



CONTROL OF MICROSTRUCTURE IN POLY-LACTIC ACID AND
THE EFFECT ON BIODEGRADATION.

by

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Abstract

Poly(lactic acid) (PLA) is a biodegradable polymer with numerous applications in tissue regeneration and repair. The degree of crystallinity governs the rate of degradation in vitro and in vivo. In this project, the effect of the microstructure on the degradation of Poly(lactic acid) (PLA) is studied.

PLA samples were crystallised to 40% crystallinity at various temperatures between 90 °C and 140 °C and then subjected to enzymatic degradation using the enzyme Proteinase K. Hot stage microscopy showed that on increasing the crystallisation temperature an increase in the growth rate of spherulites and a decrease in spherulite nucleation was observed. Short-term (five day) and preliminary long-term (ten week) biodegradation studies were conducted on samples crystallised to 40% at 90 °C, 108 °C and 118 °C. DSC and FT-IR analysis in short-term degradation studies showed little difference in samples before and after degradation. However, it is seen that as the crystallisation temperature increased there was a resulting increase in weight loss observed in samples after degradation. Preliminary long-term degradation studies found that weight loss increased after three weeks and also found weight loss to be higher in samples crystallised to 40% at 118 °C than at 90 °C. The results suggest that the microstructure of PLA does have an effect on the rate of biodegradation and further long term biodegradation studies must be conducted to explore this fully.

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Contents

1.0 Introduction	1
1.1 Biomaterials	1
1.2 Polymer Morphology	2
1.3 Crystallisation Kinetics	3
1.4 Polylactic Acid	6
1.5 Biodegradation.....	8
2.0 Literature Review	10
2.1 Crystallinity	10
2.2 PLA Degradation	11
2.3 Objectives	19
3.0 Experimental Methods	21
3.1 Materials	21
3.2 Sample Preparation	21
3.3 Sample Conditioning	22
3.4 Differential Scanning Calorimetry	22
3.4.1 DSC Theory.....	22
3.4.2 Thermal Analysis	23
3.5 Hot Stage Microscopy	24
3.6 Sample Biodegradation	24
3.6.1. Preliminary Biodegradation	24
3.6.2 Five Day Biodegradation	26
3.6.3 Ten Week Biodegradation.....	27
3.7 FTIR	29
4.0 Results and Discussion	30
4.1 PLA	30
4.2 The Effect of Crystallisation Time and Temperature on PLA	30
4.3 The Effect of Crystallisation Temperature on Spherulite Formation	41
4.4 The Effect of Crystallisation Temperature on Peak Melting Point	44
4.5 The Effect of Degree of Crystallinity on Melting Temperature	47
4.6 Biodegradation.....	51
4.6.1. Preliminary Biodegradation	51
4.6.2. One Week Biodegradation	56
4.6.3. Ten Week Biodegradation.....	61

5.0 Conclusions and Further Work..... 67

1.0 Introduction

Polymers are widely used materials that are particularly important as biomaterials. Polymers can be used in a wide range of applications and industries due to the ability to engineer varying microstructures and properties by controlling how the polymer is processed. Polylactic acid (PLA) is thought to be one of the most important bio-materials due to its biocompatibility, good mechanical properties, degradation rates and it being FDA approved. Polylactic acid is a linear aliphatic thermoplastic polymer with a high molecular weight (Nam et al, 2005).

1.1 Biomaterials

Biomaterials can be defined as materials used within the body in order to aid, treat or replace a natural function of the body. The main factor for a material to be classed as a biomaterial must be that it is biocompatible (i.e. The material must cause little to no immune response by the body). Polymers are currently widely used as biomaterials and are extensively researched for biomaterial applications. The most widely researched polymers used in biomaterials are biodegradable polymers and the main advantage of these being that they eliminate the need for further surgical procedures. Biodegradable polymers refer to polymers that degrade into non-toxic products that are often found in the body (i.e. polylactic acid degrades to lactic acid which is a natural metabolite).

