mentioned above one catalytic method is cationic in nature. Although not used in this research, both cationic and anionic polymerisations will be briefly covered to broaden the understanding of ring opening polymerisation. Cationic ROP begins with the formation of a cationic species to which the εCL can attack. The carbonyl oxygen is usually the cationic species formed through the use of the oxygen’s lone pair towards an electrophile. The positive charge is stabilised through the resonance of the carbonyl (31), forming the tertiary carbocation (32). This positive charge resonates between the carbon and the endocyclic oxygen (33). The positive charge at this position is neutralised through the nucleophilic attack of another εCL monomer at the alkyl-oxygen carbon in a $S_N2$ fashion (Scheme 8).²⁵

![Scheme 8: Mechanism for the initiation step of cationic ROP](image)

8.6.2. Anionic polymerisation

The previous catalytic method utilised a positive species but there are also examples of negative species being used. The polymerisation of lactones anionically is most common when using alkoxide based catalysts. The polymerisation is initiated by the nucleophilic attack of the negatively charged catalytic species at either the acyl carbon of the carbonyl group or attack of the alkyl – oxygen (Scheme 9). When the monomer is a β-lactone both instances can occur creating alkoxide (36) or carboxylate (37) species from which to propagate from. This is not the case however when polymerising larger lactones, e.g. δ and ε-lactones. In these cases only the acyl-carbon is attacked, leading to the alkoxide species as the sole propagator.
8.6.3. Coordination insertion

Although anionic and cationic catalysis have been discussed, the main method of technique used when polymerising lactones, is the coordination insertion approach. Coordination insertion will be the method of choice when carrying out polymerisations in this research; a brief overview will be discussed, followed by a more in-depth look towards the different catalysts used later in the thesis. Coordination insertion has been used extensively in the polymerisation of aliphatic polyesters with well-defined structures and architectures, through the use of multifunction initiators, e.g. ethylene glycol. This method of catalysis also produces polymers with narrow molecular weight distribution and mass. The general mechanism is shown in Scheme 7, where the metal oxygen bond of the catalyst (26) coordinates with that of the carbonyl bond in the monomer (27). The electrons in the carbonyl form a new metal oxygen bond (28) while the metal attached oxide migrates to the acyl carbon (30). Propagation occurs through the reformation of the carbonyl, cleavage of the ester linkage and the migration of the newly formed alkoxide end onto the metal.
8.6.4. Transesterification reactions

Before moving on to discuss the catalyst used in ROP it is important to understand that although this technique is associated with low polydispersities, when incorrect reaction conditions are used side processes can occur. Certain conditions can lead to deactivation of the catalyst, side products and loss in molecular weight together with an increase in polydispersity, with ultimately loss of polymerisation control.

A transesterification reaction is one in which polymers react with either another polymer, or itself, and usually occurs during the late stages of polymerisation at elevated temperatures. Intermolecular transesterification occurs between polymer chains when a propagating species (38) (one bound to the metal alkoxide) reacts with a polymer chain (39). This causes the metal to stop the growth of one chain (40) and begin on another (41). This in turn leads to irregularity in polymer chain length thus increasing the polydispersity of the final product. Intramolecular transesterification occurs when the propagating end of the polymer reacts with a section of itself (42). This process cyclises the polymer leaving no terminal ends from which to propagate from (Scheme 10).

Scheme 10: Inter- and intramolecular transesterification reactions
These transesterification reactions can be influenced by a variety of parameters. Kricheldorf observed the production of octanoic acid, when using tin (II) octanoate, above 100 °C.26 This may appear harmless but when combined with alcohols (used as initiators) esterification reactions can occur, liberating water. This water can coordinate with the metal forming a range of oxides and hydroxides. With the presence of a variety of catalytic species, the molecular weight would be hard to control and thus a broad distribution of weights would be observed.

Kowalski et al.27 found that upon addition of carboxylic acids, especially ethyl hexanoic acid, the ‘growing’ or propagating chain of the polymer can be put into a dormant state. The carboxylic acid inserts into the metal – polymer bond, removing the ‘living’ end of the polymer. This then leaves it dormant with a hydroxyl end as opposed to an active metal species (Scheme 11).

![Scheme 11: Formation of a dormant chain during ROP when catalysed by tin octanoate](image)

The polymerisation of lactones has been shown to proceed via a variety of mechanisms, but these pathways are all a product of which catalyst is used. These can be as varied as the types of catalysis they exhibit, with the biggest research being carried out toward co-ordination complexes of transition metals.
8.7. Catalysts used for ROP

The research into catalysts for the ROP of lactones is vast and varied, although the post-transition metal catalysts are the most common, and the one that will be used in this study, the following section will introduce a broad range of catalysts to give a broader scope to the field. They include alkali bases, metal alkoxides, alkali earth metals, enzymes and even inorganic acids. Some are far more commonly used than others, with different conditions required for polymerisation, yielding varied molecular weights.

8.7.1. Alkali-base catalysts

The first class of catalysts to be discussed, in this brief look at different catalytic systems, are alkali – based catalysts, which are compounds that are ionic in nature and proceed through an anionic catalysis route. As a result of this they experience a high degree of tranesterification with little control of polymerisation. An example of an alkali-base catalyst can be found in lithium diisoproplyamine (49) (LDA). Bhaw-Luximon et al.\textsuperscript{28} used LDA in dioxane to polymerise εCL in just 25 minutes at 25 °C, with a molecular weight of 5,700 g/mol. This catalyst initiates the reaction through the attack of the carbonyl which creates a negative charge on the oxygen (51). This negative charge can then be used to reform the carbonyl which then breaks the ester linkage, or to directly attack the next lactone monomer. The former leaves an isopropyl amine group as the end group of the polymer (53), and the latter leaves an intact ring as the end group (52) (Scheme 14).
Scheme 12: ROP of $\varepsilon$-caprolactone using LDA as a catalyst

8.7.2. Transition metal catalysts

Although not used in this research, the transition metal catalysts would be more than applicable for the polymerisations that are to be employed in this research; therefore a brief overview will be discussed about their use in this field. Transition metals are far more controlled when compared to the previous example and the types used are titanium
complexes, with zirconium based catalysts growing in popularity. Davidson et al.\textsuperscript{29} used titanium complexes using the catechol ligand, giving rise to a PCL polymer with a narrow polydispersity, indicating a controlled polymerisation. They also tried a range of titanium\textsuperscript{(IV)} and zirconium\textsuperscript{(VI)} amine bis(phenolate) ligands complexes with the titanium complexes yielding polymer only with the most bulky groups.\textsuperscript{30-31} This is not the case however with the zirconium complexes where the less bulky substituents favoured polymerisation. The synthesis of titanium\textsuperscript{(IV)} complexes with bisphenolate ligands bearing no amine functionality has also been shown to have interesting effects.\textsuperscript{32} The polymerisation is twofold, with each catalyst molecule (54) facilitating the growth of two separate polymer chains (55) (Scheme 13).

![Scheme 13: Synthesis of two polymer chains for one molecule of catalyst when using a titanium\textsuperscript{(IV)} complex](image)

Zinc complexes have also been found to be useful in εCL polymerisation. Zinc mono- and di-alkoxides have been found to initiate the ROP process with the catalysis following the coordination insertion mechanism, and giving rise to well defined range of molecular weights (polydispersity of 1.1).\textsuperscript{33}

Other zinc alkoxides bearing either alkyl or amine ligands have been shown to surpass their magnesium analogues in stability and reactivity. Both catalysts showed high throughput achieving 300 kg (mol metal)\textsuperscript{-1} h\textsuperscript{-1}, with the silyl amine bearing complex yielding polymers with high molecular weights (55,000 g/mol) with polydispersity of 2.3.\textsuperscript{34}

Other metals that have been researched include molybdenum\textsuperscript{(VI)} and vanadium\textsuperscript{(V)} compounds, with results indicating that the metals were reduced and became inactive during
polymerisations under an inert atmosphere. One potential drawback for these catalysts, from the view of biodegradable stents, is that the metals used are not compatible within the body therefore residual catalyst in polymers would be an issue.

8.7.3. Organocatalysis

The use of metals can be undesirable (due to potential toxicity for example) with ligand design and synthesis lengthening any synthetic route, organocatalysis could be an elegant alternative. These catalysts fall under the same idea expressed in the transition metal overview, with these catalysts more than applicable for the polymerisation covered in this research however not being used. For that reason they will be briefly covered to give a broader overview of the ROP of lactones.

Organocatalysis has been used in the form of a dual activation ROP using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and a thiourea (56). In this example, the thiourea molecule hydrogen bonds to the carbonyl oxygen of the lactone increasing the reactivity of the εCL monomer (69). The reactivity of the alcohol (58) is increased through the hydrogen bond to the DBU (60) (Scheme 14). This then allows initiation and ring opening of the lactone through the now more reactive alcohol. The carbonyl of another monomer is then activated using the thiourea, with the DBU activating the hydroxyl of propagating species (61).
Scheme 14: ROP through dual activation using thiourea and DBU
8.7.4. Post-transition metal based catalysts

The catalyst used in this research falls into the category of the post-transition metal based catalysts. These are the most commonly used catalysts for ROP and are either aluminium or tin based, with tin being the more reactive of the two. Aluminium catalysts, although less reactive, are used because they allow good control over the polymerisation. This is observed through work by Wang and Kunioka who screened a variety of metal triflates, with both aluminium(III) and copper(II) proving to be excellent catalysts for the ROP of εCL. Metals that did not catalyse the polymerisation were sodium(I), ytterbium(III) and magnesium(II) triflates, with samarium(III) and lanthanum(III) producing oligomers only.

Work by Duda et al. with aluminium alkoxides with formula R₂AlOR, has elucidated that the alkyl groups play no role in the ROP of lactones but the alkoxides only. It was found that with each catalyst molecule, a polymer chain is formed, giving rise to the observation that only the alkoxide groups initiate the ROP and the alkyl groups do not. The only effect the alkyl groups appear to influence is rate of polymerisation, with the larger the alkyl group the faster the reaction.

![Scheme 15: Six-coordinate complex formed when aluminium(III) isopropoxide is dissolved in εCL](image)

The most common of all aluminium catalysts is aluminium(III) isopropoxide (62), which exists as a six-coordinate complex (63) when dissolved into εCL (3). This then acts as the initiator of the ROP (Scheme 15). Kricheldorf compared the use of various metal alkoxides against aluminium isopropoxide, with Al(OiPr)₃ being the only one showing a lack of transesterification reactions, this not being the case with other alkoxides.
A tin based catalyst that is used routinely is tin\(^{(II)}\) ethylhexanoate (73), which is commonly referred to as tin octanoate (Figure 9). The catalyst is efficient, soluble in a large variety of solvents and is a cheap commercially available reagent. Although very efficient, tin octanoate requires a nucleophilic initiator such as an alcohol if a controlled polymerisation is to be achieved. The foremost drawback is that the catalyst requires elevated temperatures, which increases the amount of transesterification reactions, leading to a broadening of the molecular weight distribution.

![Figure 9: Tin (II) octanoate](image)

Solvent choice can be important when using tin octanoate, with it determining the conversion percentage of monomer. When using dioxane and toluene Bhaw and Luximon found a conversion of 40 % after 42 hr, but when using pure toluene the polymerisation was complete and in 21 hr.28 In this work ethanolamine was used as an initiator, with both the amine and alcohol used to initiate the polymerisation.

The amount of initiator is also crucial as the active catalytic species is first made by the nucleophilic attack of this initiator, giving rise to this component being the limiting factor of the polymerisation. As previously mentioned introduction of carboxylic acids into the polymerisation reactions give rise to dormant polymer chains, this is also the case when using too much initiator. Simple alcohols are the usual choice for initiators but others such as ureidopyrimidinone-alcohol (65) have been studied.42 An unexpected problem was observed when conducting the reaction at 80 °C, the initiator began to switch between a mono- and dimeric form (66) (Scheme 16). This dimer is formed through the formation of a quadruple hydrogen bond. When in this form there is no free initiator to activate the catalyst, therefore the polymerisation is postponed until the monomeric form is formed.
The use of ROP is broad and is of key importance in the manufacturing of vast amounts of polyesters. The use of this catalysis is not limited to simple monomers and their relatively simple polymers. The field of functional polymers and in particularly functional polyesters is ever growing. The following section will focus on displaying a variety of functional polymers and describe how they can be further utilised in conjunction with other chemistries to create further functionality.
8.8. Functional polyesters

The use of polyesters is very established but research into functionalising the polymer backbone is becoming more and more prominent. The main purpose of functional polymers with respect to this research is that this will be the route which is used to attach a biologically active molecule to the polymeric backbone, therefore literature in this area will be discussed. Functional PCL is a polymer with hexanoate repeat units with functional groups present along the back bone. The polymerisation of these functional polyesters is comparable with that of pure PCL, including polycondensation and ROP reactions being used. One method is based around post-modification of a PCL chain. When using a base, such as LDA, the acidic protons on the alpha position to the carbonyl are deprotonated leaving a negative charge able to attack introduced electrophiles, a halogen or acid chlorides for example.\(^{43}\) There are however problems with this method including side reactions using the negative charge.

As the last point suggests the use of post-polymerisation functionality is not desirable. The pre-polymerisation route involves the use of monomers with functionality already installed. These functional monomers are then incorporated into the same style of polymerisations previously mentioned for \(\varepsilon\)CL to form copolymers (ROP, condensation and enzymatic although not previously mentioned). The functional monomers are usually protected pre-polymerisation, so that they cannot interfere with the polymerisation conditions (e.g. an amine protected as a carbamic benzyl ester).

As with before the main type of polymerisation is ROP, with the functional monomers usually being based on \(\varepsilon\)CL ring systems (Figure 10).\(^{44}\) One of the most frequently used functionalities is halogen bearing \(\varepsilon\)CL (68, 69 and 71). This can be achieved through the use of a strong based, LDA, and a halogen containing electrophilic species. An example of this is the use of iodine monochloride as the electrophile (Scheme 17), where the polymerisation
was carried out using tin octanoate at 100 °C, with methanol as the initiator and the solvent as toluene.\textsuperscript{45}

Figure 10: Functionalised εCL monomers

The use of halogens is extremely important from a synthetic point of view, with the work of Sripha \textit{et al.}\textsuperscript{46} showing that the use of a chloride group could easily be converted to the azide, using sodium azide, with the formation of a triazole ring when reacting this with DBU in the presence of a copper\textsuperscript{(I)} salt. This however is not the only use found for the chloride functionality. Lenoir \textit{et al.}\textsuperscript{47} used this pendant chloride to graft poly(methyl methacrylate) blocks by atom-transfer radical-polymerisation (ATRP).
The choice of functionality is not limited to halogens, with research branching out into a huge variety of moiety groups. These include the use of a triethylsily group (70), which is removed by acid hydrolysis post polymerisation, to give an alcohol. This alcohol could then be used as an initiating point from which to make copolymers.

Unsaturated aliphatic polyesters have been synthesised using the unsaturated εCL monomer 6,7-dihydro-2(5H)-oxepinone (74). This monomer was synthesised by Lou et al. who were able to copolymerise with εCL using Al(OiPr)_3 in toluene at room temperature (Scheme 18).

A functional polymer with an amine pendant has been synthesised with the amine functionality being protected as a carbamic benzyl ester (78). The protecting group was removed by hydrogenolysis using a Pd/C catalyst under hydrogen gas. This amine pendant was then reacted with Biotin, also known as vitamin H, demonstrating the accessibility of these functional PCL for use with important bioactive molecules.

Although rare there has been research into the medical uses for these functional polyesters. One such use is in the delivery of therapeutic peptides/proteins. The polymer poly(L-lactide-co-hydroxymethyl glycolide) has been shown to form microspheres when using a double water-oil-water solvent-emulsion method (Scheme 19). These microspheres were used to successfully deliver lysozyme in different amounts of polymer solutions. This in comparison to the poly(lactic-co-glycolic acid) (PLGA) which showed no delivery of the enzyme, shows that there is promise in this field. It is important to note that the lysozyme released was completely enzymatically active showing no loss of structural integrity.
Another use is in the field of tissue engineering. The usual aliphatic polyesters used in tissue engineering, e.g. PCL, PLA and PLGA, have the drawbacks of hydrophobicity and lack of functionality. These attributes are disadvantageous with respect to cell adhesion, an important factor when constructing scaffolds. Work by Wang et al.\textsuperscript{52} has shown how a functional polymer, poly(glycerol-sebacate) (\textbf{90}) (\textbf{Scheme 20}), was able to culture NIH 3T3 fibroplast cells with normal morphology and an increased growth rate when compared to a pure PLGA coated petri dish.

Another example of a functional polymer was shown by Dove \textit{et al.}\textsuperscript{53} who uses functional polycarbonates bearing norbornene pendants (\textbf{Scheme 21}). The key significance in this research is the use of post-functionalisation as a simple and elegant way of introducing a wide array or different functionalities. The monomer was synthesised in four steps from 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoic acid, with the first step being the protection of the diol using 2,2-dimethoxypropane. This was then coupled to 5-norbornene-2-methanol followed by deprotection of the diol. The diol was then treated with triphosgene to create the carbonate monomer.
The monomer is polymerised through the use of the organocatalyst DBU together with a thiourea derivative as a co-catalyst. Once polymerised (91) the pendant norbornene can be reacted in a variety of ways. The thiol-ene reaction can be utilised using a photoinitiator and UV light (93), while click chemistry is exploited between the norbornene and an azide (92). The alkene of the pendant norbornene can be used together with tetrazine in an inverse electron demand Diels-Alder reaction (95). The real power of all these reactions is that they can be performed sequentially in one-pot as they all require different conditions (96).
8.9. Results and discussion

The functional \( \varepsilon \)-caprolactone molecules showed in Figure 10 shows a wide variety of versatility to synthesis of functional polymer precursors. These precursors, as mentioned in the functional polymers section, can then be reacted in several ways as a platform to generate new chemistries. A particular example of note is the amine pendant \( \varepsilon \)-caprolactone, as the molecule is polymerised as the CBz protected amine, deprotected and then biotinylated.\(^{50}\)

![Scheme 22: Biotinylation of a functional PCL polymer](image)

Biotin is a specific protein binding receptor for streptavidin with a high binding affinity (\(10^{13-15}\) M\(^{-1}\)).\(^{54}\) Yan \textit{et al.}\(^{50}\) clearly show that a functional polymer can be used to generate a polymer – drug conjugate. With the principle tested and the research concept plausible, the Christie group began looking into a route to create more polymer – drug conjugates using this technique. This was to build on previous work within the group where ciprofloxacin was successfully attached to a titanium surface, with the differing approach of a biodegradable polymer.\(^{55}\)

8.9.1. Research plan

The initial aim of the project is to create functionalised monomers from which to synthesise a functionalised PCL backbone. These polymers have the structure shown in Figure 11, where \( X \) denotes the functional tether (2). It should also be noted that the location of the tether is not limited to the one shown in the figure and can be altered depending on the initial monomer.
The initial plan for the beginning of the research would be to utilize the chemistry of Yan et al. to create the ciprofloxacin – polymer conjugate. The retrosynthetic analysis shown in scheme 23 will be the basis of the functional monomer synthesis. It was envisaged that this functional polymer could be synthesised from the commercially available trans-4-aminocyclohexanol through protection of the amine to allow further oxidation of the alcohol.

A challenge that may be needed to overcome would be the potential for rearrangement upon deprotection to the amine. The deprotected monomer could rearrange to the five membered lactam with a pendant alcohol. The way in which this could be overcome would be to copolymerise with εCL in the protected amine state and then deprotect post polymerisation as there would be far less chance of the rearrangement due to the chain conformation of the polymer.

The scope of the functionality would then be increased to include other functionalities including alcohols, to investigate their potential as polymer – drug conjugates. This is of interest as the linkage to the drug molecule will be different in nature (ester compared to amide for example) therefore giving rise to analogous materials from which to investigate further.
### 8.9.2 Primary amine monomer using CBz protection

The synthesis of the primary amine monomer began with the protection of the amine of \textit{trans}-4-aminocyclohexanol. Due to the difference in functionality, alcohol and amine, the primary amine was protected using benzyl chloroformate in a 67 \% yield, without compromising the alcohol (104) (Scheme 24).50 The $^1$H NMR spectrum showed a singlet at 5.08 and a multiplet between 7.29 – 7.40 ppm, indicating a benzyl group and successful protection of the amine.

The alcohol was then subsequently oxidised to the corresponding ketone (105) using pyridinium chlorochromate, PCC, with a high yield of 86 \%.56 The oxidation was proven with the disappearance of the hydrogen peak at 3.59 ppm in the $^1$H NMR. This signal corresponded to the hydrogen on the same carbon as the hydroxyl group and the disappearance indicated the loss of this hydrogen due to the formation of a ketone. This is further proved in the $^{13}$C NMR, where there is the disappearance of a signal at 66.6 ppm and the appearance of one at 209.5 ppm indicating the presence of a ketone (this signal is also not visible in the DEPT, again showing a new quaternary carbon centre). The ketone was then treated with $m$CPBA to form the lactone (78) in a 93 \% yield, via a Baeyer-Villiger oxidation.
The successful oxidation to the lactone is shown by $^1$H NMR spectroscopy with the appearance of two signals at 4.17 and 4.30 ppm, each corresponding to the hydrogens in the CH$_2$ next to the newly inserted oxygen atom. The $^{13}$C NMR of the ketone shows four signals for the cyclohexane ring (due to symmetry), whereas the spectra for the lactone has six clearly different signals. This would indicate the successful oxidation due to the loss of symmetry, with a signal at 65.4 ppm indicating another CH$_2$ next to an oxygen (the other being the benzyl CH$_2$) also showing the insertion of an oxygen into the ring.

8.9.3. Protected primary amine polymerisations

All polymerisations of $\varepsilon$-caprolactone, including random co-polymers with the functionalised monomers, were catalysed using stannous octanoate (64). The standard polymerisation conditions employed were 1 % catalyst loading, 130 °C for 17 h. When using only $\varepsilon$-caprolactone (3) this yielded poly(caprolactone) (16) in a quantitative yield. After obtaining PCL in such a high yield, the protected primary amine monomer was then used together with $\varepsilon$CL to create a random co-polymer (Scheme 25).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Monomer used</th>
<th>Monomer amount (%)</th>
<th>Incorporated amount (%)$^1$</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protected primary amine (68)</td>
<td>5</td>
<td>3.33</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>Protected primary amine (68)</td>
<td>10</td>
<td>6.98</td>
<td>60</td>
</tr>
</tbody>
</table>

$^1$Calculated from $^1$H NMR integration.

Table 1: Results of protected primary amine co-polymerisations
The CBz protected amine (78) was used at a 5 % level (i.e. a ratio of εCL to functionalised monomer was 9.5:0.5) (Table 1, Entry 1). This yielded the random co-polymer (106) in an 86 % yield, with the incorporation of the functionalised monomer shown in the $^1$H NMR. The NMR spectra shows a singlet at 5.07 and a multiplet at 7.29-7.37 ppm with the peak integrals indicating the polymer actually contains 3.33 % of the functionalised monomer (this could be related to the relative rates of polymerisation of each monomer, i.e. εCL polymerises faster than the protected primary amine derivative). This polymerisation was repeated but using a 10 % amount of the protected amine lactone (Table 1, Entry 2). The polymer was only isolated in a 60 % yield, which can be attributed to the crystallinity of the product. As you increase the level of co-polymerisation, the degree of crystallinity drops due to an increase in random structure (i.e. there is no consistent structure therefore less favourable to crystallise). It was found that when using the protected amine monomer at greater than 10 % there was no precipitation of solid, therefore indicating the polymer was an oil and not crystalline. This accounts for the loss of yield as not all the polymer would have been collected by filtration.
The next stage was the deprotection of the amine by hydrogenolysis. This was carried out on a H-Cube® continuous-flow hydrogenation reactor using a palladium on carbon catalyst. The initial run was carried out using CH₂Cl₂ as the solvent with system not being heated and left at room temperature. After running the machine for 4 h, the volume reduced and the product precipitated in cold hexane, no removal of the CBz group was observed. The ¹H NMR clearly showed the singlet at 5.07 ppm and multiplet in the aromatic region. The solvent was changed to THF, not only as the polymer is soluble in THF but also because the reaction could be heated to 50 °C where CH₂Cl₂ could not due to solvent evaporation. After passing the protected amine polymer through the HCube® for 4 h at 50 °C, ¹H NMR analysis again showed no removal of the protecting group with the characteristic signals of the CBz being observed again.

The reaction was then tried in a round bottom flask utilising schlenk techniques to induce a hydrogen atmosphere, with balloons of hydrogen to maintain the pressure. Palladium on carbon was used again and the reaction left at room temperature for 48 h. Unfortunately there was no deprotection observed so another set of conditions were trialled.
These conditions were the use of 48 % hydrobromic acid in acetic acid at room temperature for 24 h. This reaction would cleave the CBz group off and leave the amine protonated unable to undergo and rearrangement chemistry. As with before the $^1$H NMR showed the benzyl hydrogens at 5.07 ppm and the multiplet in the aromatic region indicating unsuccessful deprotection. It was then decided to move towards a different deprotection group, therefore the BOC group was investigated.

8.9.4. Primary amine monomer using BOC protection

Due to the unsuccessful deprotection of the CBz protected primary amine, it was decided to move towards a group which is deprotected under different conditions. The BOC (tert-butyloxy carbonyl) protecting group was chosen for two reasons. The first is that the acid conditions required for removal are far easier, cheaper and more commonly available than the previous hydrogenolysis required to remove a CBz group. Secondly upon removal due to the acid conditions the newly deprotected primary amine will be protonated. This would mean that the lone pair of the nitrogen is already tied up and no rearrangement to the polylactam would occur.