Biodegradable polymers can be either natural or synthetic. Synthetic polymers are the usual choice of polymers as biomaterials due to them being widely available and the ability to

produce polymers with a variety of different properties depending on the requirements. It is also possible to control their degradation rate within the body.

1.2 Polymer Morphology

Understanding polymer morphology is important as different morphologies will result in different polymer properties. A higher degree of crystallinity will increase the strength and stiffness of the polymers; therefore semi-crystalline polymers generally display better properties than amorphous polymers.

Polymer molecules (macromolecules) are chains of repeating units covalently bonded together. Polymers can either be amorphous or semi-crystalline. Amorphous polymers retain a disordered and entangled chain structure when 'frozen', whereas semi-crystalline polymers form partially ordered regions (see figure 1).

Linear polymers are made up of the same chemical unit that is repeated several times and two end groups. The number of repeat units within a polymer chain, also known as the degree of polymerisation, is important in controlling the properties of the polymer. The degree of polymerisation is used to calculate the molecular weight of the polymer by multiplying the molecular weight of the repeat unit and adding the molecular weights of the end groups (Woodward, 1995).

Polymers very rarely display a single structure or properties due to the variance in chain length, molecular composition and stereochemistry of the polymers. This makes it difficult to establish a single molecular weight. These variances can result in widely different properties within the polymers and polymers with the same average chain length but

varying distribution of the chains can result in the polymers having vastly different properties. This therefore means that several averages are calculated to provide information on the distribution and therefore the properties of the material. (A. Jenkins, 1972).

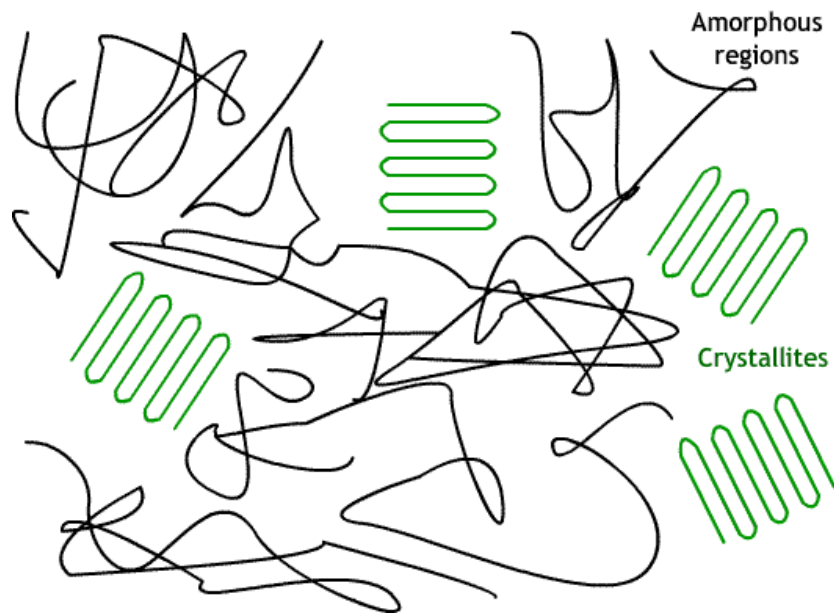


Figure 1. Chain structure in amorphous and semi crystalline polymers.

1.3 Crystallisation Kinetics

The degree of crystallinity within a polymer greatly affects the properties of the material. Crystallisation is the process of the formation of an ordered structure from a disordered phase i.e. The melt or a solution (Young et al, 1991). Crystallisation occurs in two stages, nucleation and growth. When a polymer reaches the temperature between the glass transition temperature and the melting temperature of the polymer, the random disoriented molecules within the melt or solution become aligned to form small ordered areas (nuclei). As the polymer is heated above the T_g , the chains become more mobile allowing them to become more mobile and therefore structured. This process is called

nucleation. When the nuclei of the crystalline phase of a polymer reach a critical dimension are formed by thermal changes, they provide surfaces for growth into a crystalline phase (Muthukumar). The nuclei formed during nucleation are only stable at temperatures below the melting temperature, above this point the chains are disturbed by thermal motion, i.e. The particles move too quickly and collide too much to form ordered structures. The second stage of crystallisation is the growth step. This refers to the growth of the nuclei formed during nucleation as chains are added to the small structured regions causing the nuclei to grow.