Scheme 29: Synthesis of BOC protected primary amine monomer
4-Aminocyclohexanol (103) was first protected with a BOC group using standard conditions of BOC anhydride and base (107). Care needed to be taken during work-up as when using hydrochloric acid (1 M) to remove excess base, the protecting group can be removed and carbon dioxide liberated. This would in turn regenerate the starting material, therefore a new approach was required. This was rectified by the use of citric acid (1 M) with the product being isolated in a respectable 82% yield. The product was easily identifiable from the starting material due to carbonyl peak in the IR at 1681 cm\(^{-1}\). Upon protection of the amine, the alcohol was oxidised to the ketone (108) through the use of PCC in a 72% yield. IR spectroscopy indicated the successful oxidation through the appearance of a new peak at 1714 cm\(^{-1}\), with the disappearance of the broad hydroxyl peak. The ketone was then oxidised using \(m\)CPBA to yield the corresponding BOC protected lactone (109), in 52% yield after column chromatography.

Scheme 30: Oxidation of the BOC protected amine cyclohexanone to the lactone using \(m\)CPBA

Following successful synthesis of the BOC lactone, co-polymerisation with \(\varepsilon\)CL was carried out using the same stannous octanoate loading of 1 mol% with an incorporation of 5% in the final polymer (110).

Scheme 31: Random co-polymerisation of \(\varepsilon\)CL and the BOC protected amine lactone
8.9.5. Secondary amine monomer

The aim of this monomer is to have the heteroatom as part of the polymeric chain as opposed to a tether. This would allow a secondary amine to be utilised along the backbone of the polymer and differentiate between the primary amine reactivity. This would require the amine to be part of a cyclic system from the starting material, therefore 1,4-dioxa-8-azaspiro[4.5]decane (111) was used (Scheme 32).

![Scheme 32: Trifluoroacetylation and deprotection of 1,4-dioxa-8-azaspiro[4.5]decane](image)

The spiro compound’s amine was protected using trifluoroacetic anhydride (112) in a 51 % yield.\textsuperscript{56} This was supported in the \textsuperscript{1}H NMR by the observation of a pair of triplets at 3.67 and 3.79 ppm. These represent the equatorial and axial hydrogens, respectively, on the alpha carbons to the newly protected amine. The broad singlet at 1.40 ppm is also absent, indicating the protection of the amine. It should also be noted there are two new signals in the \textsuperscript{13}C NMR at 175.5 and 155.4 ppm corresponding to the trifluoro substituted carbon and the ketone (both displaying the quartet characteristic of fluorine coupling within NMR spectroscopy). The ethylene ketal protecting group was then removed using \textit{p}-TSA in acetone and water to give the ketone (114) in a 66 % yield.\textsuperscript{56} This is shown in the \textsuperscript{1}H NMR with a disappearance of the singlet at 4.00 ppm and a chemical shift from 1.77 to 2.59 ppm for the CH\textsubscript{2} signal, indicating successful protection. There was also a new peak in the \textsuperscript{13}C NMR at 204.7 ppm again indicating the presence of the ketone.
8.9.6. Acetal monomer

This monomer was targeted so that the polymer could have an oxygen containing functionality, so when utilising these functional polymers when attaching biologically active molecules there would be an orthogonal approach compared to using the amine functionality. The retetro-synthetic pathway (Scheme 33) indicates that the commercially available 1,4-cyclohexanedione (117) as a viable starting material. The dione could be mono-protected (116), with the free ketone then expecting to undergo a Baeyer-Villiger oxidation to form the lactone (77). This lactone would then deprotected to leave the ketone functionality ready to incorporate into a co-polymer (115).

Scheme 33: Reterosynthetic analysis of ketone containing εCL monomer

The first step in the forward synthesis was the mono-protection of one of the ketone groups. This was achieved using ethylene glycol (118) and p-TSA in toluene, with the water removed using a Dean-Stark apparatus.

Scheme 34: Ketone protection using ethylene glycol

The ratio of dione to ethylene glycol was 1:1 with the intention of protecting only one ketone. The mono-protected dione was synthesised with the $^1$H NMR showing a singlet at 4.03 ppm
and triplets at 2.01 and 2.51 ppm. Unfortunately due to the statistical nature of the reaction both mono- (116) and di-protected (119) were isolated. The crude reaction yield $^1$H NMR showed there was far more di-protected than mono-protected, with singlets at 1.79 and 3.95 ppm and also a large singlet at 2.72 ppm indicating starting material. It was found that with the elevated temperatures required for the Dean-Stark to function (>110 °C), starting material was degraded. This in turn raised the ratio of ethylene glycol to dione, resulting in an increased amount of di-protected product. The amount of ethylene glycol was halved to a ratio of 1:0.5, with respect to the dione, so the reaction would only yield mono-protected and dione, and it was this reaction that yielded the mono-protected in 15 % yield. As the reactions yields were low and unpredictable, it was decided to persue different avenues.

The new approach was a direct Baeyer-Villiger of the dione to afford the 5-keto-$\varepsilon$-caprolactone. The standard reagent used to convert a cyclic ketone into a lactone is meta-chloroperoxybenzoic acid, $m$CPBA, with dichloromethane, CH$_2$Cl$_2$, as solvent.

Scheme 35: Baeyer-Villiger of 1,4-cyclohexandione using $m$CPBA

$m$CPBA, was used in CH$_2$Cl$_2$, due to the non-oxidisable nature of the solvent, for the Baeyer-Villiger of the dione. This was initially trialled using 1 mmol of starting material with no formation of product. The reaction was repeated with the use of NaHCO$_3$, to act as an in-situ base wash, with the $^1$H NMR spectrum showing a 50 % conversion (Table 2, Entry 1). The NMR spectra showed the presence of starting dione (singlet at 2.72 ppm), with multiplets at 4.42, 2.83 ppm and under the starting material signal at 2.74 ppm indicating the formation of the product. The reaction was scaled to several grams but it was found that the amount of product produced was far less, with product conversion of only 18 %, with the rest being starting material. It must also be noted that both these figures for the 1 mmol and scaled reaction are the highest of several repeat reactions. Each reaction exhibited a variety of conversions and crude yields, indicating that this reaction is not as reliable as previously thought.
The solvent was changed to chloroform to see if there was an improvement in conversion (Table 2, Entry 2). This solvent was chosen as it is also a non-oxidisable solvent and would therefore not interfere with the reaction. Although some conversion was observed (5 %), it was still far too low to be synthetically useful. Other organic solvents used were ethyl acetate (Table 2, Entry 3) and dry THF (Table 2, Entry 4), but unfortunately only the dione singlet was observed in the $^1$H NMR. Deionised water was used as solvent (Table 2, Entry 5), with only starting material isolated. To increase the solubility of the mCPBA a 1:1 mixture of acetonitrile and water (Table 2, Entry 6) was tried. Although increasing solubility, there was no conversion of product.

<table>
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<th>Entry</th>
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<th>Yield (%)</th>
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<td>40</td>
<td>50$^b$</td>
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<td>17</td>
<td>60</td>
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<td>8</td>
<td>mCPBA / BF$_3$.Et$_2$O</td>
<td>Anhydrous THF</td>
<td>17</td>
<td>-78</td>
<td>No yield</td>
</tr>
</tbody>
</table>

$^a$NaHCO$_3$ used in-situ, $^b$Conversion by $^1$H NMR.

Table 2: Baeyer-Villiger reactions using mCPBA

As the best results were observed when using CH$_2$Cl$_2$, this system was used in combination with the lewis acid, boron trifluoride etherate (BF$_3$.Et$_2$O) (Table 2, Entry 7). This addition increases the reactivity of the ketone with the intention of increasing the conversion. This however was not the case with the reaction yielding only starting material. The solvent was changed to dry THF (Table 2, Entry 8), but the result was the same with the $^1$H NMR showing only the dione singlet.
Other oxidants were then investigated, with the first being Oxone®. Potassium peroxymonosulfate, more commonly known as Oxone®, exists as the triple salt 2KHSO₅·KHSO₄·K₂SO₄ and has been used for a variety of oxidation purposes (Figure 12). This is due to the high stability, non-toxicity, cost and versatility. This versatility can be shown in Figure 12, indicating a variety of oxidation reactions. The first solvent investigated with Oxone® was CH₂Cl₂, for an extended time of 72 h (Table 3, Entry 1). Unfortunately even with the longer reaction time, only the starting dione singlet was observed in the ¹H NMR spectrum. The following reaction was carried out at room temperature so the following one reflux was used overnight (Table 3, Entry 2). Regrettably the reaction showed no conversion of the dione to the lactone, with only starting material being isolated. Solvent was then investigated with ethyl acetate initially chosen (Table 3, Entry 3), but no product was observed in the ¹H NMR. As Oxone® is a salt it is hard to dissolve in organic solvents; therefore it was then decided to use aqueous media. When using water (Table 3, Entry 4), the Oxone® was completely dissolved but still no conversion of starting material. Aqueous rich Table 3, (Entry 5) and organic rich (Table 3, Entry 6) mixtures of acetonitrile and water were then trialled. Even with these mixed solvent systems there was no formation of product.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Oxidant</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Temp (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxone®</td>
<td>CH₂Cl₂</td>
<td>72</td>
<td>25</td>
<td>No conversion</td>
</tr>
<tr>
<td>2</td>
<td>Oxone®</td>
<td>CH₂Cl₂</td>
<td>17</td>
<td>40</td>
<td>No conversion</td>
</tr>
<tr>
<td>3</td>
<td>Oxone®</td>
<td>Ethyl Acetate</td>
<td>17</td>
<td>80</td>
<td>No conversion</td>
</tr>
<tr>
<td>4</td>
<td>Oxone®</td>
<td>H₂O</td>
<td>2</td>
<td>80</td>
<td>No conversion</td>
</tr>
<tr>
<td>5</td>
<td>Oxone®</td>
<td>MeCN:H₂O (1:2)</td>
<td>17</td>
<td>100</td>
<td>No conversion</td>
</tr>
<tr>
<td>6</td>
<td>Oxone®</td>
<td>MeCN:H₂O (4:1)</td>
<td>17</td>
<td>100</td>
<td>No conversion</td>
</tr>
<tr>
<td>7</td>
<td>H₂O₂ / BF₃Et₂O</td>
<td>THF</td>
<td>3</td>
<td>25</td>
<td>No conversion</td>
</tr>
<tr>
<td>8</td>
<td>Peracetic acid</td>
<td>Acetic acid</td>
<td>17</td>
<td>120</td>
<td>No conversion</td>
</tr>
<tr>
<td>9</td>
<td>Peracetic acid</td>
<td>CH₂Cl₂</td>
<td>48</td>
<td>25</td>
<td>No conversion</td>
</tr>
<tr>
<td>10</td>
<td>Trifluoroperacetic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Anhydrous THF</td>
<td>17</td>
<td>25</td>
<td>No conversion</td>
</tr>
<tr>
<td>11</td>
<td>Trifluoroperacetic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CH₂Cl₂</td>
<td>2</td>
<td>25</td>
<td>No conversion</td>
</tr>
</tbody>
</table>

<sup>a</sup>Generated from trifluoroacetic anhydride and hydrogen peroxide in-situ.

Table 3: Use of alternative oxidising agents in the Baeyer-Villiger of 1,4-cyclohexandione
As both mCPBA and Oxone\textsuperscript{\textregistered} proved to be inefficient towards the Baeyer-Villiger of 1,4-cyclohexanedione, other oxidants were explored. This included the use of hydrogen peroxide in combination with a lewis acid, BF\textsubscript{3}.Et\textsubscript{2}O (Table 3, Entry 7). The Lewis acid increases the reactivity of the oxygen–oxygen bond, allowing the Baeyer-Villiger reaction to occur more readily. It was however found that this was not the case as there was no conversion of starting material to product. Peracetic acid was also used on its own (Table 3, Entry 8) and also with NaHCO\textsubscript{3} and NaOAc (Table 3, Entry 9). The sodium acetate was used to deprotonate the peracid, therefore increasing the nucleophilic nature of the acid. Unfortunately there was no formation of product in both cases (as indicated by the presence of only starting material in the \textsuperscript{1}H NMR). Another peracid that was used was trifluoroperacetic acid. This peracid was generated in-situ from the reaction between trifluoroacetic anhydride and hydrogen peroxide. Two solvents, THF (Table 3, Entry 10) and CH\textsubscript{2}Cl\textsubscript{2} (Table 3, Entry 11), were used but neither produced the desired product. It was decided that this route towards a ketone containing monomer needed to be approached from a different direction, therefore considering that as the mono-protected cyclohexanedione was commercially available, that was chosen as the starting point.

**Scheme 36: Baeyer-Villiger of mono-protected dione**
The Baeyer-Villiger of 1,4-cyclohexanedione monoethylene acetal (116) was carried out using mCPBA in CH₂Cl₂ (Scheme 36). This was carried out under reflux overnight and afforded γ-(ethylene acetal)-ε-caprolactone (77) in an 88 % yield. This protected ketone ε-caprolactone monomer can then be used in copolymerisation reactions. The reason for keeping the ketone protected is that the ketone can be deprotected, and then further reduced to the alcohol, post-polymerisation, avoiding possible complications the ketone may cause during polymerisation. With a functional εCL monomer synthesised the next stage was to co-polymerise to generate a functional polymer.

8.9.7. Acetal copolymers

Reacting the functional εCL monomer (77) and εCL together using polymerisation conditions of 1 % catalyst loading of stannous octanoate at 130 °C for 17 h, yielded random co-polymers containing a functional backbone (Scheme 37).

These random co-polymerisations were synthesised using 5 and 10 % of the acetal monomer (77). Both the 5 % and 10 % monomer concentrations were high yielding with the random co-polymer (120) being isolated at 90 % and 87 % respectively (Table 4, Entries 1 and 2). Their incorporation into the PCL polymer was observed in the ¹H NMR spectra as a singlet at 3.94 ppm indicating the ethylene ketal group (which was then integrated and used to find actual incorporation).
<table>
<thead>
<tr>
<th>Entry</th>
<th>Monomer used</th>
<th>Monomer amount (%)</th>
<th>Actual amount (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protected ketone (6)</td>
<td>5</td>
<td>4.82</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>Protected ketone (6)</td>
<td>10</td>
<td>8.95</td>
<td>87</td>
</tr>
</tbody>
</table>

1Calculated from 1H NMR integration.

Table 4: Results of acetal monomer co-polymerisations

The acetal polymer was treated with triphenylcarbenium tetrafluoroborate, Ph$_3$C$^+$ BF$_4^-$, to remove the protecting group and yield the ketone in a 91% yield (Scheme 38). The deprotection was observed by 1H NMR as a disappearance of the CH$_2$ signal at 3.94 ppm and a shift of all other CH$_2$ signals due to the newly formed ketone.

Scheme 38: Deprotection using triphenylcarbenium tetrafluoroborate

The ketone polymer was then treated with sodium borohydride to reduce the ketone to the alcohol. Sodium borohydride is used in protic solvents i.e. methanol or ethanol, however PCL and it’s functional forms are insoluble in these solvents. To counteract this a solvent system of CH$_2$Cl$_2$:EtOH (5:2) was used to provide solubility for the polymer, without impairing the performance of the sodium borohydride. The alcohol polymer was obtained in a 96% yield with complete conversion of the ketone group.

Scheme 39: Reduction of ketone to alcohol using sodium borohydride

The complete conversion to the alcohol was observed in the 1H NMR spectrum through the shifting of the protons either side of the previous ketone shifting further down into the
aliphatic region, and a new signal at 3.65 ppm corresponding to the CH at the base of the hydroxyl.

8.9.8. Alcohol polymer additions

With the successful synthesis of the alcohol pendant PCL polymer, additions to this functionality were then investigated. The first route chosen to attach molecules to PCL was to generate acid chlorides from which the hydroxyl group of the polymer can form an ester. The ester linkage is important as this is the same functionality that the PCL backbone has. This means that the attached molecule can be hydrolysed and ‘released’ under the same conditions as the degradation of the polymer. This will in theory be our drug delivering system. The first molecule chosen to attach was aspirin. Aspirin, or 2-acetylsalicylic acid, was synthesised through the esterification between salicylic acid and acetic anhydride (catalysed using H$_2$SO$_4$) (Scheme 40). The produced aspirin was isolated in an 80 % yield after recrystallization.

![Scheme 40: Aspirin synthesis](image_url)

Once synthesised, aspirin was dissolved in CH$_2$Cl$_2$ and thionyl chloride added to generate the acid chloride. After removal of the solvent the acid chloride was used immediately by addition to a CH$_2$Cl$_2$ solution containing the alcohol polymer, all under inert, dry conditions. The reaction was washed with sat NaHCO$_3$ to ensure any unreacted aspirin was removed before analysis. Upon precipitation in cold methanol a white solid was obtained with the $^1$H NMR spectrum showing successful attachment of the aspirin. Upon looking closer at the spectrum, a hydroxyl hydrogen could be seen indicating that the attachment was unsuccessful. The reaction was repeated with the amount of polymer being increased from 0.2 g to 1.0 g. This repeat however produced no evidence of aspirin attachment and thus
proved that the previous result was a false positive and must have occurred through insufficient washings to remove excess reagent.

Scheme 41: Addition of aspirin to alcohol polymer using thionyl chloride in solvent

The reaction conditions were then changed to have thionyl chloride as the solvent rather than used as reagent added to CH$_2$Cl$_2$. Aspirin was dissolved in thionyl chloride, reacted for an hour and the solvent removed under reduced pressure. The resulting product was immediately added to the CH$_2$Cl$_2$ solution containing the alcohol polymer. After reacting for one h, washed with sat NaHCO$_3$, and the polymer recovered through the usual hexane precipitation, no attachment to the polymer was observed through $^1$H NMR. After reviewing the conditions previously used, it was noticed that there was not any base present at the attachment stage to precipitate out any chloride ions formed through successful attachment. Therefore dry triethylamine was used, but to no avail as the $^1$H NMR spectrum again showed no successful attachment. Considering that each step of the reaction was only being allowed to react for one hour, the reaction times were increased to six hours to generate the acid chloride. The attachments were also increased to overnight reactions. Even with these prolonged reaction times, no attachment was observed through $^1$H NMR.

Scheme 42: Benzoyl chloride addition to the alcohol polymer
The next approach was to use a pre-generated acid chloride in the form of benzyol chloride. The reasoning for this was to both remove the use of thionyl chloride, and also to test purely the addition conditions. Using benzyol chloride eliminates the need for thionyl chloride and therefore improves the chance of reaction when compared to aspirin. This is because there is no need to generate the acid chloride in-situ as the reagent can be directly added to the reaction. The same conditions of an inert atmosphere, anhydrous THF, anhydrous triethylamine all at room temperature were used. Upon retrieval of the polymer through cold hexane precipitation, the $^1$H NMR spectrum yielded no evidence of successful attachment (easily observed through the lack of signals in the aromatic region). As with all initial reactions testing a new condition, only 0.2 g of polymer was used. Upon reflection it was noticed that as the polymer only contained 10% alcohol functionality, only 0.14 mmol, the use of 10 mL of solvent was creating a situation of high dilution where there was simply too much solvent for the reactants to collide and react. With this information the solvent level was dropped to 2 mL and the benzyol chloride reaction repeated. This time it would appear that the more concentrated reaction mixture had a positive effect with the $^1$H NMR spectrum indicating successful attachment of the benzyol group. This was observed through a triplet at 7.40 ppm and a doublet at 8.01 ppm, indicating aromatic protons.

This idea of carrying out the addition reactions at a high concentration was also used for methacryloyl chloride. The reaction was successful and the methacrylate polymer was obtained in a 45% yield. The attachment was confirmed by $^1$H NMR which showed the methyl singlet at 1.96 ppm and the two alkene proton at 5.57 and 6.11 ppm.

With the acid chlorides proving that attachments can in-fact take place, attention was returned towards aspirin. Aspirin was refluxed in thionyl chloride with a catalytic amount of dimethylformamide (DMF) added to aid the acid chloride generation, and then added to the
concentrated alcohol polymer solution. Unfortunately, after isolating the polymer and then analysis by $^1$H NMR, no aspirin was attached.

The next approach investigated was the condensation route to esters. The alcohol polymer and aspirin were dissolved in CH$_2$Cl$_2$ and the reaction heated to reflux. The key element of this reaction is that it was also carried out in the presence of 4 Å molecular sieves. The reason for this is that the reaction is in constant equilibrium between reactants and products, therefore removal of the water should, in accordance with the Le Chatelier’s principle, drive the equilibrium towards the product. However after allowing the reaction to be heated for 17 h, no aspirin was attached through analysis by $^1$H NMR.

![Scheme 44: Aspirin attachment using molecular sieves](image1)

The next step was to test ester coupling agents. A common coupling reagent is dicyclohexylcarbodiimide, (DCC). This reagent was first described by Wolfgang Steglich in 1978 and is therefore called the Steglich esterification. DCC is used together with a catalytic amount of DMAP to couple alcohols and carboxylic acids. Under an inert atmosphere the standard DCC conditions of anhydrous CH$_2$Cl$_2$ at room temperature were used, but as before no aspirin peaks were observed in the $^1$H NMR spectra of the collected polymer.

![Scheme 45: DCC coupling of aspirin to alcohol polymer](image2)
Another coupling reagent T3P®, propane phosphonic acid anhydride, was also tested. This reagent is usually used in peptide synthesis to couple amines and carboxylic acids; however it has been used in ester formation also. The attraction of using this reagent is that the by-product of the coupling is a phosphate salt, which is completely water soluble making work-up easier. The coupling reagent was used together with triethylamine, DMF as the solvent with the alcohol polymer being added before the aspirin. The reaction was diluted with CH₂Cl₂, so the DMF could be removed through brine washing, and the other reagents removed with HCl (1 M) and saturated NaHCO₃. After precipitating the polymer in cold hexane, it was analysed by ¹H NMR but unfortunately there were no signals in the aromatic region, therefore no aspirin attachment.

As the attempts to attach aspirin in a variety of methods were growing, with little or no success, it was decided to move on to try a different reagent in the form of the drug ciprofloxacin.

Another biologically important molecule which would be interesting to attach is ciprofloxacin. Ciprofloxacin (137) (Figure 16) is a second-generation fluoroquinoline antibiotic. It has a wide spectrum of activity including bacteria responsible for respiratory, urinary tract, gastrointestinal and abdominal infections. Although the previous list does not include cardiovascular infections/problems, ciprofloxacin has many features making it an ideal antibiotic of choice for a proof of concept study.
The main reason for using this antibiotic is that it can be easily analysed through use of the Gram staining protocol, as it responds to both gram (+) and (−) bacteria species. This means that the biological activity of any products can be easily observed in a relatively simple manner. Another key point in terms of analysis is that there are distinct hydrogen environments within the molecule making $^1$H NMR spectra simple to analyse, with the presence of the fluorine atom providing a key reference point when carrying out any surface analysis. This is because the only source of any fluorine signals is from successful attachment of the antibiotic. Another attractive feature is that it is commercially available from several sources.

Rather than trialling all the reactions used to attach aspirin, the literature provided a route towards the ciprofloxacin attachment. Sobczak polymerised εCL and lactide to generate hydroxyl terminal polymers, from which he attached ciprofloxacin. He carried this out by simply dissolving the polymers in CH$_2$Cl$_2$ and adding the ciprofloxacin together with a small amount of pyridine. Therefore this reaction was tested using a sample of the alcohol polymer. The reaction was left to react at room temperature over a weekend. As ciprofloxacin is sparingly soluble in CH$_2$Cl$_2$ (practically insoluble), upon collecting the
precipitated product there could be no way of determining if the signals observed in the $^1$H NMR spectrum were from attached or free ciprofloxacin. The product was redissolved in CH$_2$Cl$_2$ to create a cloudy white solution. The solution was then passed through a plug of celite in an attempt to remove the ciprofloxacin. The resulting solution was completely colourless indicating that the celite had filtered out the free ciprofloxacin. The polymer was precipitated out and the process repeated again to ensure all free ciprofloxacin is removed. The $^1$H NMR spectrum showed signals at 8.81, 8.06, 7.38, 3.57, 3.33, 3.13 and 1.23 ppm indicating the novel attachment of ciprofloxacin.

To conclude, although removal of the CBz group on the amine pendant polymer was problematic, success was found when switching to the alcohol variant, with a novel polymer – drug conjugate being synthesised. The research will now focus on the functionalisation of polymer films to generate polymer – drug conjugates utilising aminolysis.
8.10. Experimental

Commercially available reagents and solvents were used throughout without further purification, except for tetrahydrofuran (benzophenone/Na) which was freshly distilled. Light petroleum refers to the fraction with bp 40-60 °C. Thin layer chromatography was carried out on Merck Kieselgel 60 GF254 aluminum foil backed plates. The plates were visualized under UV light, phosphomolybdic acid stain and/or vanillin stain. Flash chromatography was carried out using Merck Kieselgel 60 H silica or Matrix silica 60, with the eluent specified. \(^1\)H and \(^{13}\)C NMR spectra were recorded using Bruker 400 MHz NMR machine (\(^1\)H 400 MHz, \(^{13}\)C 100 MHz); chemical shifts are quoted in ppm and coupling constants, \(J\), are quoted in Hz; d-Chloroform was used throughout unless otherwise stated. Spectra were calibrated to residual solvent peaks. In the \(^{13}\)C spectra, signals corresponding to C, CH, CH\(_2\) or CH\(_3\) groups, as assigned by DEPT, are noted. High and low resolution mass spectra were carried out on a Thermofisher exactive (orbi) resolution mass spectrometer. IR spectra were recorded using a Perkin Elmer FTIR Spectrometer (Paragon 100) as solutions using CH\(_2\)Cl\(_2\) as solvent. GPC was carried out on a Phenogel 10u column using polystyrene standards. Samples were run at 0.2 wt% in THF with diphenylether as a flowrate marker.