Nucleation can occur in two ways, it can either be heterogeneous or homogeneous. Homogeneous nucleation occurs when individual molecules cluster together, whereas heterogeneous nucleation occurs when nucleation occurs due to the presence of a foreign body which creates an initiation site for spherulites formation within the polymer e.g. Dust particles, small inclusions etc. The heterogeneous method of nucleation is much easier to control the crystallisation than homogeneous as the amount of nucleating sites can be controlled. Homogeneous nucleation tends to occur at lower temperatures than heterogeneous nucleation. If all factors are kept constant, the number of nuclei formed during the nucleation process is seen to be dependent on crystallisation temperature. At higher temperatures, the nuclei formed are sporadic with only a small number of large spherulites formed, whereas at lower temperatures, much smaller spherulites are formed; however there is a great increase in the number of spherulites. The space between the spherulites contains amorphous material that has not yet been crystallised and also any impurities within the polymer that are unable to crystallise (Miller, 1996).

The growth of the nuclei can occur in one, two or three dimensions and the crystals can form in rods, discs or spheres. Molecule chains join the nuclei to form lamellae, and changes in the dimensions of the lamellae cause an increase in the spherulite radius size therefore increasing the crystalline regions of the polymer. This increase in spherulite size has been shown to be linear with time until the spherulites formation is so large that they begin to touch each other. The spherulite formation radius can be calculated using a simple equation as shown below (Young et al, 1991):

$$r = vt \quad \text{equation 1}$$

Where r is the spherulite radius, v is the growth rate and t is the time.

Spherulite formation occurs as lamellar ribbons expand outwards from a single nucleus. The growth rate of the spherulite formation is greatly affected by the crystallisation temperature used. At temperatures close to the melting point, there is a decrease in the growth rate of the spherulites due to them being disturbed by thermal motion. As the crystallisation temperature decreases, the growth rate greatly increases until a peak is reached, after this point, as crystallisation temperature decreases further, the growth rate also decreases due to an increase in the viscosity of the polymer making it difficult for chain motion and therefore alignment and ordering. (Young et al, 1991). Figure 2 shows the composition of a spherulite.

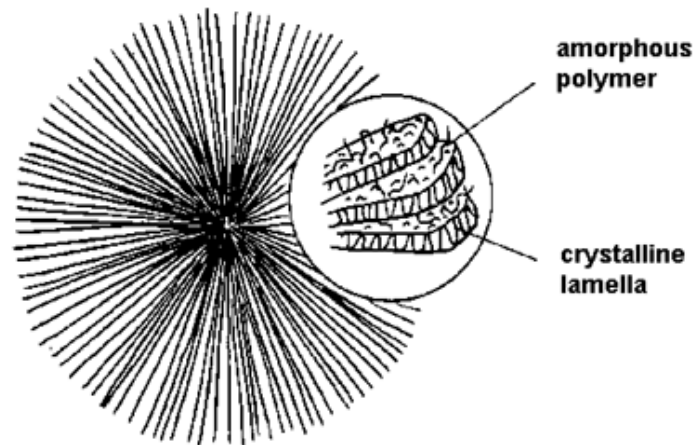


Figure 2. Spherulite formation

<http://www.doitpoms.ac.uk/tlplib/polymers/spherulites.php>

1.4 Polylactic Acid

Poly(lactic acid) (PLA) is a type of synthetic bio-erodible polymer. The bio-erosion process refers to the physical changes in a material such as size, shape and mass. This could be a result of either degradation or dissolution or both. PLA and its copolymers mainly with polyglycolic acid (PGA) are the most widely investigated and used polymers in bio-materials due to the fact that they are FDA approved. They have been used successfully in medical applications and are known to be safe, non-toxic and bio-compatible. This therefore reduces the amount of time to get new implants in the market if the material is already considered safe. It also costs less than getting a new polymer approved where biocompatibility is not entirely proven. PLA is a popular choice material for use in environmental, medical and pharmaceutical applications due to its chemical structure (Figure 3) and it therefore being biodegradable, biocompatible, easy to process and having good mechanical properties. It is relatively cheap and available in a wide range of grades. Common medical applications

include sutures, polymer scaffolds, tissue engineering, drug delivery and medical implants such as pins and screws.

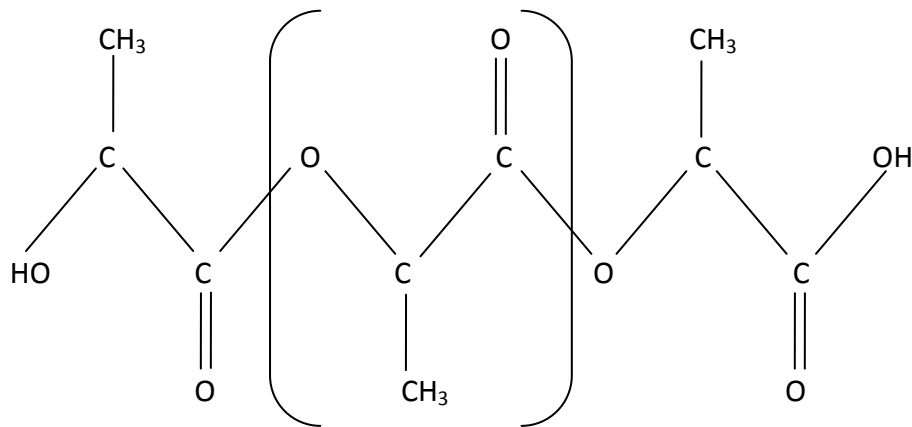


Figure 3. Chemical structure of polylactic acid

Dipoles will encourage the crystallisation of PLA, particularly if the repeat chain is straight. In acidic conditions the hydrogen from OH group is retained, this increases the dipole strength. Degradation occurs by scission of the C-O bonds.

Lactic acid is a chiral molecule with a chiral center; this refers to a molecule that cannot be superimposed on its mirror image. There are two forms, right and left isomers, which can produce four types of dimers known as D-PLA, L-PLA, DL-PLA and meso-PLA (Figure 4). D-polymers and L-polymers are semi-crystalline, whereas DL- polymers are amorphous and the 50% of each polymers (racemic form) optically inactive. These differences in crystallinity are important when choosing the applications they are used for. DL- PLA is amorphous and therefore mainly used for applications such as drug delivery. L-PLA is semi-crystalline and used mainly where high strength and toughness are needed.



Figure 4. Lactide stereo isomeric forms (http://www.futerro.com/products_lactide.html)

For a thin sample of PLA, degradation is homogeneous (same degradation rate throughout the material) for thick samples the reactions are heterogeneous (the degradation rate is not the same throughout the volume of the material). This is due to the autocatalytic phenomenon observed in this type of polymers. The lactic acid formed in the bulk of the material results in a decrease of the pH that catalyses hydrolysis causing different degradation rates throughout the volume of the material. This type of degradation results in the surface of the material degrading slower than the bulk and therefore the formation of hollow structures with very weak mechanical properties. PLA displays hydrophobic properties. Co-polymers with poly-glycolic acid however, do not follow a linear relationship, i.e. more lactic acid doesn't make them more hydrophobic. Hydrolysis occurs in the amorphous areas of the polymer before the crystalline areas.