![Benzyl 4-hydroxycyclohexanecarbamate](image)

**Benzyl 4-hydroxycyclohexanecarbamate\(^{50}\) (104)**

To a solution of trans-4-aminocyclohexanol 103 (0.57 g, 4.99 mmol) and sodium hydrogen carbonate (1.05 g, 12.5 mmol) in water (50 mL), cooled in an ice bath, was added benzyl chloroformate (0.93 mL, 6.50 mmol) over 5 minutes. The reaction mixture was heated to 45 °C for 4 h. This was then extracted using n-butanol (3 x 50 mL) with the combined organic extract washed with hydrochloric acid (0.5 M), brine, dried with magnesium, and filtered. Volatiles were removed, under reduced pressure, until 104 began to crystallise. The product was left in the freezer overnight to crystallize which, after filtration, yielded white needles.
crystals (0.84 g, 67 %). M.p. 161-164 °C (Lit 163-165 °C). IR (CH₂Cl₂) ν_max 3389, 3341, 2949, 1686, 1534, 1066 cm⁻¹. ¹H NMR (CDCl₃, 400MHz) δ (ppm): 1.19 (q, J = 12.8 Hz, 2H, CH₂), 1.39 (q, J = 12.4 Hz, 2H, CH₂), 1.46 (d, J = 4.4 Hz, 1H, OH), 1.96-2.05 (m, 4H, CH₂), 3.49-3.52 (m, 1H, CH-NH), 3.56-3.66 (m, 1H, CH-OH), 4.59 (s, 1H, NH), 5.08 (s, 2H, CH₂-Ph), 7.29-7.38 (m, 5H, ArH). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 31.1, 33.9, 49.3, 66.6, 69.7, 128.2, 128.6, 136.5, 155.6. MS-ESI found 272.1248 C₁₄H₁₉O₃N, [M+Na]⁺ requires 272.1257.

Benzyl 4-oxocyclohexylanecarbamate⁵⁶ (105)

To a solution of Pyridinium chlorochromate (2.84 g, 13.42 mmol) in CH₂Cl₂ (100 mL) at 5 °C, was added 104 (2.77 g, 11.15 mmol). The reaction mixture was stirred at room temperature for 2 h followed by 5 h at 45 °C. The resulting reaction mixture was cooled and filtered through silica gel, using diethyl ether as the solvent. Volatiles were removed under reduced pressure to yield a white solid (2.37 g, 86 %). M.p. 81-83 °C (Lit 83-85 °C). IR (CH₂Cl₂) ν_max 3329, 2950, 1699, 1534, 1043 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.65-1.75 (m, 2H, CH₂), 2.25-2.27 (m, 2H, CH₂), 2.37-2.49 (m, 4H, CH₂), 4.00 (s, 1H, CH-NH), 4.72 (s, 1H, NH), 5.12 (s, 2H, CH₂-Ph), 7.31-7.40 (m, 5H, ArH). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 32.2, 38.9, 48.0, 66.9, 128.2, 128.3, 128.6, 136.3, 155.6, 209.5. MS-ESI found 270.1092 C₁₄H₁₇O₃N, [M+Na]⁺ requires 270.1101.
γ-(Carbamic acid benzyl ester)-ε-caprolactone (78)

To a solution of 105 (0.25 g, 0.99 mmol) in CH₂Cl₂ (20 mL), was added m-chloroperoxybenzoic acid (77%, 0.45 g, 1.99 mmol). The reaction mixture was then heated to reflux for 17 h. The reaction mixture was then washed with saturated sodium sulphite, saturated sodium hydrogen carbonate, brine, dried using magnesium sulphate and filtered. Volatiles were removed under reduced pressure to yield a white solid (0.24 g, 93%). M.p. 114-116 °C (Lit 116-117 °C). IR (CH₂Cl₂) νmax 3328, 2942, 1715, 1532, 1266, 1074 cm⁻¹.

¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.58 (q, J = 12.4 Hz, 1H, CH₂), 1.70-1.80 (m, 1H, CH₂), 2.17-2.28 (m, 2H, CH₂), 2.56-2.73 (m, 2H, CH₂), 3.82-3.84 (m, 1H, CH-NH), 4.17 (t, J = 10.4 Hz, 1H, CH₂-O), 4.27-4.32 (m, 1H, CH₂-O), 4.76 (s, 1H, NH), 5.10 (s, 2H, CH₂-Ph), 7.31-7.39 (m, 5H, ArH). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 29.1, 30.5, 35.6, 51.1, 65.5, 67.0, 128.2, 128.3, 128.6, 136.2, 155.4, 174.8. MS-ESI found 286.1042 C₁₄H₁₇O₄N, [M+Na]⁺ requires 286.1050.

Polycaprolactone (29)

A flame dried round bottom flask under nitrogen was charged with ε-caprolactone 3 (5 mL, 45.1 mmol) and tin octanoate (64, 0.3 M in toluene, 1.5 mL, 0.45 mmol, 1 mol%). The reaction mixture was heated to 130 °C for 17 h. While warm the reaction mixture was dissolved in CH₂Cl₂ (20 mL), and precipitated by dropwise addition to cold methanol (250 mL). A white solid was collected via suction filtration (5.03 g, 100%). M.p. 58-61 °C (Lit 60 °C). IR (CH₂Cl₂) νmax 2945, 1725, 1190 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.34 – 1.42 (m, 2H, CH₂), 1.61 – 1.69 (m, 4H, CH₂), 2.31 (t, J = 7.6 Hz, 2H, CH₂), 4.06 (t, J
= 6.4 Hz, 2H, CH₂). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 24.6, 25.5, 28.4, 34.1, 64.2, 173.6. Mₗ = 21380 g/mol by GPC.

Poly(ε-caprolactone – co - γ–carbamic acid benzyl ester ε-caprolactone) (106)

A flame dried round bottom flask was charged with ε-caprolactone 3 (1.05 mL, 9.5 mmol) and γ-carbamic acid benzyl ester ε–caprolactone 78 (0.13 g, 0.5 mmol). The reaction mixture was heated to 50 °C for 10 min to make the reaction homogeneous. Tin octanoate 64 (0.3 mL, 0.09 mmol, 0.3 M in toluene) was added, under vigorous stirring, and the reaction mixture heated to 130 °C for 17 h. The reaction mixture was dissolved in CH₂Cl₂ (20 mL), and precipitated in cold methanol (250 mL). White solid was collected via suction filtration (1.04 g, 86 %). M.p. 53-56 °C. IR (CH₂Cl₂) ν_max 3361, 2941, 1733, 1166 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.34 – 1.42 (m, 2H, CH₂), 1.62 – 1.69 (m, 4H, CH₂), 1.71 – 1.79 (m, 2H, CH₂), 1.83 – 1.89 (m, 2H, CH₂), 2.29 (t, J = 7.6 Hz, 2H, CH₂), 2.36 – 2.38 (m, 2H, CH₂), 3.76 – 3.79 (m, 1H, CH), 4.07 (t, J = 6.4 Hz, 2H, CH₂), 4.12 – 4.15 (m, 2H, CH₂), 4.78 – 4.81 (m, 1H, NH), 5.07 (s, 2H, CH₂), 7.29 – 7.37 (m, 5H, ArH). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 24.6, 25.5, 28.3, 30.2, 30.9, 34.1, 48.6, 61.2, 64.1, 64.4, 66.7, 128.0, 128.1, 128.5, 136.5, 156.0, 173.4, 173.6. Mₗ = 8270 g/mol by GPC.
**tert-Butyl-4-hydroxycyclohexylcarbamate (107)**

*trans*-4-aminocyclohexanol 103 (1.00 g, 8.68 mmol) was added to a flame dried flask under nitrogen and dry CH₂Cl₂ (20 mL) added. The reaction mixture was cooled to 0 °C triethylamine (2.42 mL, 17.40 mmol) added drop wise over 30 min. To this solution was then added di-*tert*-butyl-dicarbonate (2.09 g, 9.55 mmol) in dry CH₂Cl₂ (5 mL) over 1 h. Reaction mixture was left to react for 17 h and allowed to warm to room temperature. Reaction mixture was washed with citric acid (1 M), brine, dried using magnesium sulphate and filtered. Volatiles were removed under reduced pressure with the resulting solid purified by column chromatography (1:1, petrol:ethyl acetate) to yield a clear oil (1.42 g, 82 %). IR (CH₂Cl₂) ν<sub>max</sub> 3419, 2939, 1682 cm⁻¹. <sup>1</sup>H NMR (CDCl₃, 400 MHz) δ (ppm): 1.16 (t, J = 12.8 Hz, 2H, CH₂), 1.57 – 1.32 – 1.57 (m, 11H, CH₃, CH₂), 1.94 – 2.01 (m, 4H, CH₂), 3.417 (bs, 1H, CH), 3.58 – 3.62 (m 1H, CH), 4.34 (bs, 1H, N-H). <sup>13</sup>C NMR (CDCl₃, 100 MHz) δ (ppm): 28.5, 31.2, 34.1, 48.9, 69.9, 79.2, 155.3. MS-ESI found 238.1413 C₁₁H₂₁O₃NNa, [M+Na]<sup>+</sup> requires 238.1414.

**tert-Butyl-4-oxocyclohexylcarbamate (108)**

To round bottom flask under a nitrogen atmosphere was added pyridinium chlorochromate (2.25 g, 10.44 mmol) in CH₂Cl₂ (50 mL). To this solution was gradually added *tert*-butyl-4-hydroxycyclohexylcarbamate 107 (1.50 g, 9.95 mmol). The reaction mixture was then heated to reflux for 17 h. The excess volatiles were removed under reduced pressure and the reaction mixture redissolved in ethyl acetate:hexane (100 mL, 1:1) and filtered through a plug
of celite and eluted with the same solution (100 mL). The eluent was washed with sodium hydroxide (1 M, 3 x 50 mL), dried using magnesium sulphate, filtered and excess volatiles removed under reduced pressure to yield a clear oil (1.28 g, 86%). IR (CH₂Cl₂) ν max 3360, 2974, 1523 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.44 (s, 9H, CH₃), 1.60 – 1.65 (m, 2H, CH axial), 2.20 – 2.23 (m, 2H, CH equatorial), 2.39 – 2.40 (m, 4H, CH₂), 3.91 (m, 1H, CH), 4.49 (s, 1H, NH). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 28.5, 32.4, 39.1, 47.7, 79.8, 155.2, 209.9. MS-ESI found 236.1255 C₁₁H₁₉O₃NNa, [M+Na]⁺ requires 236.1257.

![Image of tert-butyl (7-oxooxepan-4-yl)carbamate](image)

**tert-butyl (7-oxooxepan-4-yl)carbamate (109)**

tert-Butyl-4-oxocyclohexylcarbamate 107 (1.06 g, 4.97 mmol) was dissolved in CH₂Cl₂ (30 mL) in a round bottom flask. m-chloroperoxybenzoic acid (77%, 1.57 g, 7.00 mmol) as then added slowly over 15 min. Reaction mixture was then heated to refluxed for 17 h. The reaction mixture was then washed with saturated sodium sulphite, saturated sodium hydrogen carbonate, brine, dried using magnesium sulphated and filtered. Volatiles were removed under reduced pressure to yield a clear oil. This was purified by column chromatography (3:2, petrol:ethyl acetate) to yield a clear oil (0.60 g, 52%). IR (CH₂Cl₂) ν max 1724, 1682, 1527 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.42 (s, 9H, CH₃), 1.53 (q, J = 12.4 Hz, 1H, CH), 1.70 (q, J = 10.4 Hz, 1H, CH), 2.25 – 2.14 (m, 2H, CH), 2.57 (t, J = 12.8 Hz, 1H, CH), 2.68 (dd, J = 8.4, 8.4 Hz, 1H, CH), 3.73 (bs, 1H, CH), 4.15 (t, J = 9.6 Hz, 1H ,CH), 4.28 (dd, J = 8.4, 8.4 Hz, 1H, CH), 4.53 (bd, J = 4.8 Hz, 1H, NH). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 28.4, 29.4, 30.7, 35.9, 50.9, 65.8, 80.0, 154.9, 175.0. MS-ESI found 252.1207 C₁₁H₁₉O₃NNa, [M+Na]⁺ requires 252.1206.
Poly(ε-caprolactone – co- tert-butyl (7-oxooxepan-4-yl)carbamate) (110)

A flame dried round bottom flask was charged with ε-caprolactone 3 (1.05 mL, 9.5 mmol) and tert-butyl (7-oxooxepan-4-yl)carbamate 109 (0.12 g, 0.5 mmol). The reaction mixture was heated to 50 °C for 10 min to make the reaction homogeneous. Tin octanoate 64 (0.3 mL, 0.09 mmol, 0.3 M in toluene) was added, under vigorous stirring, and the reaction mixture heated to 130 °C for 17 h. The reaction mixture was dissolved in CH₂Cl₂ (20 mL), and precipitated in cold methanol (250 mL). White solid was collected via suction filtration (0.86 g, 72 %). IR (CH₂Cl₂) νmax 3372, 1724, 1682, 1527 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.32 – 1.42 (m, 11H, CH₃, CH₂), 1.51 – 1.67 (m, 6H, CH₂), 2.14 – 2.30 (m, 4H, CH₂), 2.57 (t, J = 12.4 Hz, 1H, CH), 2.68 (dd, J = 8.4, 8.4 Hz, 1H, CH), 3.72 (bs, 1H, CH), 4.04 (t, J = 6.4 Hz, 2H, CH₂), 4.15 (t, J = 10 Hz, 1H, CH), 4.28 (dd, J = 8.4, 8.4 Hz, 1H, CH), 4.55 (bs, 1H, NH). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 24.5, 24.6, 25.6, 28.4, 29.3, 30.7, 34.2, 35.9, 50.9, 64.2, 65.7, 80.0, 154.9, 173.6, 174.9. Mₛ = 9224 g/mol by GPC.

8-Trifluoroacetyl-8-aza-1,4-dioxa[4,5]decane⁵⁶ (113)

1,4-dioxa-9-azaspiro[4,5]decane 111 (2.56 mL, 20 mmol) was added dropwise to round bottom flask containing trifluoroacetic anhydride 112 (5.64 mL, 40 mmol) at 0 °C. The reaction mixture was then heated to 60 °C and left for 17 h. The reaction mixture was then added to ice water (50 mL) and stirred for 15 min and extracted with CH₂Cl₂ (3 x 50 mL), washed with water, dried using magnesium sulphate and filtered. Excess volatiles were
removed under reduced pressure, to yield an orange oil. Product was then purified by column chromatography (9:1 petrol:ethyl acetate) to give a clear oil (2.43 g, 51 %). IR (CH₂Cl₂) νₓmax 2892, 1691, 1178 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.75 – 1.78 (m, 4H, CH₂), 3.67 (t, 2H, J = 4 Hz, CH, equatorial), 3.77 (t, 2H, J = 6 Hz, CH, axial), 4.00 (s, 4H, CH₂). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 34.6, 35.5, 41.8, 43.7, 64.6, 106.1, 116.5, 155.9. MS-ESI found 262.0655 C₉H₁₂O₃NF₃, [M+Na]⁺ requires 262.0661.

N-trifluoroacetyl-piperidin-4-one⁵⁶ (114)

8-Trifluoroacetyl-8-aza-1,4-dioxaspiro[4.5]decane 120 (2.34 g, 9.81 mmol) was added to a round bottom flask containing a solution of acetone (50 mL) and water (10 mL). p-TSA (0.21 g, 1.12 mmol) was then added and the reaction heated to 80 °C for 48 h. The reaction mixture was concentrated and extracted with CH₂Cl₂ (3 x 50 mL). The organic extracts were combined and washed with saturated sodium hydrogen carbonate, water, dried using magnesium sulphate and filtered. Volatiles were removed under reduced pressure to give the product which was purified with column chromatography (9:1 petrol:ethyl acetate) to give a white solid (1.32 g, 66 %). M.p. 72-74 °C (Lit 75-76 °C).⁶³ IR (CH₂Cl₂) νₓmax 2919, 1727, 1691, 1176 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 2.58 (q, J = 6.4 Hz, 4H, CH₂), 3.90 (t, J = 6.4 Hz, 2H, CH equatorial), 3.98 (t, J = 6.4, 2H, CH axial). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 40.3, 41.0, 42.6, 44.0, 116.3, 155.9, 204.7. MS-ESI found 218.0395 C₇H₈O₂NF₃, [M+Na]⁺ requires 218.0399.
1,4-cyclohexanedione monoethylene acetal (116)

1,4-Cyclohexanedione 117 (0.11 g, 1.00 mmol), p-TSA (0.004 g, 0.02 mmol, 2 mol%) and ethylene glycol 118 (0.06 g, 1.00 mmol) were dissolved in toluene (20 mL) in an round bottomed flask fitted with a Dean Stark apparatus. The reaction mixture was heated to reflux and left for 1 h. The reaction mixture was then cooled and washed with saturated sodium hydrogen carbonate, brine, dried with magnesium sulphate and filtered. Removal of volatiles under reduced pressure followed by purification by column chromatography (6:4 petrol:ethyl acetate) yielded a pale orange solid (0.02 g, 15 %). M.p. 68-71 °C (Lit 70-73 °C). IR (CH₂Cl₂) ν_max 2884, 1711, 1085 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 2.01 (t, J = 6.8 Hz, 4H, CH₂), 2.51 (t, J = 7.2 Hz, 4H CH₂), 4.03 (s, 4H, CH₂). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 33.9, 38.2, 64.7, 107.2, 210.3. MS-ESI found 157.0833 C₈H₁₂O₃, [M+H]⁺ requires 157.0815.

γ-(Ethylene acetal)-ε-caprolactone (77)

1,4-cyclohexanonedione monoethylene acetal 116 (0.16 g, 1.00 mmol) in a round bottom flask was dissolved in CH₂Cl₂ (20 mL) and mCPBA (77%, 0.34 g, 1.51 mmol) added slowly, and the reaction heated at reflux for 17 h. Reaction mixture was washed with saturated sodium sulfite, saturated sodium hydrogen carbonate, brine, dried using magnesium sulphate and filtered. Volatiles were removed, under reduced pressure, to yield a white solid (0.15 g, 88%). M.p. 47-49 °C (Lit 49-51 °C). IR (CH₂Cl₂) ν_max 2891, 1732, 1158 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.91 (t, J = 5.6 Hz, 2H, CH₂), 2.01 (t, J = 4.8 Hz, 2H, CH₂), 2.71 (t, J = 5.6 Hz, 2H, CH₂), 3.99 (s, 4H, CH₂), 4.29 (t, J = 4.8 Hz, 2H, CH₂). ¹³C NMR
(CDCl₃, 100 MHz) δ (ppm): 28.8, 32.7, 39.0, 64.3, 64.8, 107.9, 175.4. MS-ESI found 195.0623 C₈H₁₂O₄, [M+Na]⁺ requires 195.0628.

Poly(ε-caprolactone – co - γ-ethylene acetal ε-caprolactone) (120)

A flame dried round bottom flask was charged with ε-caprolactone 3 (1.05 mL, 9.5 mmol) and γ-(ethylene acetal)-ε-caprolactone 77 (0.09 g, 0.5 mmol). The reaction mixture was heated to 50 °C for 10 min to make the reaction homogeneous. Tin octanoate 64 (0.3 mL, 0.09 mmol, 0.3 M in toluene) was added while the reaction mixture was stirred vigorously. Then the reaction mixture was heated to 130 °C for 17 h. After cooling the reaction mixture was dissolved in CH₂Cl₂ (20 mL), and precipitated in cold methanol (250 mL). White solid was collected via suction filtration (1.05 g, 90 %). M.p. 50-53 °C. IR (CH₂Cl₂) νₘₐₓ 2943, 1733, 1165 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.34 – 1.42 (m, 2H, CH₂), 1.61 – 1.69 (m, 4H, CH₂), 1.95 – 2.03 (m, 4H, CH₂), 2.31 (t, J = 7.6 Hz, 2H, CH₂), 2.37 (t, J = 8 Hz, 2H, CH₂), 3.94 (s, 4H, CH₂), 4.06 (t, J = 6.8 Hz, 2H, CH₂), 4.16 (t, J = 7.2 Hz, 2H, CH₂). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 24.6, 25.5, 28.4, 28.7, 32.6, 34.1, 36.0, 60.2, 64.2, 65.1, 109.4, 173.4, 173.6. Mₘ = 24570 g/mol by GPC.

Poly(ε-caprolactone – co - γ-keto-ε-caprolactone) (121)

The co-polymer poly(ε-caprolactone – co - γ-ethylene acetal ε-caprolactone) 120 (6.14 g, 3.56 mmol acetal content) was dissolved in CH₂Cl₂ (100 mL) in a round bottom flask. Triphenylcarbenium tetrafluoroborate (1.77 g, 5.35 mmol) was added and a colour change from colourless to green was observed. The reaction mixture was then stirred at room temperature for 24 h. The reaction mixture was concentrated and precipitated in cold methanol (250 mL) to yield a white solid (5.42 g, 91 %). M.p. 56-59 °C. IR (CH₂Cl₂) νₘₐₓ
2944, 1726, 1190 cm\(^{-1}\). \(^1\)H NMR (CDCl\(_3\), 400MHz) \(\delta\) (ppm): 1.34 – 1.42 (m, 2H, CH\(_2\)), 1.60 – 1.69 (m, 4H, CH\(_2\)), 2.31 (t, \(J = 7.6\) Hz, 2H, CH\(_2\)), 2.60 (t, \(J = 6.4\) Hz, 2H, CH\(_2\)), 2.75 (t, \(J = 6.8\) Hz, 2H, CH\(_2\)), 2.80 (t, \(J = 6.4\) Hz, 2H, CH\(_2\)), 4.06 (t, \(J = 6.8\) Hz, 2H, CH\(_2\)), 4.34 (t, \(J = 6.4\) Hz, 2H, CH\(_2\)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) (ppm): 24.6, 25.5, 28.3, 34.2, 37.4, 41.5, 59.1, 64.2, 64.7, 172.8, 173.4, 205.9. \(M_w = 20410\) g/mol by GPC.

![Poly(ε-caprolactone – co – γ-hydroxyl-ε-caprolactone)(122)](image)

Poly(ε-caprolactone – co – γ-hydroxyl-ε-caprolactone) (122)

Poly(ε-caprolactone – co – 4-keto-ε-caprolactone) 120 (3.52 g, 2.74 mmol ketone content) was dissolved in CH\(_2\)Cl\(_2\) (30mL) in a round bottom flask and ethanol (12mL) added. Sodium borohydride (0.21 g, 5.48 mmol) was added slowly to a stirring solution with effervescence observed. The reaction mixture was left stirring for 24 h at room temperature, before the reaction mixture was concentrated and precipitated in cold hexane (250 mL) to yield a white solid which was collected by suction filtration (3.37 g, 96 %). \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) (ppm): 1.28 – 1.44 (m, 2H, CH\(_2\)), 1.55 – 1.71 (m, 8H, CH\(_2\)), 2.34 (t, \(J = 4\) Hz, 4H, CH\(_2\)), 3.65 (t, \(J = 6.4\) Hz, 1H, CH), 4.06 (t, \(J = 6.4\) Hz, 4H, CH\(_2\)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) (ppm): 24.7, 25.5, 28.4, 32.3, 34.1, 34.2, 34.8, 62.8, 64.2, 68.5, 173.9, 174.0. \(M_w = 19570\) g/mol by GPC.

![2-Acetoxybenzoic acid (125)](image)

2-Acetoxybenzoic acid (125)

Salicylic acid 123 (5.00 g, 36.2 mmol) was dissolved in acetic anhydride 124 (6.8 mL, 71.9 mmol) in a round bottom flask and conc sulphuric acid (few drops) was added, the reaction mixture was then heated to 80 °C for two h. The reaction mixture was poured into cold distilled water (50 mL) and placed in an ice bath. White crystals were filtered off and recrystallized using hot ethanol with the addition of a small portion of cold water. This
yielded the product as white needle crystals (5.19 g, 80 %). M.p. 132-135 °C (lit M.p. 134-136 °C) IR. \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) (ppm): 2.38 (s, 3H, CH\(_3\)), 7.17 (dd, \(J = 1.2, 8\) Hz, 1H, ArH), 7.39 (dt, \(J = 1.2, 8\) Hz, 1H, ArH), 7.66 (dt, \(J = 1.6, 8\) Hz, 1H, ArH), 8.18 (dd, \(J = 1.6, 8\) Hz, 1H, ArH). \(^13\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) (ppm): 21.1, 122.2, 124.1, 126.3, 132.6, 135.0, 151.3, 169.6, 169.8. MS-ESI found 203.0315 C\(_9\)H\(_8\)O\(_4\)Na, [M+Na]\(^+\) requires 203.0315.

Poly(ε-caprolactone – co – γ- benzoyl ester-ε-caprolactone) (128)

To a dry round bottom flask under nitrogen was added poly(ε-caprolactone – co – γ-hydroxyl-ε-caprolactone) 122 (0.30 g, 0.23 mmol alcohol content) and anhydrous THF (2 mL). Dry triethylamine (0.48 mL, 3.4 mmol) was added and benzoyl chloride 127 (0.27 mL, 2.3 mmol) was drospise added to produce a cloudy white solution. After complete addition the reaction mixture was left stirring for 17 h at room temperature. Distilled water (5 mL) was added followed by extraction with CH\(_2\)Cl\(_2\) (3 x 10 mL). The organic extracts were washed with saturated sodium hydrogen carbonate, hydrochloric acid (1 M), brine and dried using magnesium sulfate. Solvent was concentrated under reduced pressure and precipitated in cold hexane (100 mL) to yield a white solid which was collected by suction filtration (0.27 g, 83 %). IR (CH\(_2\)Cl\(_2\)) \(\nu_{\text{max}}\) 1724, 1630 cm\(^{-1}\). \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) (ppm): 1.32 – 1.39 (m, 2H, CH\(_2\)), 1.58 – 1.70 (m, 6H, CH\(_2\)), 1.75 – 1.78 (m, 2H, CH\(_2\)), 2.28 (t, \(J = 8\) Hz, 4H, CH\(_2\)), 4.03 (t, \(J = 6.8\) Hz, 4H, CH\(_2\)), 4.29 (t, \(J = 6.4\) Hz, 1H, CH) 7.40 (t, \(J = 7.2\) Hz, 2H, ArH), 7.50 (t, \(J = 7.6\) Hz, 1H, ArH), 8.01 (d, \(J = 7.2\) Hz, 2H, ArH). \(M_w\) = 33690 g/mol by GPC.
Poly(ε-caprolactone – co – γ- methacryloyl ester-ε-caprolactone) (130)

To a dry round bottom flask under nitrogen was added poly(ε-caprolactone – co – γ-hydroxyl-ε-caprolactone) 122 (0.30 g, 0.23 mmol alcohol content) and anhydrous CH₂Cl₂ (2 mL). Dry triethylamine (0.48 mL, 3.4 mmol) was added and the reaction mixture cooled to 0 °C. Methacryloyl chloride 129 (0.22 mL, 2.25 mmol) was added dropwise over 1 h. The reaction mixture was allowed to warm to room temperature and was washed with hydrochloric acid (1 M), saturated sodium hydrogen carbonate, brine, dried using magnesium sulphate and filtered. The reaction mixture was concentrated under reduced pressure and precipitated in cold hexane to yield a white solid which was collected under suction filtration (0.14 g, 45 %). IR (CH₂Cl₂) ν max 1724, 1630 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.37 – 1.47 (m, 2H, CH₂), 1.63 – 1.71 (m, 8H, CH₂), 1.96 (s, 3H, CH₃), 2.33 (t, J = 8 Hz, 4H, CH₂), 4.08 (t, J = 6.4 Hz, 4H, CH₂), 4.17 (t, J = 6.4 Hz, 1H, CH), 5.57 (t, J=1.6 Hz, 1H, CH), 6.11 – 6.12 (m, 1H, CH). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 14.1, 24.4, 25.6, 28.4, 31.2, 34.2, 35.6, 45.8, 61.0, 62.9, 64.2, 125.4, 136.5, 167.5, 173.6, 173.8. Mₘ = 33300 g/mol by GPC.

Poly(ε-caprolactone – co – γ- ciprofloxacin ester-ε-caprolactone) (132)

Poly(ε-caprolactone – co – γ - hydroxyl-ε-caprolactone) (122, 0.30 g, 0.23 mmol alcohol content) was dissolved in CH₂Cl₂ (5 mL) in a round bottom flask and pyridine (5 drops,
catalytic) added. Ciprofloxacin 131 (0.38 g, 1.15 mmol) was then added and the reaction stirred at room temperature for 72 h. The reaction mixture was then passed through a celite plug, using CH₂Cl₂ as the eluent. The filtrate was concentrated and then precipitated in cold hexane to yield a white solid. The solid was then redissolved in CH₂Cl₂ and passed through a second celite plug to ensure all particles of ciprofloxacin were removed. The filtrate was concentrated under reduced pressure and precipitated in cold hexane to yield a white solid which was collected using suction filtration (0.09 g, 26%). IR (CH₂Cl₂) vₘₐₓ 1725, 1626 cm⁻¹. ¹H NMR (CDCl₃, 400MHz) δ (ppm): 1.21 – 1.26 (m, 2H, CH₂), 1.39 – 1.44 (m, 4H, CH₂), 1.65 – 1.70 (m, 8H, CH₂), 2.23 (t, J = 7.6 Hz, 4H, CH₂), 3.13 (t, J = 5.2 Hz, 4H, CH₂), 3.33 (t, J = 5.2 Hz, 4H, CH₂), 3.57 (m, 1H, CH), 3.67 (t, J = 6.4 Hz, 1H, CH), 4.08 (t, J = 6.4 Hz, 4H, CH₂), 7.38 (d, J = 7.2 Hz, 1H, ArH), 8.06 (d, J = 13.2 Hz, 1H, ArH), 8.81 (s, 1H, CH). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 8.3, 24.7, 25.3, 25.4, 28.4, 32.4, 34.2, 34.3, 35.3, 45.8, 50.1, 62.8, 64.2, 77.3, 106.4, 111.2, 111.4, 112.2, 122.1, 139.7, 148.4, 152.5, 166.5, 173.4, 173.6, 179.9. M_w = 16540 g/mol by GPC.
9. Surface modification of polyesters with biologically active molecules through aminolysis

9.1. Introduction

This section of the thesis will cover the use of surface chemistry as an alternative to generate polymer – drug conjugates. This area of research will be undertaken with the intent of utilising a simple surface modification technique, to generate a functional polymer via a process called aminolysis.

Initially literature surrounding surface modification and its applications will be discussed as an introduction to the field. This will include a brief summary of the modification of surfaces through various methods including how PCL can be modified, as this will be the polymer used in this research.

The literature review will continue in looking at how surfaces are analysed. These techniques are extremely varied and can be used to analyse a broad range of parameters. The overview will cover several techniques with XPS being used specifically in this research (although the others could become applicable in future work).

Following the section on surface analysis, aminolysis will be introduced not only from a surface point of view, but its use in organic chemistry. The principles of aminolysis will be further discussed as this will be the main technique used in the following research.

After the literature review, the aminolysis of PCL and PLA films will be shown, along with extensive XPS analysis. This will lead into the biologically important molecules of ciprofloxacin and penicillin G being successfully attached to these substrates, using amide coupling chemistry.
9.2. Types of surface modification

The area of research being undertaken in this section is centred on surface chemistry as a way of functionalising polyesters; therefore the examples of this chemistry and the ways in which they are analysed will be discussed. These modifications are incorporated into materials to create a difference in properties between the bulk material and the surface. These can range from simple roughness (to increase surface area) to hydrophilicity and biocompatibility.

![Figure 14: Polymer brush formation; (A) physisorption, (B) chemisorption and (C) surface initiated](image)

One of the most utilised and researched surface modification techniques is the use of polymer brushes. Polymer brushes are chains of polymer that create an ultra-thin coating, with a chain end tethered directly to the surface of a substrate. There are two methods of attaching these
polymers to the surface, the *grafting to* and *grafting from* approaches.\(^6^6\) The former involves the attachment of pre-existing polymers through either physisorption or chemisorption (i.e. a covalent bond). In the *grafting from* approach, the polymerisation is initiated from an initiator-functionalised surface allowing accurate manipulation of the resulting polymer. To create a dense polymer brush the surface initiated approach is usually used,\(^6^7\) due to the steric hindrance found when trying to manipulate pre-existing polymers.\(^6^8\)

The previously mentioned technique is used predominantly in the functionalisation of silicon or metal based materials, by utilising the chemistry of silicon – oxygen bonds,\(^6^9\) or the reactivity of the metal involved (e.g. the reaction between gold and thiols) (Scheme 48).\(^7^0\)

![Scheme 48: Surface initiated polymerisation from a gold substrate](image)

The previous example of surface chemistry focused on the activation of metal based materials, and this is not so applicable to polymers. For polymers, such as polyesters, surface activation is highly desirable in the field of tissue engineering. Tissue engineering is a multidisciplinary field consisting of chemists and material scientists’ right through to cellular biology and genetic engineers. A challenge that is faced by such teams is to find materials that are compatible within the body, do not cause or trigger an immune response, but that contain enough reactivity as to be further utilised for specific applications.

A whole range of natural materials are already employed in tissue replacement such as collagen, laminin and fibronectin because they exhibit exceptional biodegradability, biocompatibility and cell adhesion.\(^7^1\) The major drawback of such materials is that when placed in an environment where physical properties are required, they are very weak. This is where synthetic polymers such as polyesters can be used to give better physical
characteristics. Another problem that presents itself when solely using polymers, is that they themselves are not favourable surfaces from which to propagate cells due to their hydrophobicity, whereas hydrophilic surfaces have been shown to be advantageous.

There are several techniques employed throughout the literature focusing on increasing the hydrophilicity of polyesters. Although the previous polymer brush examples used existing surfaces that then reacted, in the case of polymers, their surface are relatively inert and need to be activated. Once such method is the use of plasma to generate radical species on the surface, these are then reacted with air to produce peroxides from which to react from.

This process used by Cheng et al. utilised argon plasma to generate surface radicals, which upon standing in air, generated peroxides (Scheme 49). These peroxides were then grafted with acrylic acid using UV light to generate a hydrophobic layer of carboxylic acid functionality. These acid moieties were then activated using a water solubile carbodiimide (WSC) and then treated with a collagen solution to create a biologically active surface. The ability of the surface to increase the rate of proliferation was then tested using a human dermal fibroplast culture, with the biologically activated films showing a far greater level of proliferation compared to the pure unreacted film (Figure 15).
Although successful in increasing cell proliferation, treatment using plasma has potential side effects. Due to the radical nature of the plasma (i.e. argon radicals in the previous example) undesired side reactions can occur due to the uncontrollable formation and reaction of radicals. One such side reaction is the crosslinking of the treated material. This is shown in work by Tajima et al.\textsuperscript{75} who utilised this crosslinking nature when using octafluorocyclobutane (C\textsubscript{8}F\textsubscript{8}) plasma. By exploiting the crosslinking nature of plasma, they were able to form fluorocarbon films onto polyethylene substrates (Figure 16). Although the crosslinking nature of plasma treatment has been exploited in this example, in the previous work by Chang et al. this crosslinking would be detrimental therefore for the purposes of this research plasma will not be used.
A far simpler approach is to use the swelling nature of a polymer to modify the surface. In certain solvents polymers do not dissolve but rather swell as the molecules of solvent diffuse into the polymer structure. This allows the development of pores from which to embed other molecules. This method was first investigated by Desai and Hubbell who were using polyethylene terephthalate (PET) and embedding other polymers within the swollen surface to increase hydrophilicity and by extension, cell viability. They chose trifluoroacetic acid as the solvent as although it dissolves PET, when used at a more dilute level the swelling can be controlled. This dilution was made using a solution of water soluble polymers (such as polyethylene glycol) in distilled water. A range of these soluble polymers were embedded and then analysed using contact angle measurements. Once surface chemistry has been utilised a variety of techniques can be used to determine the nature and characteristics of the newly created surface. A sample of these techniques will now be discussed to give a brief overview of how surfaces can be analysed.
9.3. Surface analysis

There are several ways in which scientists can monitor or analyse a surface. These techniques are varied and if used together they can provide a detailed look towards what, if any, changes are occurring on a materials surface. The main method utilised in this research is XPS however, several different techniques will be discussed to give a broader scope to how surfaces can be analysed, as they could potentially be used in future work.

An example previously alluded to in the swelling polymer modification type was the use of contact angles (Figure 17). Although not used in this research, due to the hydrophobic nature of PCL and the increase of hydrophilicity when aminolysing the surface, this technique alongside XPS would indicate successful functionalisation of the polyester surface, therefore the principles will be discussed. A contact angle is the angle between a solid surface and the tangent of a droplet. It is used to determine the ‘wettability’ of a surface, i.e. how much the surface likes water (hydrophilicity). Small contact angle means that the droplet has flattened and covered a greater area on the solid surface (i.e. it has a high affinity for the surface, hydrophilic), whereas a large contact angle shows a greater degree of repulsion from the surface indicating a more hydrophobic nature. The limitation of contact angle measurements is that although they allow an indication that surface modification has taken place, it does not indicate the reason why, i.e. it cannot indicate what particular chemistry or characteristic of the surface has changed, only that it has.

![Figure 17: Contact angle (θ) diagram and Young's equation](image)

A key part of any surface reaction or chemistry is how much of the surface is available to react. This is governed by the total surface area of the material and is generally called surface roughness. Topography of the surface can be measured using two main techniques, scanning electron microscope (SEM) or atomic force microscopy (AFM). The research following this
introduction could utilise surface imaging as a way of investigating the effect of aminolysis, and whether or not it affects surface roughness, therefore a brief overview of each technique will covered.

A commonly employed technique used to analyse surface roughness is atomic force microscopy (AFM). This method uses a cantilever and atomic precise tip to measure the profile of a surface. It does this in two ways. The first is called the contact mode and measures the force required to move this tip across the surface. When moving along a flat surface the force required is constant, however when there is a depression or a rise in topography, there is a change in force to reflect this. The change of this together with the mapping of the stage creates a depth profile and therefore topography of the surface (Figure 18).

![AFM images of an unmodified polyurethane film (a) and a polyurethane film treated with 5 minutes of UV ozone (b)](image)

However if the sample’s surface is ‘soft’ or brittle it could be damaged using the previous mode of AFM. This is because force applied while moving the tip across the surface could damage the sample. An alternate approach is called the tapping mode. This mode oscillated the tip and uses the frequency of the oscillation to guage the depth profile of the sample and thus the topography. On a flat surface the oscillation frequency is constant, but upon reaching a peak or depression in the sample the frequency increases or decreases respectively mapping the surface. This is because when the tip reaches a peak the distance of the oscillation becomes smaller and therefore the frequency rises (and vice versa for the
depression). This reduces the stress on the surface and therefore there is no damage to the sample.

The other method commonly employed to analyse surface roughness is scanning electron microscopy (SEM). SEM relies on the emission of electrons when a surface is subjected to a focused beam of electrons. The focused or high energy electrons used by this method collide with electrons within the energy shells of an atom and ejects one from the shell effectively replacing it. The electron ejected from the atom (known as the secondary electron) has far lower energy and can be analysed using a detector. The relative intensity of these secondary electrons is what creates the surface image. The contrast of this image is determined from the location the secondary electron is emitted from. The closer the location is to the detector the brighter the contrast on of the produced image, and conversely the darker the image in a depression. This creates a three dimensional representation of the surface’s topography and an indication of surface roughness. A limitation of this technique is the sample itself. The technique requires the sample to be conductive, which in the case of conducting metals is not an issue; however for non-conducting materials such as polymers sample preparation is required. This is usually carried out by deposition a layer of gold, or a gold/palladium alloy, on the surface of a sample to generate the conductivity. 

![Figure 19: SEM images of a clean Ti Surface (left) and an HCl/HNO₃ etched Ti surface (right). Bar indicated 10μM](image)

A very simple method of increase the surface area or roughness is to either physically or chemically etch a polished metal surface. Boyan et al. used titanium discs for use in observing the effect of surface roughness on the cellular response/propagation of cells. They utilised a variety of methods to increase surface area. The most simple of these methods was
to etch the surface using acid. This was carried out using a hydrochloric/nitric acid mixture. A physical method they employed was to sandblast the surface using 0.25 – 0.50 μM corundum grit at 5 bars of pressure. Both techniques were also employed together to see if the effect could be cumulative.

What is clear to see in Figure 19 is that the surface topography has clearly changed, and that the roughness has greatly increased. This increase in surface area is also reflected in the responses of the tested cells. MG63 osteoblast-like cells (those involved in agglomerating and depositing calcium in bone formation) were cultured on the clean and etched surfaces, and then treated with a hormone solution to gauge the response and therefore proliferation of the cultured cells. There was a surface-roughness dependant increase in hormone activity where an increase in surface roughness gave a higher activity. This shows the importance of surface topography when trying to utilise surfaces for biological applications.

Although surface roughness has been shown to increase cell proliferation, come biomedical applications require a smooth surface, such as stents that are being forced along arteries, to avoid cellular damage. In the work by Vaithilingam et al. surface roughness of a titanium surface was mechanically polished using a series of silicon carbide grits (200, 400, 600, 800 and 1200 μm) followed by diamond paste of 6 and 1 μm. This created a smoother surface from which to functionalise from (Figure 20).

![Figure 20: SEM micrographs showing an untreated titanium surface (a and b, 50x and 500x magnification respectively), and the mechanically polished surface (c)](image)

The titanium surface was further functionalised through the use of a carboxylic acid terminated phosphonate. Due to the structure of the phosphonate (amphiphilic) a self assembled monolayer (SAM) was formed generating carboxylic acid functionalised surface. Acid chloride chemistry was then utilised to generate the mixed anhydride with the
ciprofloxacin (Scheme 50). This work shows that the route of surface functionalisation is a viable approach to polymer–drug conjugates.

Scheme 50: Functionalisation of a titanium surface with ciprofloxacin

Another key feature of a surface is elemental composition. A key technique in this field is X-ray photoelectron spectroscopy (XPS) and will be used extensively in this research in looking for successful aminolysis of polymer surfaces but also for drug attachments. This technique uses the unique binding energies of electrons within different elements to determine what elements are present on a surface. An X-ray beam is directed at the sample and electrons from different orbital shells are ejected from the atoms of the surface. The energy of these electrons are then measured at the detector and correspond to the exact binding energies of electrons found in the orbitals of specific atoms. This means that when looking at the produced spectrum the composition can be deduced from the integrals of the peaks and their element identified from the specific binding energy (Figure 21). This information can be especially useful when looking at polymers where the specific bonding modes of atoms can
be deduced (as the electrons in the bonds have different energies, for example the difference between the electrons in a carbonyl or C-C bond).

![Figure 21: XPS spectra of the binding energies of the C1s electrons within different bonding environments of PLA](image)

Following on from surfaces and how their chemistries can be analysed is a brief look at how amines can alter the surface functionality for polymers, through a process known as aminolysis.

### 9.4. Aminolysis

Aminolysis is the chemical reaction in where a compound is reacted with an amine and is cleaved into two new species. This process is commonly used in the production of amides from carboxylic acids or derivatives of. As the polymers being used in the following research are polyester based, aminolysis is an elegant way of introducing functionality and will therefore be discussed as this technique will be the heavily used in this project. Such examples are show in the work by Lima et al. who use DBU as a catalyst during peptide synthesis, with the reaction being carried out neat to give an indication towards how preferable this method can be (Scheme 51).
The use of amine to convert esters to amide has also been utilised in the field of polymers. The previous example using methyl benzoate was aminolysed using a mono functional amine, giving rise to the corresponding amide. The other functionality found on the amine is a benzyl group but if there was another amine (to create a diamine) then there would be a free amine from which to react. This style of aminolysis via the use of a diamine is common place in polyester surface modification.

A commonly used polymer for this aminolysis reaction is PCL. The reason that this is a target for aminolysis is because it has desirable biocompatibility and biodegradation, but it is very hydrophobic in nature. The aim of the aminolysis reaction is to generate a more hydrophilic surface (through the introduction of polar amine groups) and increase the compatibility with proliferating cells.

Although the choice of material is an important one, equally so is the choice of the diamine used in the aminolysis stage. Although any diamine would give the corresponding boost in hydrophilicity desired from the reaction, the size of the diamine plays an important role. Zhu et al.\textsuperscript{85} found that a decrease in the chain length of the diamine (i.e. going from 1,8-octanediarnine to 1,2-ethanediarnine) resulted in a higher density of amine groups. The likely cause of this increase is due to the lack of steric hindrance found in the smaller chain diamines, giving rise to a greater depth penetration into the surface (Figure 22, left). Another important note is that having far more amine density might seem like an advantage, however after a certain length of time the structural integrity of the material suffers as a function of aminolysis (Figure 22, right). This is due to the very nature of the aminolysis reaction where you split a molecule in two using an amine, therefore what is effectively happening is for each successful reaction you are reducing the molecular weight of the polymer.
Figure 22: The relative increase in amine density when altering the diamine (left) and the observed weight loss of a PCL film during extended aminolysis experiments (right)

The most common diamine used in the aminolysis of PCL is 1,6-hexamethylenediamine (HMDA). This is shown in work from Gao et al.\textsuperscript{86} who employ HMDA in an isopropyl alcohol solution to aminolyse PCL films prepared by slow evaporation in a petri dish. Once aminolysed their approach was to react the free amino group with glutaraldehyde (a di-aldehyde) from which to immobilise their biomacromolecule. They did this so that the free amine would form the imine and a free aldehyde. This aldehyde can then further react with the free amine found on a biomacromolecules (Scheme 52).

\begin{center}
\textbf{Scheme 52: Use of HMDA and glutaraldehyde to attach macromolecules to a PCL surface}
\end{center}
An alternate approach used by Mattanavee et al.\textsuperscript{87} was to use an activating group rather than adding another reactive moiety. The activating agent used was N,N’-disuccinimidylcarbonate (DSC) which when reacted onto the free amine, produces an activated carbonyl group giving rise to a more susceptible centre for nucleophilic attack from biomacromolecules (\textbf{Scheme 55}).

\begin{center}
\textbf{Scheme 53: Use of DSC as an activating agent in the attachment of biomacromolecules to a PCL surface}
\end{center}

A novel twist on the aminolysis of PCL is used in work from Zhang et al.\textsuperscript{88} who manufacture a 3D scaffold of PCL from which to aminolysate and functionalise with RGD. The structure was created using an inverse wax mold manufactured on a 3D printer. This mold was placed in molten PCL at just below 80°C (just below the melting point of the wax but above the PCL), followed by allowing the construct to cool overnight. The wax was removed using ethanol (which PCL is insoluble in). The 3D scaffold of PCL was then treated with HMDA in IPA to aminolysate the surface. This aminolysed surface was activated in a similar fashion as discussed in previous work, followed by a solution of the RGD peptide to bind it to the surface.
What is also important to consider is that although the previous examples have focused on PCL, other polyesters are also applicable. Work by Zhu et al.\textsuperscript{89} utilise PLA instead of PCL in the synthesis of a polymer-drug conjugate. PLA undergoes the same aminolysis pathway yielding a free amine from which to react further. In this research both the newly formed amine and alcohol are reacted with $\alpha$-bromoisobutryl bromide (BIBB) to generate a terminal bromide. This bromide is then used in a polymerisation mechanism called atom-transfer radical polymerisation (ATRP) together with methacrylic acid (MAAS). The pendant carboxylic acids are then esterified using gelatin to form the polymer-drug conjugate (Scheme 54).

![Scheme 54: Aminolysis of PLA and further synthesis to achieve a polymer-drug conjugate](image)

**Figure 23:** 3D PCL scaffold aminolysed using HMDA and stained using ninhydrin
Aminolysis has been shown to give rise to free amine groups when reacted with polyesters. However due to the variety of reactions that amines can undergo, other functionalities can be aminolysed in a similar fashion to polyesters. Work by Horning et al. has utilised aminolysis to yield thiol bearing polymers through the cleavage of dithioesters used in reversible addition-fragmentation chain transfer (RAFT) polymerisation. During the RAFT process the polymer is always capped with the thioester group, which in this research was reacted with an amine to afford the corresponding thio terminus polymer and the thioamide by-product (Figure 24).

Figure 24: Aminolysis of a polymer utilising RAFT chemistry to yield thiol terminal polymers
9.5. Results and discussion

The literature discussed in the aminolysis section previously, shows how a polyester surface can be aminolysed to yield pendant amine groups. These groups can then subsequently be further functionalised to incorporate biologically active molecules. This gives a clear indication that aminolysis can be utilised in the formation of polymer – drug conjugates. The route in which these polymer – drug conjugates are synthesised also shows that they can be made fairly straightforward utilising well understood chemistry.

With the literature proving that aminolysis is a viable method, focus is shifted towards work being carried out within the Christie group. As previously mentioned in the introduction, ciprofloxacin was successfully attached to a titanium surface. The aim of this research is to transfer that idea to a fully biodegradable system using a polyester. What can be gained from this previous work is that there is sufficient sensitivity when using XPS to detect successful attachment (Figure 25). The key to using ciprofloxacin is that it can be the only source of fluorine, giving rise to a distinctive signal in the XPS spectrum.

![Figure 25: XPS spectra showing the successful attachment of ciprofloxacin to a titanium surface](image-url)
9.5.1. Research Plan

The work by Gao et al.\textsuperscript{86} used HMDA to aminolyse a PCL film, followed by glutaraldehyde to further functionalise the aminolysed surface, whereas Mattanavee et al.\textsuperscript{87} also used HMDA for the aminolysis however chose to use DSC to activate the pendant amine. Imide chemistry was used in the first work and an activating group for the latter, however both created an extra synthetic step towards attaching the biomacromolecule of choice.

\begin{center}
\includegraphics[width=\textwidth]{scheme_55.png}
\end{center}

\textit{Scheme 55: Proposed route to a polymer - drug conjugate through amide coupling chemistry}

In this research the knowledge of aminolysis using HMDA will be utilised however rather than either inserting extra functionality or activating the amine, amide coupling chemistry will be used. This will allow the use of a well established field in the form of peptide synthesis along with shortening the steps needed to attach a drug molecule.
9.5.2. Aminolysis of a PCL film

Firstly, the PCL was synthesised using the same method as previously used earlier in this thesis (Scheme 56). This produced PCL in quantitative yields and was achieved on a multigram scale.

![Scheme 56: Polymerisation of εCL to PCL](image)

The next stage in this research was to create a surface from which to aminolyse. This was carried out by the manufacture of PCL films. The films were produced by the slow evaporation of solvent from a polymer solution (typically 10% polymer), which was poured into a petri dish. These petri dishes were then heated to 37 °C overnight and the solvent slowly removed, to yield a uniform PCL film. Common solvents that dissolve PCL are dichloromethane and THF however upon slow evaporation at 37 °C from a 10% PCL solution, the produced films were broken and instead of a uniform film, large pieces of brittle PCL were collected. A potential reason for this could be due to the volatility of these solvents. Both dichloromethane and THF are highly volatile and the broken films would suggest that rather than allowing the PCL to distribute itself evenly across the petri dish, the solvent evaporated too quickly leading to aggregates of PCL and therefore no film. Another potential cause of this could lie with water that has condensed due to the fast evaporation, leading to defects and holes. This problem was overcome with the use of chloroform. Although still volatile in its own right, chloroform does not exhibit the evaporation speed shown by either dichloromethane or THF. This allowed the even distribution of PCL not only through the solution, but more importantly it retained this during evaporation. Due to the robustness of the polymerisation technique (ie, the fact it can be multi-gram) a large number of films could be produced in a single batch.
A key technique that was mentioned in the literature review (especially when concerning surfaces) is XPS, or X-ray photoelectron spectroscopy. **Figure 26** shows the survey XPS spectrum of a pure PCL film. A survey spectrum contains all the data over a wide range of binding energies. This gives a general overview of what elements are present. The large peak at 533 eV indicates the presence of oxygen and the peak at 285 eV is that of carbon. What the ‘1s’ donates is that the electron which has been detected comes from the 1s shell orbiting the atoms nucleus.
Figure 26: XPS survey spectrum of pure PCL
The following figures show the respective high resolution scans of both the carbon and oxygen regions. What these scans show is a data collected over a more narrow range of binding energies, but with far higher sensitivity, giving rise to a greater level of detail.