1.5 Biodegradation

Biodegradation refers to the chemical breakdown of materials by living organisms, which leads to a change in physical properties. Degradation can either occur naturally by hydrolysis, or by enzymatic hydrolytic degradation with the aid of an enzyme.

One enzyme used in enzymatic hydrolysis of PLA is Proteinase K, which is particularly useful in the degradation of aliphatic polymers. Proteinase K works by initiating cleavage of the peptide bond at the carboxyl groups. An advantage of Proteinase K is that it is stable over a wide range of pH, 4-12; however the optimum pH is between 7.5 and 12. The optimum temperature for maximum activity is at 37 °C; however the enzyme is functional between 20 and 60°C. The molecular weight of Proteinase K is around 28, 930 Daltons and it is soluble in water (Sigma Aldrich).

2.0 Literature Review

2.1 Crystallinity

PLA is available in a wide range of different grades, with a range of different levels of crystallisation; however, higher crystallisation levels generally provide better properties. The percentage of crystallinity strongly affects the physical properties and therefore the performance of the PLA (Ahmed et al, 2009). PLA can be amorphous, semi-crystalline or a combination of the two, which means that many different thermal and physical properties can be seen in PLA. Ahmed et al (2009) investigated the thermal properties (melting, crystallisation and glass transition) of PLA as a function of molecular mass, isomer and microstructure. They reported that higher T_g values were found in poly-L-lactic acid (PLLA) than in poly-DL-lactic acid (PDLLA) samples. They also found that semi-crystalline samples display a higher T_g than amorphous samples with similar molecular weights. Samples with lower molecular weights did not show melting and crystalline peaks, whereas higher molecular weight samples showed a drop in crystallisation temperature. They concluded that the microstructure, molecular weight and type of isomers present, all affect the glass transition temperature, melting point and crystallisation behaviour of PLA. SII Nanotechnology Inc (2007) also supported these findings showing that the higher the L-isomer ratio the easier it was for crystallisation to occur.

He et al crystallised PLA at various temperatures between 90°C and 125°C. They found that at lower temperatures (105, 110 and 115°C), a double melting peak was observed, whereas at higher temperatures (120°C and 125°C) single melting peaks were observed. He et al suggested, that the formation of double melting peaks at lower temperatures is due to the

melt-recrystallisation mechanism i.e. Small and imperfect crystals gradually becoming more stable and perfectly formed. Yasuniwa et al (2002) and Di Lorenzo (2005) also supported this finding observing double melting peaks at lower temperatures and heating rates.

Di Lorenzo (2005) conducted a study into the crystallisation behaviour of poly-lactic acid under both isothermal and non-isothermal conditions. They found that the crystallisation rate greatly increased between 100 and 118°C and this was said to be due to an increase in the spherulite growth rate as opposed to a change in spherulite appearance. Di Lorenzo also found that at lower temperatures during crystallisation small defective crystals are formed. As these isothermally crystallised samples are then heated through the melt, the imperfect crystals melt to form an endothermic peak, recrystallisation and reorganisation of the crystals then occurs which melt at a higher temperature causing a second endothermic peak. At higher crystallisation temperatures however, recrystallisation and reorganisation were not observed resulting in a single melting peak.

2.2 PLA Degradation

There are many different factors that influence the biodegradation of polymers such as size, molecular weight and density of the polymer, the availability of the functional groups, the amount of crystalline and amorphous regions within the sample, the structural complexity of the polymer, the types of bonds present within the polymer such as easily breakable ester bonds, the molecular composition of the sample and the form the polymer is in e.g. Films, pellets, powder etc. (Arutchelvi et al, 2007)

It is possible to degrade PLA by both enzymatic and non-enzymatic hydrolysis; the biodegradation rate is strongly influenced by the molecular weight and crystal structure of

the PLA (Di Lorenzo, 2005). MacDonald et al (1996) showed that the degradation rate decreases as crystallinity level increases.