![High resolution XPS spectrum of the carbon region in pure PCL](image)

**Figure 27: High resolution XPS spectrum of the carbon region in pure PCL**

<table>
<thead>
<tr>
<th>Environment</th>
<th>C-C/H</th>
<th>C-O</th>
<th>C=O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic %</td>
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<td>13.59</td>
<td>9.7</td>
</tr>
<tr>
<td>Ratio</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Table 5: Atomic % and ratio for the carbon region of pure PCL*

The blue line is the raw data with the yellow lines correspond to those that are peak fitted. These peaks take into account that the shoulder present on a peak is there because it is in fact two peaks overlapping. The red line is the sum of the fitted curves which when compared to the blue, shows a good fit for the peak fitted data. The peak fitting is carried out because of the broad peaks often observed when using XPS compared to those with other techniques, e.g. NMR, which have far better resolution. As with NMR the binding energy (chemical shift in the NMR example) is affected by what the atom in question is bonding to. This is because...
the change in electronegativity between the atom and heteroatoms alters the binding energy of
the electrons within the atom. The high resolution spectrum of the C1s signal shows four
distinct carbon environments. Three of these are equal in area with the other being roughly
three times as large. This is because the larger peak (284.6 eV) represents the three aliphatic
carbon atoms present in the PCL backbone. The other peaks are shifted due to their
proximity to oxygen. The least effects by oxygens presence is the carbon alpha to the
carbonyl (285.2 eV) followed by the carbon attached to the ester oxygen (286.2 eV). The
final peak belongs to that of the carbonyl (288.7 eV), all corresponding to literature values.  

![High resolution XPS spectrum](image)

**Figure 28: High resolution XPS spectrum of the oxygen region in pure PCL**

<table>
<thead>
<tr>
<th>Environment</th>
<th>O=(\text{C})</th>
<th>C-(\text{O})</th>
</tr>
</thead>
<tbody>
<tr>
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<td>9.38</td>
</tr>
<tr>
<td>Ratio</td>
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<td>1</td>
</tr>
</tbody>
</table>

**Table 6: Atomic % and ratio for the oxygen region of pure PCL**
The above figure is the high resolution spectra showing the oxygen region. As expected there are two peaks of equal area with one belonging to the carbonyl oxygen (531.9 eV) and the other to that of the ester (533.2 eV).

Figure 29: High resolution XPS spectrum of the nitrogen region in pure PCL

<table>
<thead>
<tr>
<th>Environment</th>
<th>N1s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic %</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Table 7: Atomic % for the nitrogen region of pure PCL

The figure above (Figure 29) shows the nitrogen 1s region in the same high resolution previously shown for carbon and oxygen. The key point to note is that when compared to the previous high resolution spectra the counts are extremely low. This would indicate that there is only trace nitrogen present in the pure PCL film, as the peak is for all intents and purposes in the baseline noise. These trace levels could be attributed to could be the incorrect handling of the sample and therefore the potential presence of a fingerprint (protein signal).
Once the pure PCL films had been successfully cast into films and their surface chemistry analysed, aminolysis reactions were investigated to transform the polyester surface into a functionalised one capable of further manipulation. The mechanism behind aminolysis is that a primary amine reacts with an ester linkage to form the corresponding amide bond and generate a free alcohol terminated chain. The challenge that PCL films present is that solvents such as dichloromethane or THF cannot be used as the film would dissolve, and carrying out reactions at elevated temperatures would result in the film melting and losing its structural integrity (and therefore far more difficult to analyse surface chemistry) as the melting point of PCL is roughly 60 °C. For this reason IPA was chosen with the reaction temperature not exceeding 37 °C. Another consideration is that when you start to react the ester bonds of the PCL and begin creating smaller chains through the mechanism of aminolysis, you degrade the polymer’s physical properties. This results in loss of the PCL film thickness and therefore reaction times need to be considered or else there will be no film left to analyse (or it will become too brittle to handle and effectively use it in subsequent stages and reactions).

Scheme 57: Aminolysis of a pure PCL film using HMDA in IPA at 37 °C for one h

The final consideration to make is which diamine to use. The smaller the alkyl chain between the primary amines, the greater the penetration depth and therefore the higher degree of aminolysis. The initial investigations were carried out using hexamethylene diamine 151 (HMDA). This was used at 10 % (w/w) in IPA, with the film being immersed for 1 h at 37 °C (Scheme 57). The film was then washed using copious distilled water to both remove any alcohol solvent and more importantly any residual diamine.

A quick method to see if any aminolysis has taken place is the use of a ninhydrin stain. Ninhydrin solutions are used in organic chemistry during TLC analysis as a way of staining amines. The aim of a TLC stain is to provide a visual aid when determining a compound
purity (or for use in chromatography to aid in finding a compound among fractions of a column), with ninhydrin the characteristic colour is purple for primary amines and yellow for secondary amines. The purple colour (known as Ruhemann’s purple) is the result of the primary amine acting as a linker between 2 ninhydrin molecules creating an extended chromophore and a distinctive purple colour (Scheme 58).  

![Scheme 58: Formation of Ruhemann's purple using ninhydrin](image)

The ninhydrin stain of the aminolysed PCL film was compared to that of a pure unreacted PCL film, and the results clearly show the presence of free amine groups. The ninhydrin test first involves the bathing of a film in a 1M ninhydrin in ethanol solution before being dissolved in 1,4-dioxane and heated to drive the formation of the stained compound and therefore the purple colour. As expected, a film that has not been aminolysed shows a clear solution compared to that of the aminolysed film which shows the purple colouration (Figure 30).

![Figure 30: Pure PCL film (left) and aminolysed PCL film (right) after ninhydrin treatment](image)
Another quick analytical method to qualitatively identify if aminolysis occurred is attenuated total reflectance infra-red (ATR-IR). In Figure 31 there is a strong carbonyl signal for the pure PCL film at 1720 cm\(^{-1}\) due to the ester bond, however there is no amide peak. The ATR-IR of the aminolysed film, clearly showing the amide peaks present at 1637 cm\(^{-1}\) and 1562 cm\(^{-1}\), together with the ester peak at 1720 cm\(^{-1}\)(comparison also shown in Figure 32).

![Figure 31: ATR-IR spectrum of an aminolysed PCL film](image)

![Figure 32: ATR-IR comparison of a pure PCL film (blue) and an aminolysed film (red) in the carbonyl region](image)
The aminolysed films were then analysed using XPS to further investigate whether aminolysis had occurred (to confirm what is observed in the FTIR).

Figure 33: High resolution XPS spectrum of the oxygen region in HMDA aminoyled PCL in IPA for 1 h at 37 °C

<table>
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<tr>
<th>Environment</th>
<th>O=C</th>
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<tr>
<td>Atomic %</td>
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</table>

Table 8: Atomic % and ratio for the oxygen region in HMDA aminolyed PCL in IPA for 1 h at 37 °C
Figure 34: High resolution XPS spectrum of the carbon region in HMDA aminolysed PCL in IPA for 1 h at 37 °C

<table>
<thead>
<tr>
<th>Environment</th>
<th>C-C/H</th>
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<th>C=O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic %</td>
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</tr>
<tr>
<td>Ratio</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 9: Atomic % and ratio for the oxygen region in HMDA aminolysed PCL in IPA for 1 h at 37 °C
There is no real change when looking at the oxygen and carbon high resolution spectra however there is now a clear peak in the nitrogen region. The high resolution spectrum clearly shows one peak far larger than the other. In XPS the area of a peak is proportional to its content within the sample, indicating that in this scenario there is a difference in the abundance of one nitrogen environment with respect to another. If the film had been successfully aminolysed then there would be a peak for the newly formed amide and also a peak for the free amine, at equal intensity. The two peaks do correspond to the literature values (399.4 and 401.1 eV) however due to the difference in peak area, not all of the nitrogen can be associated to successful aminolysis.\(^8\) Contamination of the film was a possibility, i.e. incomplete washing of the film to remove the excess HMDA. This was investigated using the ninhydrin test previously discussed. The presence of the Ruhemann’s
purple gives a visual indicator of the presence of free amine (qualitative), utilising this colour with UV spectroscopy can quantify the amount of free amine. The key wavelength to use is 538 nm, with the film being prepared as previously discussed. The absorbance obtained when aminolysing a PCL film using HMDA in IPA at a 10 % (w/w) concentration was 0.458 Abs. This method of anaylsis has been used in the literature with a calibration graph giving the amount of free amine using this result as being orders of magnitude higher than in the reported literature. This can be interpreted as confirming the hypothesis that free HMDA has not been washed off, and that the colour obtained from the ninhydrin test cannot prove that aminolysis has actually taken place.

The ‘aminolysed’ film was then placed in a beaker of water overnight (using a shaker to encourage cleaning), in an attempt to remove the excess HMDA. In this case angle resolved XPS was utilised to investigate the penetration of the aminolysis. Angle resolved XPS (ARXPS) uses the exact same principles of XPS but alters the angle of the stage on which the sample sits. The penetration depth of the x-rays used in an experiment is <10 nm, by altering the angle of the sample stage, this penetration depth decrease. This is because when the X-ray is pointed direct at the stage (with 0° from perpendicular), the depth profile is directly down into the sample. This gives data from further within the sample to give bulk information. When the angle is increased through to 80°, the X-ray penetrates far less, giving data concentrating on the surface. Comparison of these data sets allows the difference in bulk and surface elemental composition to be determined (shown in Figure 36 with the example of an oxide layer).
The key piece of information observed when using ARXPS is that as you increase the angle, the intensity of the signal drops. This is, as previously mentioned, due to the loss in penetration. The relative loss in intensity between different angles and different elements gives an indication of the composition of the bulk and surface. As expected the intensity of the carbon and oxygen reduce as the angle increases (due to the material being made of PCL). What these spectra show is that the decrease in nitrogen is far more pronounced, shown not only by the increase in noise (showing a far more reduced abundance of nitrogen, and only partial aminolysis), but by the fact that at 60° there is almost none present. This coupled with the fact that the silicon signal is still strong at this angle, indicates that the bulk contains more nitrogen and the surface contains more silicon (possible contamination from silicon in the lab like grease for joints). This translates to there being a large surface contamination, meaning that only a small amount of aminolysis can take place below this layer. This could also be the reason why the aqueous washing post aminolysis was less effective and why the prolonged water soak (overnight) removed the excess HMDA.

Figure 37: ARXPS spectrum showing the carbon, oxygen, nitrogen and silicon regions. Angles used were 0° (red), 30° (green) and 60° (blue)
It was then decided to try a cleaning regime prior to aminolysis in an attempt to remove the contaminent layer. Films were soaked in an ethanol:water (1:1) solution at 37 °C for 1 h and then dried using a flow of nitrogen. After further looking at the aminolysis process, it was decided to run the reaction in water at pH = 11.5. This is because this ensures that the diamine exists as the amine and can therefore react. The theory being that if the pH was not adjusted, the amine would protonate using hydrogens from water (due to the water being acid at pH 7, relative to the amine). This would in turn create an ammonium ion which would be unreactive towards the ester due to its lone pair not being available.

Scheme 59: Aminolysing conditions including the aqueous ethanol clean

The newly cleaned films were submerged into a solution of HMDA (10 % in water adjusted to pH 11.5). This was carried out immediately after the films were cleaned to avoid contamination between conditions. The films were left as before for 1 h at 37 °C, before being washed with water and dried under a flow of nitrogen. The films were analysed using XPS to give the nitrogen high resolution spectrum shown in Figure 38.
Figure 38: High resolution spectrum of the nitrogen region of a PCL film, when using an aqueous ethanolic wash and aminolysing using HMDA 10% in water adjusted to pH 11.5

<table>
<thead>
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<td>Atomic %</td>
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<tr>
<td>Ratio</td>
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<td>1</td>
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</table>

Table 11: Atomic % and ratio for the nitrogen region of a PCL film, when using an aqueous ethanolic wash and aminolysing using HMDA 10% in water adjusted to pH 11.5

What is clear to see is that the intensity of the peaks match giving rise to two different nitrogen environments in equal abundance, leading to the conclusion of all the nitrogen content coming from a successful aminolysis reaction.
The nitrogen region gives a clear indication of aminolysis as the only source of nitrogen can come from successful aminolysis. Another indicator is the oxygen region and the presence of a third peak. For every successfully aminolysed ester bond you generate the corresponding amide and free amine, but also the free alcohol generated from the ester cleavage. This peak is clearly visible in the spectrum shown in Figure 39, whereas the peak is absent when looking at the pure PCL film.

With a successful aminolysis regime in place a different aminolysing reagent was used. Another readily available diamine is ethylene diamine (EDA). This diamine was chosen as the shorter alkyl chain length grants greater penetration depth and theoretically a larger

---

**Figure 39**: High resolution XPS of the oxygen region of a PCL film when using an aqueous ethanolic wash and aminolysing using HMDA 10% in water adjusted to pH 11.5

<table>
<thead>
<tr>
<th>Environment</th>
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<th>O-H</th>
</tr>
</thead>
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<tr>
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<td>Ratio</td>
<td>1</td>
<td>1</td>
<td>0.16</td>
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</tbody>
</table>

Table 12: Atomic % and ratio for the oxygen region of a PCL film, when using an aqueous ethanolic wash and aminolysing using HMDA 10% in water adjusted to pH 11.5
degree of aminolysis. The conditions used for aminolysis are identical to those of the HMDA aminolysed films (including the cleaning of the films). XPS data shows that aminolysis has successfully occurred (although the difference in peak intensity would indicate some residual EDA, potentially due to the increase in penetration depth and therefore the more difficult to wash).

Another indicator of successful aminolysis when using the oxygen region was also noticed with the appearance of the hydroxyl peak (Figure 41).

**Figure 40:** High resolution XPS of the nitrogen region of a PCL film when using an aqueous ethanolic wash and aminolysing using EDA 10% in water adjusted to pH 11.5

<table>
<thead>
<tr>
<th>Environment</th>
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<tr>
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<td>Ratio</td>
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</table>

**Table 13:** Atomic % and ratio for the nitrogen region of a PCL film, when using an aqueous ethanolic wash and aminolysing using EDA 10% in water adjusted to pH 11.5
Figure 41: High resolution XPS of the oxygen region of a PCL film when using an aqueous ethanolic wash and aminolysing using EDA 10% in water adjusted to pH 11.5

<table>
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<td>Ratio</td>
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<td>0.33</td>
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</table>

Table 14: Atomic % and ratio for the oxygen region of a PCL film, when using an aqueous ethanolic wash and aminolysing using EDA 10% in water adjusted to pH 11.5

The theory that EDA can penetrate the films deeper than HMDA and therefore increase the degree of aminolysis, is shown in Figure 42. In both comparisons there is a clear increase in the degree of aminolysis. The intensity difference between the green (HMDA) and blue (EDA) lines shows that more aminolysis has taken place when using EDA. The unfortunate side effect of this increase in aminolysis, is that films lose too much of their physical properties and become too brittle to work with, often crumbling under the force of a nitrogen flow while driving. With this in mind, it was decided to utilise the HMDA aminolysing conditions to further manipulate the PCL films in drug attachment reactions.
Figure 42: Comparison of the nitrogen (top) and oxygen (bottom) regions. Pure PCL (red), HMDA aminolysed (green) and EDA aminolysed (blue)
Table 15: Comparison of the atomic % of nitrogen and oxygen species of PCL films used in various aminolysis conditions

<table>
<thead>
<tr>
<th>Environment</th>
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<th>O-H</th>
</tr>
</thead>
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<td>0.27</td>
<td>-</td>
</tr>
<tr>
<td>HMDA aminolysed</td>
<td>1.67</td>
<td>1.34</td>
<td>1.59</td>
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<tr>
<td>EDA aminolysed</td>
<td>2.5</td>
<td>3</td>
<td>3.4</td>
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</table>

Following the successful aminolysis of the PCL surface using both a short (C2) and longer (C6) chain diamine, other aminolysing reagents were investigated. Two new reagents, ethanolamine and butylamine were chosen. The reasoning behind using ethanolamine was to synthesise the aminolysed product and generate an alcohol tether from which to functionalise from (in contrast from an amine tether). The hexylamine was chosen as it would only generate an alkyl chain length. This was important as it was unknown how the ethanolamine would react during the ninhydrin stain, and therefore the hexylamine was used to see if a hydroxyl group could be differentiated using the test. PCL films were used as before, including the same cleaning regime, and treated with a 10% solution of each respective aminolysis reagent at 37 °C for one h. The ninhydrin test provided evidence of aminolysis for both the new reagents, which could lead to ethanolamine being utilised in a similar fashion to the amine tether (ester formation over amide linkages).

Figure 43: Ninhydrin stains when using different aminolysis reagents. Left: butylamine, middle: hexamethylene diamine, right: ethanolamine
9.5.3. Ciprofloxacin attachment to a PCL film

The biologically important molecule chosen to attach to the PCL film was ciprofloxacin. The key reason for this choice is because of the presence of an element that is not carbon, oxygen or nitrogen. Ciprofloxacin has a fluoride atom attached to a benzene ring which can be used as an indicator of the drug’s presence on the film using XPS analysis.

Another feature of ciprofloxacin is that there is a carboxylic acid moiety meaning that amide coupling chemistry can be used to attach to the free amine on the aminolysed surface. One such coupling agent is 1-propanephosphonic anhydride (T3P®) which is used in conjunction with N,N-diisopropylethylamine (Hünig’s base or DIPEA) (Figure 45).

Both the coupling agent and the base are commercially available with T3P® being available in a solution of either ethyl acetate or acetonitrile. Ethyl acetate was chosen purely based on availability within the lab, as both solvents have similar properties with respect to PCL solubility. Literature methods use ~3 mg/mL solutions when trying to attach molecules to aminolysed films, therefore this amount was utilised in drug attachment reactions. The first attachment was trialled using ciprofloxacin, T3P® and Hünig’s base in ethyl acetate at room
temperature under an inert atmosphere of N₂. As previously mentioned the amount of ciprofloxacin used was 3 mg/mL in ethyl acetate, with 1.1 equivalents coupling agent and 2 equivalents of base (calculated using 5 mL of ciprofloxacin solution, enough to completely submerge the PCL film in a 25 mL flask).

Scheme 60: Ciprofloxacin attachment using T3P®

Films were washed with ethanol (to remove any ethyl acetate), then with copious water (to remove any ethanol) before being washed in fresh water overnight (to diffuse out any residual solvents or reagents). After being dried using a flow of N₂ the films were analysed using XPS. Specifically looking at the fluorine region (679 – 699 eV), no peak was observed.

Figure 46: High resolution XPS showing the fluorine region of a ciprofloxacin attached film when using T3P®
It is clear from the high resolution XPS spectrum that the attachment was not successful (Figure 46). The attachment was then carried out using ciprofloxacin hydrochloride (CIP.HCl). The reason behind this switch was the presence of the secondary amine on the ciprofloxacin molecule. Although the primary amine of the surface should be more reactive, the aminolysed film is added to the reaction last. This is because in the mechanism of coupling when using T3P® requires the carboxylic acid, coupling agent and base to be stirred prior to the introduction of the amine. This could lead to the coupling of ciprofloxacin with itself, which would be prevented if the secondary amine was present as the hydrochloride salt. The attachment reaction was repeated using the hydrochloride salt however no peak was observed in the XPS fluorine region. Regardless of how successful using T3P® was, a new approach was required as it was noticed that the structural integrity of the films was being compromised through the use of ethyl acetate. The films were extremely brittle and this made extracting them from reaction flasks a tedious and destructive process, damaging and sometimes destroying potential samples for analysis.

A different approach was to use water as the solvent to increase the solubility of the ciprofloxacin hydrichloride. This would mean changing the coupling agent as T3P® hydrolyses in aqueous conditions. A common motif found in some coupling agents is the carbodiimide, with such a reagent being 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). EDC is used in combination with an activating reagent called hydroxybenzotriazole (HOBt), and is available as the hydrochloride (EDC.HCl) for use in aqueous media.

![Figure 47: EDC and HOBt](image)

The next iteration of the attachment reaction was the use of CIP.HCl, EDC.HCl, HOBt in water at 37 °C overnight. The films were analysed using XPS to show the spectrum shown in Figure 48.
Figure 48: High resolution XPS showing the fluorine region of a ciprofloxacin attached film when using CIP.HCl, EDC.HCl and HOBt in water at 37 °C overnight.

![High resolution XPS spectrum showing the fluorine region of a ciprofloxacin attached film](image)

<table>
<thead>
<tr>
<th>Environment</th>
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</thead>
<tbody>
<tr>
<td>Atomic %</td>
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</table>

Table 16: Atomic % and ratio for the fluorine species of a ciprofloxacin attached film when using CIP.HCl, EDC.HCl and HOBt in water at 37 °C overnight.

The high resolution XPS spectrum clearly shows the presence of fluorine and therefore successful attachment of ciprofloxacin to the aminolysed surface (Figure 48). The next stage of investigation was to test that the coupling agents are the reason for this result; therefore conditions without these reagents were conducted. The conditions used to compare the coupling result were using water at 37 °C overnight and the same but at rt.
Figure 49: Comparison of the ciprofloxacin attachment conditions; water at rt overnight (red), water at 37 °C overnight (green) and using EDC.HCl, HOBt at 37 °C overnight (blue)

<table>
<thead>
<tr>
<th>Environment</th>
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<tbody>
<tr>
<td>Room temperature</td>
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</tr>
<tr>
<td>37°C</td>
<td>0.2</td>
</tr>
<tr>
<td>37°C, EDC.HCl and HOBt</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Table 17: Comparison of the atomic % of fluorine species of PCL films used in various ciprofloxacin attachment conditions (all overnight)

The comparison shows that there is certainly a need to have the attachment at 37 °C as no coupling has taken place at room temperature (Figure 49). Although some attachment has occurred without coupling agents at 37 °C, it is clear to see that the use of those agents (EDC and HOBt) has a pronounced effect with the observation of a far stronger fluorine signal observed in the XPS spectrum. To demonstrate that previous synthetic steps had not contributed to the fluorine, and furthermore proving that the signal could have only come from successful attachment of ciprofloxacin, a direct comparison at each synthetic stage was conducted.
Figure 50: Comparison of the fluorine high resolution XPS region of each synthetic stage in the attachment of ciprofloxacin; pure cleaned PCL film (green), aminolysed film (blue) and the attached film using EDC.HCl and HOBT (red).

<table>
<thead>
<tr>
<th>Environment</th>
<th>F1s</th>
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<tbody>
<tr>
<td>Pure PCL</td>
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<tr>
<td>Aminolysed PCL</td>
<td>0</td>
</tr>
<tr>
<td>37°C, EDC.HCl and HOBT</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Table 18: Comparison of the atomic % of fluorine species of a pure PCL, an aminolysed film and a ciprofloxacin attached film

What the comparison shows is that there is no contamination of fluorine leading up to the coupling reaction, giving greater confidence that the signal is from successful coupling (Figure 50).
9.5.4. Penicillin G attachment to a PCL film

Following the successful attachment of ciprofloxacin, another biologically active compound was chosen to test the robustness of the newly developed coupling method. Penicillin G was chosen as it has the same structural motif required for coupling (a carboxylic acid) and also an element not present in any other step, sulfur.

The exact same methods used to cast, clean and aminolyse (HMDA 10 % in water at pH 11.5) the PCL films were employed. Previously the hydrochloride salt was utilised in an attempt to aid water solubility, however when using penicillin G the sodium salt was commercially available and was therefore used.

The produced films were as before washed with copious distilled water to ensure that all of the reagents were removed, before being analysed in the same way in which the ciprofloxacin films were. The high resolution XPS spectrum of the sulfur region clearly shows attachment of the penicillin G.
Figure 52: High resolution XPS showing the sulfur region of a penicillin G attached film when using PEN Na salt, EDC.HCl and HOBT in water at 37 °C overnight

<table>
<thead>
<tr>
<th>Environment</th>
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</thead>
<tbody>
<tr>
<td>Atomic %</td>
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</tr>
</tbody>
</table>

Table 19: Atomic % and ratio for the sulfur region of a ciprofloxacin attached film when using CIP.HCl, EDC.HCl and HOBT in water at 37 °C overnight

The high resolution XPS spectrum of the sulfur region clearly shows the presence of sulfur and of the successful attachment of penicillin G (Figure 52). Following on from this positive result, the same validation analysis as previously conducted for the ciprofloxacin attachment was undertaken. These were as before to compare the attachment conditions and to make sure that the source of sulfur was from successful attachment and not carried through to from previous steps.
Figure 53: Comparison of the sulfur high resolution XPS region of each synthetic stage in the attachment of penicillin G; pure cleaned PCL film (green), aminolysed film (blue) and the attached film using EDC.HCl and HOBt (red)

<table>
<thead>
<tr>
<th>Environment</th>
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<tbody>
<tr>
<td>Pure PCL</td>
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<tr>
<td>Aminolysed PCL</td>
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<tr>
<td>37°C, EDC.HCl and HOBt</td>
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</tr>
</tbody>
</table>

Table 20: Comparison of the atomic % of sulfur species of a pure PCL, an aminolysed film and a ciprofloxacin attached film

The XPS spectrum shows that there was no contamination carried through from casting the films and then aminolysing them, indicating that the source of sulfur is the successful coupling of penicillin G (Figure 53). The spectrum below (Figure 54) again shows the requirement for the presence of EDC.HCl and HOBt during the coupling reaction.
Figure 54: Comparison of the penicillin G attachment conditions; water at room temperature overnight (red), water at 37 °C overnight (blue) and using EDC.HCl, HOBr at 37 °C overnight (green)

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<th>Environment</th>
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</tr>
<tr>
<td>37°C, EDC.HCl and HOBr</td>
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</table>

Table 21: Comparison of the atomic % of sulfur species of PCL films used in various penicillin G attachment conditions (all overnight)

9.5.5. Aminolysis of a PLA film

After successfully attaching two biologically active molecules to a PCL surface, a change of substrate was investigated. Another common and readily available polyester is PLA. The key difference between the two polymers is the increase in steric hindrance around the ester linkages, with PLA having a methyl group alpha to carbonyl compared to the linear PCL backbone. This tertiary centre and small repeat unit means that the PLA polymer backbone is more tightly packed and could prove to be a more challenging substrate to aminolyse.
A readily available source of PLA is plastic cups so a sample was analysed using NMR to see how pure and therefore suitable this was for use in further research. The $^1$H NMR showed a clear doublet and quartet corresponding to the CH$_3$ and CH respectively with no noticeable impurities; therefore films were cast using this material.

A solution of 10 % PLA in chloroform was used to cast films by pouring into a petri dish and evaporating slowly overnight at 37°C to yield a uniform film. These films were treated before aminolysis using the same 1:1 aqueous ethanol solution as used for the PCL films (3 7°C for 1 h). Once the films had been dried using a nitrogen flow they were analysed using XPS.
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<td>Ratio</td>
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<td>1</td>
</tr>
</tbody>
</table>

Table 22: Atomic % and ratio for the carbon region of pure PLA

![High resolution XPS spectrum of PLA showing the oxygen regions](image)

Figure 57: High resolution XPS spectrum of PLA showing the oxygen regions

<table>
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<tr>
<th>Environment</th>
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<th>O-C</th>
</tr>
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<tbody>
<tr>
<td>Atomic %</td>
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<td>15.5</td>
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<tr>
<td>Ratio</td>
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<td>1</td>
</tr>
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</table>

Table 23: Atomic % and ratio for the oxygen region of pure PLA

The spectrum of the carbon region shows three clear peaks corresponding to the methyl, CH and carbonyl (285, 287 and 289 eV respectively), and the spectrum of the oxygen region gives rise to two peaks corresponding to the carbonyl and ester (532 and 533 eV respectively).
Figure 58: High resolution spectrum of the nitrogen region of a PLA film, when using an aqueous ethanolic wash and aminolysing using HMDA 10% in water adjusted to pH 11.5

<table>
<thead>
<tr>
<th>Environment</th>
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</table>

Table 24: Atomic % and ratio for the nitrogen region of a PCL film, when using an aqueous ethanolic wash and aminolysing using HMDA 10% in water adjusted to pH 11.5

The cleaned PLA film was then subjected to the same aminolysis conditions as before (HMDA 10% in water adjusted to pH 11.5 at 37°C for 1 h), and analysed using XPS to give the spectrum shown in Figure 58. The two peaks show successful aminolysis of the polyester.
9.5.6. Ciprofloxacin attachment to a PLA film

Scheme 62: Attachment of ciprofloxacin (HCl) to an aminolysed PLA film using EDC.HCl and HOBr in water at 37 °C overnight

Following the aminolysis of the film, investigation into coupling reactions was conducted. The success of using the EDC.HCl and HOBr combination was used in conjunction with ciprofloxacin hydrochloride to see if a coupling would occur when using PLA.

Figure 59: High resolution XPS showing the fluorine region of a ciprofloxacin attached film when using CIP.HCl, EDC.HCl and HOBr in water at 37 °C overnight
Table 25: Atomic % and ratio for the fluorine species of a ciprofloxacin attached film when using CIP.HCl, EDC.HCl and HOBT in water at 37 °C overnight

<table>
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</thead>
<tbody>
<tr>
<td>Atomic %</td>
<td>1.32</td>
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</table>

The XPS spectrum in Figure 59 shows a clear fluorine signal, indicating successful coupling of the ciprofloxacin to the PLA surface. The same validation analysis to prove that the fluorine signal was from successful attachment, were carried out. The first spectrum shows that there was no contamination of fluorine during the casting or aminolysis stage (Figure 60). The second spectrum shows the difference in conditions when attaching the ciprofloxacin. The comparison again clearly shows the need for the coupling reagents, with only a small amount of coupling being observed at the higher temperature (37 °C) and none when conducting the coupling at room temperature (Figure 61).

Figure 60: Comparison of the fluorine high resolution XPS region of each synthetic stage in the attachment of ciprofloxacin; pure cleaned PLA film (green), aminolyzed film (blue) and the attached film using EDC.HCl and HOBT (red)
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<tr>
<td>Aminolysed PCL</td>
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<tr>
<td>37°C, EDC.HCl and HOBt</td>
<td>1.32</td>
</tr>
</tbody>
</table>

Table 26: Comparison of the atomic % of fluorine species of a pure PLA, an aminolysed film and a ciprofloxacin attached film

![Graph](image)

Figure 61: Comparison of the ciprofloxacin attachment conditions; water at room temperature overnight (blue), water at 37°C overnight (green) and using EDC.HCl, HOBt at 37°C overnight (red)

<table>
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Table 27: Comparison of the atomic % of fluorine species of PLA films used in various ciprofloxacin attachment conditions (all overnight)
9.5.7. Penicillin G attachment to a PLA film

The next stage was to investigate whether or not penicillin G could be attached to the aminolysed PLA surface. The same set of conditions as the PCL films were used, however no substantial coupling was noticed. The previous conditions that have given the best attachment results, ie the EDC.HCl and HOBr at 37 °C overnight, did show the most promise however the signal for the S1s peak was still very close to the background level (when compared to all previous attachments), therefore it was not conclusive if the attachment was successful (Figure 62).

![High resolution XPS showing the sulfur region of an aminolysed PLA film when using penicillin G, EDC.HCl and HOBr in water at 37 °C overnight (red) and pure PLA (blue)](image)

Table 28: Comparisons of the atomic % of fluorine species of a pure PLA, an aminolysed film and a ciprofloxacin attached film

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<td>0.2</td>
</tr>
</tbody>
</table>
To conclude, PCL and PLA films were successfully cast and aminolysed using HMDA. Ciprofloxacin was successfully attached utilising amide coupling chemistry in the form of HOBt and EDC. Penicillin G was also attached to PCL using the same conditions previously mentioned, however translating this to PLA proved ineffective. All reactions were analysed using XPS showing effective aminolysis and attachment products. The research will now move away from polymer – drug conjugates however still remain with the idea of functional polyesters, utilising it in the field of 3D printing.
9.6. Experimental

General

Commercially available reagents and solvents were used throughout without further purification, unless otherwise stated. ATRIR spectra were recorded using a Perkin Elmer FTIR Spectrometer (Paragon 100). XPS was carried out on a Thermo Fisher K-Alpha using an aluminium monochromatic X-ray source with survey scans at 200 eV and high resolution scans at 50eV pass energies. GPC was carried out on a Phenogel 10u column using polystyrene standards. Samples were run at 0.2 wt% in THF with diphenylether as a flowrate marker.

Film synthesis

Firstly PCL was synthesised as previously described in this thesis. A flame dried round bottom flask under nitrogen was charged with ε-caprolactone 5 (5 mL, 45.1 mmol) and tin octanoate (53, 0.3 M in toluene, 1.5 mL, 0.45 mmol, 1 mol%). Reaction mixture was heated to 130 °C for 17 h. The reaction mixture was dissolved in CH$_2$Cl$_2$ (20 mL) while warm, and precipitated in cold methanol (250 mL). White solid was collected via filtration (5.03 g, 100 %, $M_w = 21380$ g/mol by GPC).

PCL (1 g,) was dissolved in chloroform (9 mL, 10 % solution) and poured into a glass petri dish. The petri dish was placed into a vacuum oven at 37 °C overnight (not under vacuum). Once the solvent had evaporated the films were cut into 2 x 2 cm squares from which to further functionalise.

PLA films were synthesised using PLA plastic cups ($M_w = 398500$ g/mol by GPC) and were created using the sample procedure replacing for PCL at the same weights, times and temperatures.

Aminolysis of films

Both PCL and PLA films were aminolysed using the same conditions. Briefly, PCL films were first washed in an aqueous ethanol solution (1:1) for 1 h and dried using a N$_2$ flow. The
washed films were immediately placed into a solution of hexamethylene diamine in distilled water (10 %) adjusted to pH = 11.5 at 37 °C. No agitation was used and the PCL films were incubated for 1 h, washed with copious distilled water and dried under a N₂ flow.

**CIP/PEN attachment**

Freshly aminolysed films were submerged into distilled water at 37°C containing EDC.HCl (5 mg/mL), HOBt (5 mg/mL) and ciprofloxacin hydrochloride (5 mg/mL) overnight. Films were then washed with copious distilled water before being dried under an N₂ flow.

PCL and PLA films were treated using the same procedure, times and temperature with penicillin G sodium salt replacing ciprofloxacin hydrochloride where applicable.
10. UV curing semi-biodegradable ink for 3D printing technology

10.1. Introduction

This section of the thesis utilises functional polyesters but applied it in the field of 3D printing. This will be again using PCL synthesised with UV cureable terminus, allowing crosslinking from a liquid to a solid. When combined with 3D printing technology this will provide a material that can be printed into a variety of constructs while maintaining a degree of degradability.

As 3D printing will be the final use for the produced material in this research, a brief overview of printing techniques will be discussed. Although this will include a wide range of techniques, those involving UV light will be of key importance, as these are techniques which may be applicable for the following research.

The compounds produced in this research will utilise radical chemistry to generate a crosslinked material. Therefore the field of radical chemistry will be discussed focusing on how radicals can be generated using UV light and polymerisations that also utilise radicals. Polymerisation techniques will be discussed as although synthesising a polymer is not of importance in this research, the UV curing terminus attached to the PCL in this work can undergo polymerisation in a variety of ways. These are the polymerisation techniques that will be discussed.

Following the literature review will be the research into synthesising UV cureable polyesters. This will be achieved through acid chloride attachment to PCL diol and subsequent UV curing using suitable photoinitiators. Ratios of these initiators and their type will be varied to find the optimal system for use in inkjet printing. 3D printed constructs made using material from this project will also be shown, demonstrating their viability in this field.
10.2. Additive manufacturing

Additive manufacturing (AM) will be utilised as a way of testing materials made in this research therefore an overview of this technique and its varients will be discussed. AM is a method in industry which builds complex models using a layer-by-layer approach and is otherwise known as rapid prototyping or 3D printing.\textsuperscript{93} This method of manufacturing operates on the principle of adding material to a build, rather than the conventional route of the removal of material from a bulk mass. The term 3D printing, refers specifically to the jetting type of manufacturing, but is used interchangeably with other techniques in the field. This technique was first employed in the 1980’s and has grown in its ability to create extremely complicated designs.\textsuperscript{94} These designs are initially made using computer aided design (CAD) where prototype is visualised through a software program until a finished design is reached. The CAD design is then subjected to slicing program which analyses the model and dissects it into a series of cross-sections. This dissected model is then turned into the prototype by ‘printing’ each layer in sequence.\textsuperscript{95}

10.2.1. Types of additive manufacturing

Although all the methods discussed in this section are involved with the addition of material in a layer by layer fashion, each carries this out in a unique manner. The first and most accessible technology is fused deposition modelling (FDM) which uses a filament that is extruded via a nozzle to generate designs. Although not used in this research, it could be utilised in the previous section (aminolysed films), as both PCL and PLA are possible feedstocks for this process allowing parts made to be potentially aminolysed. A typical schematic of the instrumentation used in FDM is shown below (Figure 63). The nozzle is heated to extrude the filament which is guided using three axis build tray according to a predesigned path generated from a CAD file. Upon reaching the build the melted material solidifies generating the desired design.\textsuperscript{96}
A recent development is that the diameters of filaments have greatly reduced, increasing the resolution of the technique. Filaments with diameters in the range of 250 – 700 nm have been successfully produced using a process called electrospinning. Electrospinning is a process of utilising an electric force to draw out polymer strands from a solution. In the work shown by Croisier et al. PCL was dissolved in a 1:1 THF:DFM solution at 15 wt% and extruded from a syringe at 1 mL h⁻¹ onto an aluminium plate. When the plate is charged (in this case at 12 kV) charged material is drawn out of solution onto the plate forming fibres of a few hundred nanometers (Figure 64).
The reason for FDM’s popularity is that it is a very inexpensive process but has the limitation of having a limited choice of feedstock (a thermoplastic with a processable viscosity when melted); however another highly developed technique is stereolithography (SLA). This method utilises a bath of photocureable resin as its feedstock and then polymerises it using one of two methods. As the feedstock for this technique is a UV cureable resin, although not used in this research it could be viable in the future. The first method employs the use of a focused UV laser which cures the resin into a solid form through photoinitiated polymerisation. The UV laser traces the CAD generated design layer by layer, lowering the photocureable resin after each successful layer, building on the previously cured material generating a three dimensional structure. The second method is a more modern take on the technology commonly employing a projected image rather than a UV laser. A CAD design is split into several layers and each layer is ordered using a program than displays images in a sequential fashion (such as PowerPoint). These slides are then sequentially projected into the same photocureable resin. After every slide the stage is lowered allowing the image to be built up in layers (Figure 65).
Another rapid prototype method which uses a photocuring resin is jet printing. This will be the technique used to test the viability of the material produced in this research. A polymeric resin is ejected from a print head, moving in the X and Y axis, onto a support material, or build tray, acting as the platform for the build. Between layers the photo-polymer is cured using UV light to form a solid construct from which to build the next layer from. Once a layer is complete and fully cured, the build tray is lowered in the Z direction to allow the prototype to be constructed in three-dimensions (Figure 66).
A key example of how 3D printing is being used in real world biomedical application comes from work by Probst *et al.* who utilise a PCL filament in FDM. Firstly a 3D scaffold was designed using a CT scan from a patient. This was then printed using an FDM machine using a PCL-calcium phosphate filament, before being implanted onto a calvarial defect. The implant stimulates the growth of bone along the scaffold before consolidation of the defect is observed six months from implantation (Figure 67).

![Figure 67: A calvarial defect treated using a 3D printed scaffold. (A) 3D printed scaffold using FDM (B) Calvarial defect (C) implantation of the scaffold (D) Consolidation of the defect 6 months after implantation](image)

The methods of stereolithography and inket printing are dependant on the UV curing nature of the resins used. The following section will discussing the UV curing process involving radical chemistry, radical generation and processes which utilise these properties.
10.3. Radical polymerisation

10.3.1. Characteristics of radical polymerisation

Radical chemistry is extensively used in the polymerisation industry for a variety of polymers. For example, all commercial poly(methyl methacrylate) is produced using radical polymerisation. All radical polymerisations are governed by the same 3 steps; initiation, propagation and termination. Initiation is the step involved with the formation of the radical. The two most common types of initiators are those that generate radicals by thermal decomposition or photolysis (the latter being discussed later on). Thermal decomposition initiators are those that produce radicals as a function of heat. An example would be the thermal decomposition of AIBN, above 60 °C, to yield two 2-cyanoprop-2-yl radicals.\textsuperscript{102} Propagation is the process in which a polymer spends most of its time in a reaction. This is the stage involved with increasing chain length and molecular weight. The propagation of an alkene proceeds through the repetitive attack of a radical to one carbon atom of the alkene (179). This then not only creates a new bond but also creates the next radical from which to propagate (180). This reacts with another alkene and the process continues (182).

Termination is the combining of radicals at the end or during of the propagating step. The recombination of the radicals can be between different species. For example the combination of two propagating species results in the doubling of the molecular weight and an element of
symmetry. Other cases include the reaction of a propagating species and an initiator, or even with inhibitors such as oxygen. The final method of termination is called disproportionation, where two radical species react to form two non-radical species. Rather than the joining of two radical species, in this instance one radical is an acceptor and the other the donor.

Another important factor to consider especially when looking at the previous example is tacticity. This is the description of the relative stereochemistry of adjacent chiral centres in a polymer and is generally not very well controlled in free radical polymerisations. In Scheme 64 every time a new monomer is attacked by the propagating radical a new chiral centre is produced. The order of these stereocentres is what tacticity describes. There are three types of tacticity; isotactic (184), syndiotactic (185) and atactic (186) (Figure 68). Isotactic refers to polymers which have all stereocentres of either the R or S configuration. Syndiotactic polymers have perfectly alternating stereocentres, i.e. R, S, R, S, R, S, etc. Both of these polymers are formed from catalytic cycles specifically designed to give these structures (Kaminsky catalyst for example).

![Illustration of tacticity](image)

10.3.2. Controlled radical polymerisation

Controlled radical polymerisations are those whose mechanism involves the specific control of molecular weights, giving rise to polymers with low polydispersions. A very relevant and important paper with respect this research is the work carried out by Atzet et al.\textsuperscript{103} as they utilised a methacrylate bearing PCL to generate crosslinked networks, a key target in the following research. Oligomeric PCL diol was reacted with methacryloyl chloride to yield a
radical crosslinker. What was also intriguing about this research was the synthesis of a polycaprolactone radical initiator. The same PCL diol was used however instead of an acid chloride, $\alpha$-bromoisobutryl bromide (BIBB, 187) was used. This generated terminal bromide groups from which to initiate the controlled radical process known as ATRP (Scheme 64).

Scheme 64: Synthesis of a PCL crosslinking initiator

The approach shown in Scheme 64 has also been employed using a PEG backbone. The work by Ercole et al. use acrylate terminated polymers to act as a crosslinking agent compared with the PCL group previously shown. What was key in this research was not only were the terminus acrylate motifs, but were also bound to a UV degrading group. The degradation of o-nitrobenzyl groups is well understood, and is shown at the bottom of Scheme 65 allows the diacrylate macromonomer (91) to not only crosslink but also to degrade under certain UV conditions.
The PCL initiator shown in Scheme 64 can initiate a type of controlled radical polymerisation called atom-transfer radical polymerisation (ATRP). ATRP, discovered independently by Sawamoto et al.\textsuperscript{106} and Matyjaszewski in 1995,\textsuperscript{107} is involved with the transfer of a monomer (195) to a propagating species (197), usually using a transition metal complex (196). There is some key optimisation that is required when carrying out ATRP as it is the rate constant between active (197) and dormant species (195) that controls the narrow polydispersity of the product.

Scheme 65: Synthesis and degradation pathway of a diacrylate bearing PEG macromonomer

Scheme 66: ATRP equilibrium
In simplistic terms the fact that the rate constant to left, $k_{\text{deact}}$ (Scheme 66), is greater than the activating one ($k_{\text{act}}$) means that there are only ever a few propagating species at any one time (197). This means that the chance of terminating via propagation chain combination is very low (199, $k_t$). As the propagating species is only short lived, the chain length grows at a steady rate until all monomer is used. This allows the molecular weight to be controlled directly by the amount of monomer in a controlled manner, generating low polydispersity.

### 10.3.3 UV photopolymerisations

UV photopolymerisations are extensively used in this research therefore how these reactions are initiated and propagate will be discussed. UV light can also be used to generate radicals and therefore initiate polymerisation. This occurs through the absorbance of UV light into the bonds of photoinitiators. A photoinitiator is a molecule that when excited by UV light forms radicals, either by the breaking of a bond or by the excitation of a functional group. There are two types of photoinitiators, type-I and type-II. A type-I photoinitiation is a unimolecular process involving the homolytic bond cleavage upon absorption of light. A typical type-I photoinitiator is benzoin (200) (and derivatives containing the same structural motif), with the bond between the ketone and hydroxyl carbons being the one cleaved (Scheme 67).

![Scheme 67: Photolytic cleaving of benzoin](image)

The type-I photoinitiators cleave by what is called a Norrish type I reaction. When benzoin is irradiated with UV light, the ketone group absorbs the energy and is excited into a singlet state. From here the excited electron can change into a triplet state (unpairing of electron
spin) with both of the excited states resulting in cleavage of the α-carbon of the ketone. This gives rise to the initiating radical species (201, 202).

Type-II photoinitiators act in a biomolecular fashion as opposed to a unimolecular one. The other component of a type-II photoinitiating system is an electron transfer reagent (in addition to the UV absorbing molecule). Motifs can include derivatives of benzophenone, camphorquinone, and thioxanthone, however the example below will utilise a benzophenone (Scheme 68).

![Scheme 68: An example of a type-II photoinitiator](image)

The initial stage involves the UV light being absorbed by the benzophenone (203) to create a photoexcited state (204). Radicals are generated by the electron transfer (or hydrogen transfer) to a suitable accelerator (205) to generate the hydroxyl bearing radical centre (Scheme 68, 206, 207).

The main drawbacks when using photopolymerisations is the high sensitivity to oxygen. This is because of the ability for oxygen to generate radicals, therefore if dissolved in a sample they can become radical scavengers, reacting with radicals at all stages of radical polymerisation. The main way in which oxygen sensitivity is reduced is through deoxygenation with nitrogen and carrying out the experiment under an inert atmosphere.

The examples of UV initiator above, regardless of type I or II, only generate radicals once. Work by Fisher et al. initiate radical polymerisations using biacylphosphine oxides (BAPO, 208). These photo initiators undergo fast cleavage to create the first round of radicals, followed by the cleavage of the remaining phosphine oxide to generate a second set of radicals (Scheme 69).
Scheme 69: Photoinitiation of BAPO to generate two sets of radicals
The literature previously discussed in this section showed that a degradable material can be synthesised and successfully crosslinked using UV light. The work by Ivirico et al.\textsuperscript{115} shows that this idea can be implemented using a PCL based material utilising the UV reactivity of methacrylates. This work demonstrates that the methacrylated PCL can in fact crosslink with itself, but also allows the incorporation of other UV reactive molecules such as HEA to generate extended crosslinked systems. Although this work investigated the use of these crosslinked systems in water uptake, it demonstrates the versatility of the PCL dimethacrylate as a crosslinkable reagent for potential use in inkjet printing, where UV curing is essential in creating constructs.

\begin{center}
\includegraphics[width=\textwidth]{Scheme70.png}
\end{center}

\textbf{Scheme 70: Synthesis and crosslinking of a PCL based oligomer}
Further to the previously mentioned research is work carried out by Atzet et al.\textsuperscript{103} who use the same material to generate hydrogels. In this work they again utilise the incorporation of a comonomer to the crosslinking process in the form of HEMA, and investigate the degradation of the crosslinking networks (i.e. the PCL content). They found that there was degradation of the crosslinked network not only when using NaOH, but also \textit{in vivo} conditions using lipase solutions. This gives credibility to the hypothesis that when constructs are made using a 3D printing inkjet technique, they would be degradable and therefore the material made during this research could be classified as a biodegradable UV curing ink for 3D printing technology.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{mass_loss_degradable_pHEMA_vs_time.png}
\caption{Mass loss of a PCL pHEMA crosslinked network as a function of time using lipase solutions}
\end{figure}
10.4.1. Research plan

The aim of this area of research is to investigate and evaluate the use of PCL based UV curing oligomers in the field of 3D printing. The work previously mentioned by both Ivirico and Atzet both synthesise a UV curing PCL bearing oligomer, and successfully crosslinked the material. Their work will act as a starting point from which to investigate both the oligomer and the UV curing reaction. However before synthesising the PCL derivatives a commercially available PEG diacrylate will be used to establish a model for investigating further oligomers.

![Figure 70: UV photoinitiator used in this research; Irgacure 2959 and DEXT/EDB](image)

After the commercially available material has been investigated, the PCL derivative will be both synthesised and testing in similar fashion. The initial UV photoinitiator to be used is Irgacure 2959 (219) which is an example of a type I photoinitiator. The research will then investigate the use of type II photoinitiators. This is because previous research discussed so far does not require the same fast curing speeds that 3D inkjet printing requires; therefore the curing rates of both the commercially available and synthetically made materials will be of upmost importance throughout the research.
10.4.2. UV crosslinking material

The use of UV radicals in polymerisation has previously been discussed in the introductory chapter with particular attention towards the use of alkenes. When a short chain monomer contains two alkene units and is subjected to UV photo polymerisation conditions, a highly cross-linked network is created (Figure 71). These cross-linked polymers are extremely stiff and brittle and are insoluble in solvent. This research proposes that, using the correct chemicals and methods, a liquid ‘ink’ containing a UV photo polymerisable monomer and a UV photoinitiator can be jetted into a specific pattern an inkjet printer. This pattern could then be cross-linked using a UV light source and built upon to create a 3D structure using a layer by layer approach.

![Representation of a crosslinked material](image)

Figure 71: Representation of a crosslinked material

It is important to consider what type of material to use, as both mono- and diacrylated components have the ability to UV cure and potentially make a crosslinked structure. Diacrylates are more attractive than a mono-acrylated monomer because there is a higher concentration of alkene groups from which to crosslink. This reasoning is founded on one of Carother’s equations regarding gel point. Gel point, or the point at which a material is classed as crosslinked, is dependent on the conversion of the reactive species and the number of bonds created by each monomer during the polymerisation process.
For instance, when using only one acrylate group, two new carbon–carbon bonds are formed, therefore $f_{\text{avg}} = \text{two}$. This would mean that the percentage conversion required to generate a crosslinked material, $P_c$, equals one or 100%. As chemical reactions almost never go to complete conversion this makes it almost impossible to create a crosslinked material using only a mono-acrylated polymer. However when you increase the number of acrylates to two, the amount of new carbon–carbon bonds increases to four, meaning that you only need 50% conversion of monomer to generate a crosslinked material ($P_c = 0.5$). The importance of this crosslinking parameter is because once a material becomes crosslinked, it quickly solidifies. This is extremely important in the field of inkjet printing where the quicker an ink solidifies, the faster the solid construct can form and therefore accept the subsequent layer.

![Figure 73: Poly(ethylene glycol) diacrylate (PEGDA)](image)

A commercially available molecule that contains two alkene groups is poly(ethylene glycol) diacrylate (Figure 73, 222). Before moving towards any 3D printing machine, tests on the bench were carried out using PEGDA and the UV photoinitiator Irgacure 2959 (Figure 69, 219). Irgacure 2959 was chosen because of its non-toxic nature and ease of availability.\textsuperscript{117} Although irgacure 2959 has been identified for use in 3D printing,\textsuperscript{118} the novelty of this work surrounds its use with diacrylated PEG and PCL.

The UV absorption profile for Irgacure 2959 (219) is shown below (Figure 74), with the main absorption occurring at ~275 nm.\textsuperscript{119} There is a small absorption at 365 nm which is important as most commercially available UV sources operate at this wavelength. A sample of PEGDA of average molecular weight of ~250, containing 3% (w/w) of Irgacure 2959, was placed in a small sample vial and irradiated under UV torch (one Watt, 365 nm). The
sample had to be warmed slightly to ensure complete dissolution of the photoinitiator. Although being irradiated for an hour under the UV torch, only a small film was observed on the surface with no crosslinked material observed in the bulk of the material. The reasoning behind this lack of curing was the presence of dissolved oxygen within the sample, essentially acting as radical traps (ie the generated radicals intended for the acrylate monomers was instead reacting with the oxygen and therefore no crosslink was observed).

It was then decided to deoxygenate the sample before irradiation using nitrogen for 15 min, coupled with maintaining a nitrogen blanket during the exposure to the UV light. The deoxygenation process involved the bubbling of nitrogen through the sample for the stated amount of time using a needle and nitrogen supply. A film of cross-linked polymer was observed in a matter of minutes with the entire sample being observed to be cross-linked (hard to the touch) within 1 h.
At this stage the amount of catalyst was investigated to gain a better understanding of what amount gave the best curing condition. It was also noted that although the one hour of curing time was more than ample in a laboratory situation, if this process was the utilised in a 3D printing scenario the time would have to be significantly reduced. This is because in the laboratory example the UV source is irradiating the whole sample for the duration of the experiment, whereas this is not the case in the inkjet scenario. The UV light is attached to the inkjet printhead meaning the deposited sample has a far less irradiation time, therefore requiring a far faster curing response. This required a new approach to carrying out the UV curing. All credit for the development of a new curing method and preliminary work goes to Kang Wu (Materials department MSc Student). The curing method utilised a UV lamp found in high street nail salons. This lamp produced 365 nm light at 36 watts, which is far more powerful than the one watt torch.

Once the sample was irradiated (for the specified time) it was immediately soaked in acetone to identify the quantity of material that was cured. The thought was that the unpolymerised material would dissolve in the acetone, leaving only the crosslinked material behind. The acetone soak was carried out for 24 h followed by the decanting of the solvent and drying the cured material under vacuum. Using the weight of the sample used initially and the amount of cured material left after drying, a percentage conversion could be calculated.

![Figure 75: % Cure of PEG DA after 1 minute irradiation as a function of Irgacure 2959, under air and deoxygenated conditions](image-url)
The first investigation was to alter the catalyst loading of Irgacure 2959 from 1 – 5 % to see if there was an optimum amount. All conditions had an irradiation time of 1 min (as compared to the 1 h of the previous experiments). The curing was carried out in air and also with the 15 min deoxygenation (N₂ flow bubbled through the sample), using nitrogen to illustrate the importance of an oxygen free sample (Figure 75).

Although there was a medium level of curing when using a sample without the deoxygenation process (indicating the power of the UV source compared to before), the levels achieved under the deoxygenation scenario are far higher almost achieving a perfect cure. The best level of curing was achieved when using Irgacure 2959 at a 4 % loading, and when deoxygenating the sample before irradiation using a nitrogen bubble.

It was decided to compare a type I and II system and see if there was an improvement in curing levels. The type II system that was chosen was the initiator DETX (2,4-Diethyl thioxanthone, 220) and accelerator EDB (Ethyl-4-(dimethylamino)benzoate, 221) (Figure 70), based on literature stating that EDB was a successful accelerator (in combination with ITX) and that DETX had increased absorbance at 365 nm compared to ITX. 120, 121

Initial tests using the type II system were to find the best loading of the initiator and accelerator. This was conducted by keeping one component at a fixed level and varying the other. The first investigation kept the accelerator EDB at 3 % while varying the DETX loading between 1 – 5 %.
The data shown above (Figure 76) shows the expected trend when increasing photoinitaitor loading, an increase in the level of cured material. This was the case for both the air and nitrogen conditions, with the nitrogen deoxygenate samples showing a far higher percentage of cured material, again showing the importance of oxygen removal. A key difference between the use of the type I Irgacure 2959 and the type II DETX and EDB system is that there is a slight increase in the level of cured material when conducting the experiment in air. This is due to the slightly higher tolerance to dissolved air within the sample. The conditions giving the most cured material was found to be using the photoinitator DETX at 3 % loading, as that gave the highest curing percentage of 98 %. This value was also found when using 5 % photoinitiator however due to the strong colour associated with the inititor, having the lowest loading leads to a less coloured cured material (a possible consideration when thinking about the asthetics of 3D printed parts).

The next investigation is the reverse of the previous one in which the accelerator loading is varied with a constant level of photoinitiator. The amont of photoinitiator is kept at 3 % for every sample and the accelerator is varied between 1 – 5 %.

Figure 76: % Cure of PEG DA after 1 minute irradiation as a function of DETX loading while maintaining 3 % of EDB, under air and deoxygenated conditions.
Figure 77: % Cure of PEG DA after 1 minute irradiation as a function of EDB loading while maintaining 3 % of DEXT, under air and deoxygenated conditions

The experiments have found that you again get a near complete cure when undertaking the irradiation after conducting a nitrogen deoxygenation. The best of these conditions is found when using 4 % EDB as a 98 % cure was achieved. The key information found from this investigation is that when the UV irradiation was carried out in air, there was a considerable decrease in the curing amount (Figure 77). This is potentially due to the rapidly reacting EDB with the abundance in oxygen. Tertiary amines are known to react with radical peroxides formed when oxygen reacts with a propagating radical. These inactive peroxide radical are reacted with the tertiary amine to yield active alkylamino radicals. However if there is an abundance of these radicals along with the those generated under standard UV conditions, the rate of termination could potentially be higher and therefore leading to a lower cure rate.

With the preliminary results using PEG DA showing promise, the idea of a semi-biodegradable cross-linked structure was pursued. PCL, as previously discussed, is widely used for its biodegradability and was chosen as the starting material for this research. Unfortunately the diacrylate of PCL is not commercially available as in the PEG case, so this was synthesised through the use of PCL diol (at average molecular weight of ~ 530) and the corresponding acid chloride.
Scheme 71: Synthesis of poly(caprolactone) dimethylacrylate (PCLDMA)

The reaction of the PCL diol (187) and methacryloyl chloride (129), in the presence of anhydrous triethylamine (to precipitate the triethylammonium chloride salt), produced the dimethacrylate product (217), with $^1$H NMR indicating the methacrylate protons at 1.91, 5.52 and 6.06 ppm (Scheme 71). The reasoning behind the methacrylate usage was that initial reactions using acryloyl chloride proved problematic whereas the methacryloyl addition proceeded successful. The only issue with NMR analysis is that due to the polymer containing an undisclosed ‘n’ value, the integrations in the $^1$H NMR spectrum are non indicative of chain length as they would change depending on the value of n. What the spectrum does confirm is the presence of the methacrylate, not the relative chain length of the polymer.

After successful synthesis of the polycaprolactone dimethacrylate (PCL DMA), the initial experiments using Irgacure 2959 were carried out, with the loading ranging from 1 – 5 %.
Figure 78: % Cure of PCL DMA after 1 minute irradiation as a function of Irgacure 2959 loading, under air and deoxygenated conditions

What the graph shows is the same relationship between the presence and lack of oxygen with the nitrogen deoxygenation showing a far higher cure (Figure 78). What is noticeable is that the usual mid–high 90% cure that is observed with the PEG DA is not present. This could be the result of the reactivity difference between an acrylate group and a methacrylate. An acryloyl group is far more reactive than a methacryloyl group due to the lack of a tertiary centre on one end of the alkene.

A sample of PCL DMA was given to collaborators (Yinfeng He, Nottingham University) who utilised the UV curing polymer in 3D printing. The technology chosen was inkjet printing where the setup was available to directly UV cure a deposited layer before printing the subsequent one.
A key parameter that is crucial in inkjet printing is the viscosity of the ink. The PCL DMA was found to be too viscous therefore PEG DA was used as a reactive diluent. PEG DA was chosen based on its low viscosity and high UV curing ability which would aid the ink in curing during printing. The use of PEG DA in the final ink reduces the biodegradability of any produced constructs, therefore the higher ratio of PCL DMA:PEG DA is preferable. The optimal printing ratio with the highest PCL DMA content was found to be 70:30 by wt (PCL DMA:PEG DA). To test the ink a 10 layer construct was printed, with each layer being cured before printing the next using the attached UV unit (Figure 79).

The variable UV initiator levels previously tested using PCL DMA and PEG DA were then investigated using the 70:30 mixture. What Figure 80 shows is that as expected the samples that have not undergone deoxygenation were far poorer in performance than their nitrogen deoxygenate counterparts.
Our collaborators noticed that upon receiving synthesised samples the viscosity was different between batches. The reproducibility of the jetting experiments required a constant viscosity to be met therefore this issue was investigated to ensure a consistent viscosity between batches.

When looking at a stored sample in the lab, a clear oil, together with a sediment of white solid was observed at the bottom of the vial. This sediment was filtered in an attempt to isolate and analyse the solid, however it proved too fine to collect by filtration with the solid being removed only through the use of a celite plug. In the mass spectrum of the sample (ie not specifically the solid) it was noted that each peak did not fit the exact mass of the methacrylated polymer. The extra mass was attributed to the polymer ionising with a triethylammonium cation (Figure 81), as the peaks were 102.13 mass units greater than the what was expected for the repeating PCL units.

Figure 80: % Cure of PCL DMA:PEG DA (70:30) after 1 minute irradiation as a function of Irgacure 2959 loading, under air and deoxygenated conditions
This was due to not all of the salt being removed during work-up. Due to the oligomeric and also viscous nature of the produced polymer, the work-up procedure following the reaction was problematic, with emulsions being a common problem when trying to wash with aqueous media. The reaction is carried out in anhydrous THF with the solvent being removed and replaced with dichloromethane for the work-up. This was chosen because THF is miscible with water whereas dichloromethane is not; making it theoretically possible to carry out aqueous base washes. The emulsions occurred because of the large amount of salt being dissolved into the aqueous media. This would increase the density of the aqueous layer and therefore ‘fall’ into the organic layer causing the emulsion. This issue was resolved by replacing the organic solvent from dichloromethane to diethyl ether. The reason for this
choice is that diethyl ether is again not miscible with water but also is less dense and creates a layer above the aqueous phase rather than below, allowing the aqueous layer to increase in density without compromising the organic phase and therefore the separation. Another way in which the triethylammonium salt was removed was through columning the polymer using a standard silica system.

![Graph of Figure 82](image-url)

**Figure 82: % Cure of PCL DMA purified by three different methods after 1 minute irradiation as a function of Irgacure 2959 loading under deoxygenated conditions**

What the data shows in Figure 82 is that although there was almost 75 % curing observed using the dichloromethane work-up method (at 5% Irgacure 2959), changing to an ether work-up greatly improved the curing results. It was also observed that the product produced in switching to ether was clearer and more stable over time, leading to the conclusion that the triethylammonium salt was being removed. When looking at the column method, only a small increase was noticed above the ether work-up product. Considering that to get to the column purification stage requires to have worked-up the reaction, for only a small increase in performance, it would not be an attractive option when considering the scale our colleagues required (100 g +).

Our collaborators with the newly optimised material began the process of generating more complicated architecture in their products. They chose to generate square mesh of varying dimensions to investigate the accuracy of the newly developed ink.
The SEM images in Figure 83 show that regardless of wall thickness the edges of each internal square were not well defined. This could be due to the ink not curing very fast as rather than hardening, the droplet of ink has time to spread out (both due to gravity and the movement of the printing platform) and subsequently becomes cured in a flatter, less defined, final position. This can be clearly observed in image (c) (Figure 83) where the increased wall thickness requires the deposition of more ink with resulting in a more pronounced deviation between layers. There are several ways to increase the rate of curing and therefore increase the definition during printing. In Figure 79 the UV unit is shown attached to the printhead, with every layer of deposited material receiving one pass of UV light. A more constant source of UV could potentially help the curing rate. Oxygen inhibition has been
shown in the curing studies to have a dramatic impact to curing levels. Although the ink is deoxygenated and sealed before use, jetting in an inert atmosphere would increased the efficiency of the photoinitiator and allow a faster rate of curing.

Both of the previous examples would require modification to the existing equipment therefore the next logical step was to utilise the stronger curing potential of a di-acrylate as opposed to the di-methacrylate in conjunction with PCL diol. This was achieved by using acryloyl chloride instead of methacryloyl chloride when using PCL diol (Scheme 72).

![Scheme 72: Synthesis of poly(caprolactone) diacrylate (PCL DA)](image)

Initial trials using anhydrous THF and slow addition of the acryloyl chloride (in an ice bath) yielded an orange solid during addition (potentially acrylate oligomerisation). This effect was lessened when using anhydrous toluene however there was no acrylate addition to the PCL diol. Success was found using the conditions of Jaiswal et al.\textsuperscript{123} by using a slightly elevated temperature (45 °C) in anhydrous toluene, and adding the acryloyl chloride in one charge rather than over an hour through slow addition. This material was then tested using the same Irgacure 2959 loadings as before to compare against the methacrylate version.
The same dramatic increase in curing percentage shows that a nitrogen gas deoxygenation is essential for curing conditions (Figure 84). What the graph does show is an increase on performance from the PCL DA however not reaching that of the PEG DA. The main reasoning behind this would likely be as a result of the molecular weight and the abundance of acrylate groups between the two compounds. Although the acrylate groups would react very similarly on each oligomer, the Mw of PCL diol initially used is ~530 whereas the PED DA used has a Mw of 250. This therefore means there is a far higher molar concentration of acrylate groups when using the PEG DA when compared to the PCL DA, with a high concentration giving rise to a fast curing rate.

At this stage each material has been evaluated against each other using Irgacure 2959 with the irradiation time and catalyst levels remaining constant. The next investigation would be into the kinetics of these materials and how they perform over far shorter irradiation times. This is important as this will better reflect conditions found during inkjet printing.
The data from both graphs confirms the results already observed in this study so far. The PEG DA achieves the highest cure percentage with the PCL DMA achieving the lowest. This is due to the reactivity of acrylates vs methacrylates and the molar amounts previously discussed. The cured amount found in the PCL DA and the PCL DMA:PEG DA at 70:30 are closer however due to the presence of acrylates as the faster curing group, with the results from the two graphs showing comparable curing amounts (Figure 85).
A final use of the 70:30 PCL DMA:PEG DA ink was to test a curved structure architecture (similar to figure 76, but utilising a curved motif). 50 layers were printed to observe the effect of a far larger difference in the top and bottom printed layers (Figure 79). Although different motifs can be used, the same problem of definition was observed using optical microscopy.

Figure 86: (a) Curved printing pattern, (b) Sample after being removed from printing platform, (c) Surface depth profile
10.4.3. Acrylate monomer synthesis

The jetting research was carried out using end functionalisation of polymers to achieve the UV curing properties. As previously shown functionality can be introduced to the PCL backbone through the copolymerisation of functional monomers. Work therefore began on the synthesis of an acrylate containing εCL monomer.

The reasoning behind this project was that the increased number of acrylate groups on the crosslinking precursor. If the oligomer backbone had an acrylate group every repeat unit, the percentage conversion required to generate a crosslinked material would be far lower than in previous work (due to $f_{\text{avg}}$ being far higher, Carother’s equations (Figure 71). The work previously shown in the first section of the thesis, has several post polymerisation reactions before the step allowing addition of a cureable group (deprotection then reduction). The approach followed in this section allows the large scale synthesis of the UV curing monomer, which would only require one polymerisation step to become viable for crosslinking tests.

The starting material would be the commercially available 1,4-cyclohexanediol. This was treated with one equivalent of acryloyl chloride to yield the monoacrylated alcohol in a 27% yield. The reason the yield was poor is the statistical nature of the reaction. It could be clearly observed through thin layer chromatography that starting material, mono- and di-acrylated were in the product. The di-product is undesirable as it would not allow further oxidation to the ketone in subsequent reactions. The reaction was analysed to show a clear carbonyl peak in the IR at 1719 cm$^{-1}$, with the distinct $^1$H NMR signals of an acrylate group present also present.

![Scheme 73: Synthesis of acrylate εCL monomer](image)

The monoacryloyl alcohol was then treated with pyridinium chlorochromate (PCC) to convert the alcohol to the ketone. The reaction yielded the ketone in a 77% yield as shown
by the disappearance of the hydroxyl peak at 3398 cm\(^{-1}\), and also by the loss of the CH multiplet between 3.70 – 3.82 ppm. The next stage of this synthesis would be to carry out a Baeyer-Villiger reaction and then co-polymerise with PCL to form a UV cureable biodegradable polymer which is purely polyester based (compared to the inclusion of polyether of PEG DA as shown earlier).

To conclude, UV cureable groups were successfully attached to PCL diol in the forms of methacrylate and acrylate. They were successfully cured under UV light, using both type I and II photoinitiators to generate solid crosslinked materials. The initiator ratios were altered to provide an insight into the optimal loadings. Materials were then jetted using an inkjet printer to generate a variety of solid constructs, demonstrating that they are viable materials for use within this field.
10.5. Experimental

General

Commercially available reagents and solvents were used throughout without further purification, except for tetrahydrofuran which was freshly distilled (over benzophenone/Na). Light petroleum refers to the fraction with bp 40-60 °C. Thin layer chromatography was carried out on Merck Kieselgel 60 GF254 aluminum foil backed plates. The plates were visualized under UV light, phosphomolybdic acid stain and/or vanillin stain. Flash chromatography was carried out using Merck Kieselgel 60 H silica or Matrix silica 60, with the eluent specified. $^1$H and $^{13}$C NMR spectra were recorded using Bruker 400 MHz NMR machine ($^1$H 400 MHz, $^{13}$C 100 MHz); chemical shifts are quoted in ppm and coupling constants, $J$, are quoted in Hz; d-Chloroform was used throughout unless otherwise stated. Spectra were calibrated to residual solvent peaks. In the $^{13}$C spectra, signals corresponding to C, CH, CH$_2$ or CH$_3$ groups, as assigned by DEPT, are noted. High and low resolution mass spectra were carried out on a Thermofisher exactive (orbi) resolution mass spectrometer. IR spectra were recorded using a Perkin Elmer FTIR Spectrometer (Paragon 100) as solutions using CH$_2$Cl$_2$ as solvent. 36 W UV box was used for the curing of samples with light at 365 nm.

Crosslinking procedure

Samples were prepared in a glass vial (~ 5mL) and warmed to encourage the initiators and accelerator to dissolve. A flow of nitrogen was passed through the sample to deoxygenate prior to irradiation. Immediately after deoxygenation, the vial lid was placed on top of the sample and the vial places under the UV lamp for one min. Acetone (3 mL) was then added to the vial to dissolve and uncured material. This solution was allowed to stir for 24 h before being decanted followed by drying of the cured material under vacuum at room temperature for 24 h (vacuum applied gradually to reduce the chance of material loss).
Poly(ε-caprolactone) dimethacrylate (217)

A flame dried round bottomed flask under nitrogen was charged with dry THF (100 mL), PCL diol (187, 110 mL, 20.2 mmol) and dry triethylamine (8.46 mL, 60.7 mmol). The reaction mixture was cooled to 0 °C and wrapped in tin foil (to avoid crosslinking in ambient light). Methacryloyl chloride (129, 5.92 mL, 60.7 mmol) was added slowly over one hour using an automatic syringe pump. The syringe containing the methacryloyl chloride was similarly wrapped in tin foil. The reaction mixture was left for 17 h and allowed to warm to room temperature after complete addition of the acid chloride. The solvent was removed on the rotary evaporator and diethyl ether (100 mL) and distilled water (100 mL) added and allowed to stir for 15 mins. The layers were separated and the organic layer washed water (3 x 100 mL). Organic layer was dried using magnesium sulphate, filtered and volatiles removed under reduced pressure to yield a pale yellow oil (13.187 g, 98 %). IR (CH₂Cl₂) νmax 1729, 1637 cm⁻¹. ¹H NMR (CDCl₃, 400MHz) δ (ppm): 1.42 – 1.45 (m, 2H, CH₂), 1.60 – 1.71 (m, 4H, CH₂), 1.93 (s, 3H, CH₃), 2.28 – 2.37 (m, 2H, CH₂), 3.67 – 3.75 (m, 2H, CH₂), 4.05 (t, J = 6.4 Hz, 2H, CH₂), 4.21 – 4.31 (m, 2H, CH₂), 5.54 (s, 1H, CH), 6.08 (s, 1H, CH).

Poly(ε-caprolactone) diacrylate (225)

A flame dried flask under nitrogen was charged with dry toluene (5 mL), PCL diol (187, 1 mL, 2.02 mmol) and dry triethylamine (0.62 mL, 4.45 mmol). The reaction mixture was stirred for 20 minutes at room temperature and wrapped in tin foil (to avoid crosslinking in ambient light). Acryloyl chloride (224, 0.36 mL, 4.43 mmol) was added and the reaction heated to 45 °C for 6 h. The volatiles were removed under reduced pressure and the resulting white semi solid was redissolved in diethyl ether (50 mL). The reaction mixture was washed with water (3 x 50 mL), dried using magnesium sulphate, filtered and volatiles removed to
yield a pale yellow oil (1.26 g, 98%). IR (CH$_2$Cl$_2$) $\nu_{\text{max}}$ 1733, 1636 cm$^{-1}$. $^1$H NMR (CDCl$_3$, 400MHz) $\delta$ (ppm): 1.32 – 1.43 (m, 2H, CH$_2$), 1.59 – 1.71 (m, 4H, CH$_2$), 2.27 -2.36 (m, 2H, CH$_2$), 3.66 – 3.75 (m, 2H, CH$_2$), 3.04 (t, $J = 6.4$ Hz, 2H, CH$_2$), 4.19 – 4.23 (m, 2H, CH$_2$), 5.80 (dd, $J = 1.6$, 10.4 Hz, 1H, CH), 6.09 (dd, $J = 10.4$, 17.2 Hz, 1H, CH), 6.37 (dd, $J = 1.6$, 17.2 Hz, 1H, CH).

\[
\text{HO} \quad \text{C-H}_2 \quad \text{O} \quad \text{O} \\
\]

4-Hydroxycyclohexyl acrylate (cis:trans mixture) (228)

1,4-Cyclohexanediol (226, 5.00 g, 43 mmol) was added to a flame dried 3-neck flame under nitrogen. Dry CH$_2$Cl$_2$ (100 mL), dry THF (50 mL) and dry triethylamine (6 mL, 43 mmol) were added and the reaction cooled to 0 °C. The reaction was covered in tin foil and acryloyl chloride (224, 4.17 mL, 52 mmol) was added slowly over one h using an automatic syringe pump. The syringe containing the acryloyl chloride was similarly wrapped in tin foil. The reaction mixture was left for 17 h and allowed to warm to room temperature after complete addition of the acid chloride. Reaction was washed with HCl (1 M), distilled water, dried using magnesium sulphate and filtered. Volatiles were removed under reduced pressure to yield a yellow semi-solid. Collected product was purified using column chromatography (petrol:ethyl acetate, 6:4) to give a clear oil (1.95 g, 27 %). IR (CH$_2$Cl$_2$) $\nu_{\text{max}}$ 3398, 1719, 1636 cm$^{-1}$. $^1$H NMR (CDCl$_3$, 400MHz) $\delta$ (ppm): 1.36 – 1.53 (m, 2H, CH$_2$), 1.60 – 1.78 (m, 4H, CH$_2$), 1.88 – 2.04 (m, 2H, CH$_2$), 2.21 (bs, 1H, OH), 3.70 – 3.81 (m, 1H, CH), 4.78 – 4.96 (m, 1H, CH), 5.81 (dd, $J = 1.6$, 5.2Hz, 1H, CH), 6.10 (dt, $J = 10.4$, 17.2Hz, 1H, CH), 6.38 (dd, $J = 1.2$, 7.6Hz, 1H, CH). MS-ESI found 193.0834 C$_9$H$_{14}$O$_3$, [M+Na]$^+$ requires 193.0834.
4-Oxocyclohexyl acrylate (228)

4-Hydroxycyclohexyl acrylate (227, 0.49 g, 2.90 mmol) dissolved in CH$_2$Cl$_2$ (50 mL). Addition of PCC (0.75 g, 3.48 mmol) produced an orange solution. The reaction mixture was left for 17 h at room temperature. Reaction mixture was filtered through a silica plug using ethyl acetate as eluent. The reaction was washed with NaOH (1 M) to remove any residual chromium followed by brine. Organic layer was dried with magnesium sulphate, filtered and volatiles removed under reduced pressure to yield a pale yellow oil (0.42 g, 85%). IR (CH$_2$Cl$_2$) $\nu_{\text{max}}$ 1720, 1637 cm$^{-1}$. $^1$H NMR (CDCl$_3$, 400MHz) $\delta$ (ppm): 2.06 – 2.21 (m, 4H, CH$_2$), 2.37 – 2.44 (m, 2H, CH$_2$), 2.55 – 2.63 (m, 2H, CH$_2$), 5.25 – 5.30 (m, 1H, CH), 5.90 (dd, $J = 1.2$, 10.4Hz, 1H, CH), 6.18 (dd, $J = 10.4$, 17.2Hz, 1H, CH), 6.47 (dd, $J = 1.2$, 17.2Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ (ppm): 30.4, 37.3, 68.7, 128.5, 131.2, 165.5, 209.8. MS-ESI found 167.0680 C$_9$H$_{12}$O$_3$, [M-H]$^+$ requires 167.0703.
11. Conclusions and further work

To conclude this project was successful in synthesising polymer – drug conjugates through the use of the functional monomer approach, and also the surface modification route. The functional monomer route was also utilised in generating a UV curable PCL derivative.

A series of functional εCL monomers were synthesised, including a CBz and BOC protected amine and also an acetal protected ketone. These were synthesised from the commercially available trans-4-aminocyclohexanol for the amine examples, and 1,4-cyclohexanedione monoethylene acetal for the protected ketone derivative. The amine examples utilised a similar synthetic route where the amine was first protected, followed by subsequent oxidation of the alcohol to the ketone, using PCC, and then the lactone using mCPBA (with the acetal variant only requiring oxidation to the lactone using mCPBA).

The above monomers were also successfully polymerised together with εCL using stannous octanoate to yield random copolymers. Although the CBz group was unsuccessfully removed, the acetal protected ketone was converted to the ketone and then subsequently reduced to the alcohol. From this alcohol a polymer – drug conjugate was successfully synthesised using ciprofloxacin.
Future work in this area would include the removal of either the CBz or BOC group to yield a free amine from which to attach ciprofloxacin using amide coupling chemistry. The removal of the CBz group would yield a free amine which could be an issue regarding rearrangement, however when removing the BOC protecting group the acidic environment would yield the ammonium ion therefore potentially being a more attractive. Once synthesised both the amide and ester linked ciprofloxacin polymer–drug conjugates, could be tested using cell culture experiments.

Following the functional monomer approach was the successful synthesis of polymer–drug conjugates through the technique of aminolysis. Both PCL and PLA films were successfully cast and subsequently aminolysed using HMDA, to yield the corresponding aminolysed and amine functional surfaces.
Figure 89: High resolution spectrum of the nitrogen region of a PCL film, when using an aqueous ethanolic wash and aminolysing using HMDA 10 % in water adjusted to pH 11.5

The aminolysed films were then further functionalised through amide coupling chemistry to yield ciprofloxacin and penicillin G attached PCL surfaces, and also the ciprofloxacin PLA conjugate.

Work in this area should continue with the testing of these biologically active surfaces in cell culture studies. Pending these results, 3D printed constructs generated using either PCL or PLA feedstock could be aminolysed and then biologically activated, with the resulting structures not only being biologically tested but also physically to understand the impact that aminolysis and functionalisation has on such properties.

The route of functional monomers was also investigated in the field of 3D printing, with a UV crosslinking monomer synthesised using PCL diol and either methacryloyl or acryloyl chloride.
These monomers along with the commercially available PEG DA were investigated using both type I (Irgacure 2959) and II (DETX/EDB) photoinitiators, to compare the speeds at which the neat and mixed materials cured under UV light. The ratio of photoinitiators was investigated and the combination of 3 wt% DETX and 3 wt% EDB found to give the best curing speed. This data along with cureable material were given to collaborators who successfully printed 3D constructs demonstrating the potential for these materials in the field of 3D inkjet printing.

As curing speed was of key importance during this work, a further investigation of photoinitiators could be conducted. This would focus around the use of BAPO, a
photoinitaitor capable of forming more radicals upon UV irradiation. This could lead to a faster curing rate, increasing the resolution of 3D printed constructs.

![Figure 91: Biarylphoshine oxide (BAPO)](image)

A key piece of further investigation would be the inclusion of a biologically active molecule into the UV curing ink. After the successful printing of a 3D construct, the biological activity could be probed to investigate whether this could be a viable route to a drug eluting, customisable 3D printed construct.
12. References

3 http://www.cardiology.md/procedures.htm (accessed 05/08/12).


(accessed 29/08/12).
(accessed 29/08/12).


Young, T. *Phil. Trans.*, 1805, 95, 65.


13. Appendix: Research Papers

13.1. A Biodegradable Polycaprolactone based ink developed for 3D Ink Jetting

13.2. Immobilisation of an antibacterial drug to Ti6Al4V components fabricated using selective laser melting

13.3. Functionalisation of Ti6Al4V components fabricated using selective laser melting with a bioactive compound
13.4. Publication contributions

In this section the contribution towards the three publications by the thesis author will be discussed.

The contribution towards the paper titled ‘A Biodegradable Polycaprolactone based ink developed for 3D Ink Jetting’ was through both synthesis of the polycaprolactone ink as well as the testing of curing conditions. The main contribution came with the synthesis of polycaprolactone dimethacrylate (a component of the jetted ink), with scale-up and optimisation of the synthesis allowing the author to successfully tested a variety aspects of both the ink and jetted materials, as without a robust source of UV curable material the research would have not been possible.

The following two papers (10.2. and 10.3.) were contributed towards through the synthesis of the biologically active surfaces. This involved the conversion of the carboxylic acid to the corresponding acid chloride and then the further reaction with ciprofloxacin and paracetamol (respectively). Although the carboxylic bearing surfaces were previously prepared by the publication’s author, knowledge of inert atmospheric chemistry set-up and coupling reactions from the thesis author, allowed a biologically active molecule to be attached to the surface. Without this key synthetic step, subsequent analysis and testing (XPS, cell culture, etc) would not have yielded successful findings.
A Biodegradable Polycaprolactone based ink developed for 3D Ink Jetting

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Abstract: Biomedical applications are one of the driving forces for Additive Manufacturing, however to extend the range of applications and markets new materials are required. A new type of biodegradable Polycaprolactone (PCL) based ink that is suitable for 3D inkjet printing was successfully developed. UV curable PCL was synthesized and mixed with Poly(ethylene glycol) di-acrylated (PEGDA) to prepare an ink with suitable viscosity for inkjet printing. Their mechanical properties as well as the printing accuracy were measured by nano-indentation and scanning electron microscopy. Post curing was applied to printed samples in order to study how post curing may influence sample properties. It was found that within 30min post-curing period, the sample’s surface which is direct illuminated by UV light increased from 31.22MPa to 70.20MPa while the bottom surface showed less incensement from 34.9MPa to 39.8MPa.

Introduction

3D Printing or Additive Manufacturing (AM), as a disruptive manufacturing technology, has been attracting increasing attention in recent years. Many studies have been carried out to enable AM to become a process that is able to manufacture end-use products. One of the potential applications for AM is in the making of biodegradable or bioresorbable medical products with the potential of tailored drug release functions. Some biomedical products require complex and strict pore structures to achieve enhanced cell adhesion and growth [1-3]. These requirements in pore structure and sizes are difficult to be controlled with current manufacturing techniques (such as foaming). However with AM technology, the sizes and locations of each pore can be accurately controlled and this lends the possibility of being able to manufacture bespoke products.

Polycaprolactone (PCL) is widely used in biomedicine as it can gradually decompose by random hydrolytic chain scission of the ester groups [4-5]. Some researchers have attempted to build PCL structures with additive manufacturing methods such as Powder Bed Fusion (i.e. laser sintering) or material extrusion [6-8]. However, little work has been done in processing PCL by 3D inkjet printing. Compared with other AM techniques, 3D inkjet printing has the potential of producing multi-material artefacts within a single process cycle with controlled material distribution. This facility can be used to manufacture products with localized drug distribution or degradation speeds. Therefore, developing a biodegradable material which is
suitable for 3D inkjet printing could bring the manufacturing of biomedical product into a brand new field.

3D inkjet printing requires a low viscosity ink, which solidifies quickly after deposition hence photo-curing has been a popular method of achieving fast solidification for commercial materials. Although pure caprolactone does not cure under UV irradiation, groups (normally acrylated group) can be grafted onto the end of a PCL polymer chain to make it UV curable [9-10]. As printers have very strict requirements on ink viscosity, a diluent is also a necessary component to help adjust the inks viscosity. PolyEthyleneGlycol (diacyrlated) (PEGDA), can be used as such a diluent, and has been widely used for UV curable biocompatible materials [11-13]. Some authors have suggested that a copolymer of PCL and PEG can help develop products with different surface function and modify the drug release profile of a material because PCL is hydrophilic and PEG is hydrophobic [14-15].

In this paper, the printability of UV curable PCL: Polycaprolactone di-methacrylated (PCLDMA) was assessed. Rheological data were collected with different PEGDA proportions in a temperature range from ambient temperature to 60°C to help select suitable processing conditions and PEGDA concentration for printing. The synthesised UV curable PCL's viscosity was adjusted by adding 30wt% of PEGDA and successfully printed by using a Dimatix DMP2800 inkjet printer. 3D structures were then created with this ink and characterized to help understand the properties of this material.

**Methodology**

**Ink preparation**

PCLDMA (synthesised) and PEGDA (Sigma-Aldrich average Mn~250) were added into an 8ml amber vial and stirred at room temperature for 15mins at 800rpm using an IKA RCT Basic IKAMAG Magnetic Stirrer (with Temperature Controller). 3wt% of photo-initiator (2,4-Diethyl-9H-thioxanthen-9-one( DETX), sigma-aldrich, 98%) and 3wt% of accelerator (Ethyl 4-(dimethylamino)benzoate(EDB), sigma-aldrich, 99wt%) were added into the PCLDMA:PEGDA mix and stirred at 85°C for 5mins until all the solutes are fully dissolved. Before printing, the prepared ink required a degassing procedure to remove dissolved oxygen and to help minimize the 'oxygen inhibition' effect [17-18]. The degassing procedure was carried out by purging the mixed ink with nitrogen gas for 15minutes. This procedure created lots of Nitrogen bubbles within the ink which seriously reduces the droplet formation stability of the ink. Therefore the ink was prepared 24hrs prior to printing; the degassed ink was then settled to release the bubbles.

**Printability Assessment**
The key parameters for determining “printability” are viscosity and surface tension. Normally, viscosity is the most fundamental parameter used to decide whether an ink is printable or not. The printing viscosity range varies dependent on the printheads being used. Surface tension should also be taken into consideration. It has been reported that the inverse Ohnesorge number (Oh⁻¹) can be used as the printing indicator (Z) to help predict an ink’s printability. Z takes both viscosity and surface tension into consideration and is shown following equation[10].

\[ Z = \frac{\sqrt{\rho \gamma}}{\mu} \]

Where \( \rho \) is density, \( r \) is characteristic length, \( \gamma \) is surface tension of the fluid and \( \mu \) is viscosity of ink.

The viscosity of the PCLDMA:PEGDA mixture (under shear rates of 100s⁻¹ and 1000s⁻¹) was measured by a cone plate rheometer (Malvern Kinexus Pro) to identify the PCLDMA:PEGDA proportion and the processing temperature that would give a suitable viscosity for inkjet printing. Each measurement started at 25°C with 5°C increments up to 60°C. A protocol of waiting 300s after reaching the test temperature was set to ensure the ink was in a steady state condition. At each temperature point and shear rate, the viscosity was recorded at 5s intervals within a 180s test time. Surface tension was measured by a pendant drop method using a Kruss DSA100S. The shape was captured at the equilibrium state and used to calculate the surface tension.

**Sample properties assessment**

The ink with the final composition was then injected into a print cartridge. The injection procedure was carried out in the dark to prevent light irradiation and careful attention was paid to avoid bubble formation within the ink. The cartridge was wrapped with foil tape to make sure the ink was not cured inside the cartridge by ambient light. About 2ml of the prepared ink was injected into a disposable cartridge and printed by DimatixDMP-2800 material printer. The printed ink was cured by real-time UV curing; a UV curing unit was mounted directly on to the printing unit (Figure 1) to move in conjunction with the print direction. A further UV LED unit (intensity of 1000mW/cm²) was used to examine the influence of different post-curing time (10 minutes, 20 minutes and 30 minutes) on the mechanical properties of the printed parts.

The mechanical properties of printed sample were characterized by nano-indentation at room temperature (Micro Materials, NanoTest NTX with hot stage and inert gas cabinet). Both the top and bottom surface were characterized. Load-depth curves were recorded on 5×5 grid with 100μm separation between each indentation. The
applying force was set to 5mN with a 0.25mN/s loading and unloading rate and a spherical indenter with 50μm radius was used. The Hardness is calculated by:

\[ H = \frac{P}{A} = \frac{P}{\pi a_r^2} \]

Where \( P \) is applied load, \( a \) is the radius of the circle of contact. The radius of the circle of contact is calculated by follow equation:

\[ a_r = \frac{\sqrt{4R(h_t + h_r)} - (h_t + h_r)}{2} \cdot c \]

Where \( R \) is the radius of the spherical indenter, \( h_t \) is the total penetration depth, \( h_r \) is the residual depth and \( c \) is correction constant for piling-up or sinking-in effect [19].

The indentation modulus \( E \) can be calculated by:

\[ E = \frac{3}{4} \frac{P}{a_r h_e} \]

Where \( P \) and \( a \) are applied load and radius of circle of contact respectively, \( h_e \) is the elastic deformation depth.

Mesh structures were printed to help understand the manufacturing accuracy that PCLDMA:PEGDA (70:30) ink could achieve. Printed mesh structures were sputter coated with Platinum and examined by SEM (XL30 ESEM Philips).

**Results and Discussion**

**Printability Assessment**

The viscosity results of PCLDMA with different PEGDA proportions were measured (Table 1). As PEGDA is a diluent and only biocompatible after curing, when choosing the composition, the amount of PEGDA needs to be as low as possible within the printable viscosity range, in order to maximise the biodegradability of final product. PCLDMA with 30wt% of PEGDA at 80°C was chosen as the final proportion in this paper based on the rheological tests and printing requirements. However, as different printheads have different printing viscosity ranges, this does not apply to all the piezo based printheads. The viscosity distributions of PCLDMA:PEGDA mixture with various proportions under different environment temperatures are given in Table 1, which can help decide the optimum composition for the other printheads.

As PEGDA is only bio-compatible but not biodegradable material, it will reduce the biodegradability of the final product. Therefore, when choosing the final composition, the one with higher PCLDMA concentration but still within printable range is
preferred. Based on this principle, PCLDMA:PEGDA (70:30) was chosen as the final composition, which will be used for following printing.

The printability indicator, Z, for PCLDMA:PEGDA (70:30) was then calculated (Table 3). It has been suggested that when the value of the printing indicator is between 1 and 10, the ink will normally be printable [16]. From Table 2, it can be seen that based on these calculations, the Z values of PCLDMA:PEGDA (70:30) at 60 °C were in the printable range.

The viscosity of the ink was measured throughout the whole ink preparation procedure to monitor viscosity variations (Table 3). It can be seen that after adding photo-initiators and being degassed, the viscosity of the ink increased by 5-10%. This was due to two reasons: the adding of solid content and a small amount of curing during the degassing procedure. As one may expect, adding high viscosity content will increase the viscosity of the ink. The photo-initiator and accelerator used in our experiment are both solids and they occupied 0.6wt% of the whole ink which led to viscosity increase. Also, the ink became quite reactive during degassing and as the degassing procedure was not carried out in a completely dark environment there will be small amount of curing which also increased the prepared ink’s viscosity.

Real-time curing and Post-curing effect

Five square specimens, 5mm (W)*5mm (L), were prepared for nano-indentation testing. The printing pattern and samples are shown in Figure 3 where 100 layers were printed. The final thicknesses of these square samples were ~500μm.

The hardness and indentation modulus of printed samples with different post curing time were measured by nano-indentation (Table 4). The measurements were carried out on the sample’s top and bottom surface respectively. As the samples were produced by stacking up layers of material, those layers printed in the early stages will inevitably be repeatedly exposed to UV illumination when following layers are printed. This will lead to a printed sample which has a gradient of UV exposure time from bottom to top. As UV exposure time is normally related to curing level, this may eventually result in property deviation and therefore, both surfaces were measured separately.

The indentation results (Table 4) showed the top and bottom surface of the sample has very similar properties before any post-curing treatment. The hardness and indentation modulus on both surface have variations however these are within the testing deviation range.

The mechanical properties of the sample’s top surface, which was directly illuminated by UV light, had a significant increase after 10mins of post-curing (Table 4 and Figure 4). However additional post-curing for 20mins and 30mins did not further influence these properties. The rise of hardness and modulus on the top surface after post-curing was mainly due to and increase of cross-link density. Prior work has shown that the hardness and modulus of a crosslink material is positive in relation to its crosslink density [20, 21]. At low crosslink density, polymer chains are
less restricted, therefore it can easily deform with an applied manifesting as a low hardness and modulus. As crosslink density increases those free segments are connected with each other building an increasingly dense network. The mobility of the polymer chain segments becomes restricted and the specimen will then show stronger resistance to an applied force. When a specimen was printed, the conversion of the C=C group into the covalent crosslink cannot normally reach 100%. During the post-curing procedure UV illumination will provide extra energy to help the residual C=C group form new crosslink and therefore, further increase the crosslink density and hence its hardness and modulus.

Meanwhile, the properties of the sample’s bottom surface did not show a notable change with the increase of post-curing time. This could be because during the post-curing procedure, samples were illuminated from the top surface and the UV irradiation needs to penetrate the whole sample before reaching the bottom surface. During the penetration procedure, the intensity of the UV light would be reduced by absorption from the sample and result in only a small quantity of radiation reaching the bottom surface. From the nano-indentation results, the bottom surface did not receive enough energy to achieve further crosslinking and therefore the properties remain unchanged. UV absorbance spectrums with a different thickness of cured ink films are currently under characterization to affirm this hypothesis and also help discover the penetration depth.

Printing and Characterization

Mesh structures were then printed and a schematic figure of the printing pattern was shown in Figure 5 (a). Meshes with three different wall thicknesses (150μm, 300μm and 500μm) were printed onto glass slides for further SEM examination. The distance between each wall was set as 1mm to allow each printed vertical or horizontal wall to be separated from each other. Ten layers of PCLDMA:PEGDA (70:30) were printed and the sample appearance is shown in Figure 6(b).

The mesh structures were then observed under SEM (Figure 6). The actual printed wall thickness was measured and calculated to help analyzing the dimensional variation between the printed structure and the original design. From the results in Table 5, it can be noticed that the printed mesh structure with larger designed wall thickness had less deviation percentage compared with the meshes with thinner walls. However the deviation was always around 40μm which would indicate that the processing accuracy of Dimatix material printer for PCLDMA:PEGDA (70:30) is around 40μm, when using printhead with 21μm nozzles.

Figure 6 also showed that under the print conditions the PCLDMA:PEGDA (70:30) ink could not form accurate and sharp edges. Rectangular gaps were designed inside the mesh structure. However in the actual printed structure, the gap morphed into rounded rectangular shapes. Meanwhile, dislocation of printed ink droplets can be observed from the SEM pictures and causing rounded rectangular gaps as well
as curving walls. These might be due to the slow curing speed of the printed ink. Although, the ink was illuminated by UV light immediately after being printed, the illumination time was quite limited in a single scan. This is because the UV curing unit was attached and moving with the printhead. So the energy provided in a single scanning may not be enough to allow freshly deposited ink to become fully cured immediately. Therefore, merging and dislocation of the uncured ink droplet could happen due to gravity, movement of the platform and merging with the ink deposited in subsequent printing cycles. All these will lead to rounded edge and curing walls.

However, as the illumination area of UV curing unit were larger than the printed area of each printing cycle, the previously printed ink can still receive UV illumination during the following printing. So the ink will receive discontinuous UV illumination and finally be cured after obtaining enough energy. But the curing time will be enlarged compared with continuous UV illumination. Increasing the curing speed could help printed ink cure in a shorter period of time, hence reduce the chance of merging and dislocation happening, improving the print quality. This could be achieved by either increasing the intensity of UV illumination or creating an oxygen free environment.

Figure 6 (d) is a SEM image showing a cured mesh surface in high magnification. Wrinkles (about 1 to 2μm) can be observed on the entire printed mesh structure's surface. A similar self-wrinkling effect was observed by Chandra et al [22]. They suggested that this effect was mainly due to oxygen inhibition which caused crosslinking speed variation from the top to the bottom. When UV curable films were exposed to UV illumination within the presence of oxygen, a thin layer at the top surface will remain uncured due to the oxygen inhibition. A crosslink gradient would be formed through the depth direction because of oxygen concentration gradient formed at the surface by diffusion. This situation will lead to in-plane stress and cause surface wrinkle. In Chandra et al.'s work, they also concluded that by controlling the oxygen concentration in the environment, the size of surface wrinkle could also be controlled.

Figure 7 is a printed curving mesh structure with PCLDMA: PEGDA (70:30) ink. 50 layers were printed and surface profiling data Figure 7(d) showed the total height of the structure was around 250μm. Figure 8 shows optical microscopy images of a printed curving mesh structure. Similar effects were also observed that the ink did not fully cure immediately after deposition and droplets at the edges falling down to the substrate forming coarse structures at the base.

Conclusion

A PCLDMA: PEGDA ink that is suitable for 3D inkjet printing to produce biodegradable 3D structures has been demonstrated for the first time. For different printers, the proportion of PEGDA and processing temperature can be varied, which should be decided based on the given rheology database.
In this paper, PCLDMA: PEGDA (70:30) was chosen and observed to be suitable for a Dimatix DMP-2800 when printed at 60°C. The prepared ink can be cured sufficiently to retain expected structures during printing and stable products can be produced. The hardness of printed samples was around 5MPa with an indentation modulus of 30MPa. These properties increased when a post-curing procedure was applied. However, only the mechanical properties at the top surface were improved. From SEM examination, it was found that print quality was influenced by curing speed and wrinkles were observed on the surface of the cured structures. For future work, the curing efficiency and printing qualities of PCLDMA:PEGDA (70:30) ink with different photo-initiator / accelerator ratios should be investigated. Oxygen inhibition effects will also be studied by performing printing under different oxygen concentration levels to investigate the impact on printing quality and mechanical properties of cured structure.

References


[8] Eshraghi S., Das S., Mechanical and Microstructural Properties of Polycaprolactone Scaffolds with One-dimensional, Two-dimensional and Three-


Figure1: Structure of printhead and UV curing unit.
Figure 2: Viscosity distribution plot of PCLDMA: PEGDA with different proportions between 25 °C to 60 °C when shear rate equals to 1000s⁻¹.

Figure 3: Printed square samples for nano-indentation test: (a) Printing pattern, (b) Top view of printed square samples, (c) Side view of printed square samples.
Figure 4: Plots of nanoindentation data for samples with different postcuring time. (a) Hardness, (b) Indentation modulus.
Figure 5: Printed mesh samples for processing accuracy check (a) Schematic diagram of printing pattern design (b) printed sample with different wall thickness (150µm, 300µm and 500µm from left to right)

Figure 6: SEM pictures of printed mesh structure with different wall thickness: (a) 150µm, (b) 300µm, (c) 500µm, (d) winkle found at sample surface
Figure 7: Curving mesh structure printing: (a) Printing pattern, (b) Sample appearance after taking off from glass slide, (c) Top view of printed sample, (d) Surface profiling of printed curving mesh structure.
Table 1: Viscosity of PCLDMA: PEGDA with different proportions between 25 °C to 60 °C when shear rate equals to 1000s⁻¹

<table>
<thead>
<tr>
<th>Temperature</th>
<th>50:50</th>
<th>60:40</th>
<th>70:30</th>
<th>80:20</th>
<th>90:10</th>
<th>100:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 °C</td>
<td>35.85±1.03</td>
<td>43.14±1.02</td>
<td>49.71±1.02</td>
<td>63.09±1.04</td>
<td>87.81±1.00</td>
<td>122.64±0.98</td>
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<tr>
<td>30 °C</td>
<td>29.22±1.00</td>
<td>34.97±1.00</td>
<td>40.07±0.97</td>
<td>50.45±0.98</td>
<td>69.68±0.95</td>
<td>96.14±0.92</td>
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<tr>
<td>35 °C</td>
<td>24.44±1.01</td>
<td>29.00±0.99</td>
<td>33.00±0.99</td>
<td>41.25±0.96</td>
<td>56.47±1.00</td>
<td>76.96±0.93</td>
</tr>
<tr>
<td>40 °C</td>
<td>20.90±1.00</td>
<td>24.44±1.00</td>
<td>27.58±1.00</td>
<td>34.21±0.99</td>
<td>46.45±0.99</td>
<td>62.64±0.94</td>
</tr>
<tr>
<td>45 °C</td>
<td>18.24±1.02</td>
<td>20.99±1.00</td>
<td>23.41±1.01</td>
<td>28.81±0.98</td>
<td>39.15±0.98</td>
<td>51.78±0.97</td>
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<tr>
<td>50 °C</td>
<td>16.18±1.02</td>
<td>18.33±1.03</td>
<td>20.15±1.03</td>
<td>24.51±1.00</td>
<td>33.05±0.99</td>
<td>43.28±1.00</td>
</tr>
<tr>
<td>55 °C</td>
<td>14.56±1.02</td>
<td>16.25±1.02</td>
<td>17.60±1.03</td>
<td>21.14±0.99</td>
<td>28.24±1.01</td>
<td>36.62±1.00</td>
</tr>
</tbody>
</table>
Table 2: Physical properties and printing indicator value of PCLDMA: PEGDA (70:30) mixture at temperature of 25°C and 60°C

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Nozzle Diameter (µm)</th>
<th>Density (g/cm³)</th>
<th>Viscosity (cp)</th>
<th>Surface Tension (mN/m)</th>
<th>PI (Oh⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>21</td>
<td>1.08</td>
<td>49.71</td>
<td>37.26</td>
<td>0.58</td>
</tr>
<tr>
<td>60°C</td>
<td>21</td>
<td>1.08</td>
<td>15.63</td>
<td>32.31</td>
<td>1.73</td>
</tr>
</tbody>
</table>

Table 3: Viscosity monitoring of PCLDMA: PEGDA=70:30 sample between 55°C to 60°C when shear rate equals to 1000s⁻¹

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Without PI and AC</th>
<th>With PI and AC</th>
<th>With PI and AC degassed</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C</td>
<td>20.15±1.03</td>
<td>20.44±1.02</td>
<td>22.27±1.01</td>
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<tr>
<td>55°C</td>
<td>17.60±1.03</td>
<td>18.35±1.01</td>
<td>19.54±1.01</td>
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<tr>
<td>60°C</td>
<td>15.63±1.03</td>
<td>16.57±1.02</td>
<td>17.55±1.04</td>
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</tbody>
</table>

Table 4: Hardness and indentation modulus for printed PCLDMA:PEGDA (70:30) before and after postcuring.

<table>
<thead>
<tr>
<th>Curing Time</th>
<th>Hardness (MPa)</th>
<th>Indentation Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0min</td>
<td>5.01±0.42</td>
<td>32.28±3.99</td>
</tr>
<tr>
<td>10mins</td>
<td>6.13±0.08</td>
<td>71.97±1.47</td>
</tr>
<tr>
<td>20mins</td>
<td>6.27±0.09</td>
<td>72.17±1.04</td>
</tr>
<tr>
<td>30mins</td>
<td>6.01±0.14</td>
<td>70.2±1.78</td>
</tr>
<tr>
<td>Bottom Surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0mins</td>
<td>4.84±0.44</td>
<td>28.44±1.87</td>
</tr>
<tr>
<td>10mins</td>
<td>5.07±0.20</td>
<td>34.90±1.27</td>
</tr>
<tr>
<td>20mins</td>
<td>5.09±0.19</td>
<td>35.01±1.84</td>
</tr>
<tr>
<td>30mins</td>
<td>5.75±0.08</td>
<td>39.88±2.01</td>
</tr>
</tbody>
</table>
Table 5: Comparison of actual printed wall thickness and designed wall thickness

<table>
<thead>
<tr>
<th>Designed Wall Thickness</th>
<th>Printed Wall Thickness (Average)</th>
<th>Deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150μm</td>
<td>194μm</td>
<td>29.3%</td>
</tr>
<tr>
<td>300μm</td>
<td>344μm</td>
<td>14.7%</td>
</tr>
<tr>
<td>500μm</td>
<td>463μm</td>
<td>-7.4%</td>
</tr>
</tbody>
</table>