Investigation into the processing techniques for PLA has been conducted by Lim, Auras and Rubino (2008). They investigated the structural composition, thermal properties, crystallisation behaviour, rheological properties, thermal degradation and processing of PLA. They reported that all the above are strongly dependent on the composition of the L and D enantiomers (chiral center molecules). PLA polymers with L content higher than 90% display a crystalline structure whereas those with lower than 90% display an amorphous structure. Also, as the percentage of L content decreases there is a corresponding decrease in the T_m and T_g of the polymer.

Lim, Auras and Rubino (2008) also reported on the thermal properties of PLA. PLA displays a relatively high T_g and T_m when semi-crystalline in comparison to other polymers. They stated that PLA T_g is increased in polymers with a higher composition of L-lactide than in a polymer with the same amount of D-lactide. These findings were also backed up by Tsuji and Ikada (1996). MacDonald et al (1996) also looked into how the stereo isomeric forms affect the degradation process. They found that degradation rates were dependent on the L-lactide content. They also found that Proteinase K has a high level of tolerance for D-lactide. Another observation from this study was that the degradation rate was significantly slower (43%) in amorphous films from L-D Lactide and L-meso lactide copolymers than in films with L-lactide and D-lactide copolymers thus showing that the polymer repeat unit distribution has a significant effect on degradation rates using proteinase K.

PLA can either be semi-crystalline or amorphous and this is determined using differential scanning calorimetry. Thermal history can be modified in PLA by the way in which it is treated, for example by quenching PLA in the melt phase, the polymer will be amorphous. Increased crystallinity in PLA can be induced by annealing at temperatures above the T_g but below the T_m , thus improving thermal stability. Crystallinity can also be induced by incorporating nucleating agents in the polymer.

Lim, Auras and Rubino (2008) also reported on the rheological properties. These properties are dependent on temperature, molecular weight and shear rate. They reported that semi-crystalline PLA has a higher shear viscosity than amorphous PLA. Kopinke et al (1996) proposed that above 200°C, PLA degrades via intra/inter-molecular ester exchange, cis-elimination, radical and concerted non-radical reactions. However, McNeill and Leiper (1985) proposed opposing views and suggested that PLA degradation occurs by non-radical ester interchange reactions. Lim, Auras and Rubino (2008) found that the product of the reaction is dependent on the location of the reaction on the backbone. The product can either be a lactide molecule, an oligomeric ring or acetaldehyde plus carbon monoxide. Similar findings were also reported by Kopinke et al (1996).

Jenkins and Harrison (2006) studied the effect of molecular weight on the crystallisation of another well-known polyester, polycaprolactone (PCL). They used differential scanning calorimetry (DSC) to study the degree of crystallinity of the PCL and then a modified Avrami equation to analyse the exotherms provided by the DSC. They found that as the degree of crystallinity and primary composites decreased, the molecular weight increased. The reason for these results can be explained by the reptation-nucleation theory i.e. long chains have longer reptation times and form loops that obstruct the reptation process which results in a

thickened lamella. This therefore reduces the end degree of crystallinity and therefore affects the biodegradation rate. Jenkins and Harrison (2008) followed their previous work with a study on the effect of crystalline morphology on the degradation of PCL in a solution of phosphate buffer and lipase. They combined DSC with electron microscopy to characterise the degradation of PCL. The results showed that increases in the molecular weight or the crystallinity reduced degradation rates. They found that up to around 10 hours, the degradation process displays rapid weight loss, this then plateaus. However, after 70 hours another rapid weight loss phase is visible. The first rapid weight loss is due to high enzyme concentration this then plateaus as the enzyme is consumed. The second rapid weight loss is due to bulk erosion of the sample. This has been backed up by Pitt et al (1981). Jenkins and Harrison (2008) proposed that the time for the start of the bulk erosion phase increases as the molecular weight increases. However, Gan et al's (1997) findings did not agree with these observations. The reason for these differing findings is most likely due to the difference in PCL processing and Gan et al produced PCL films by solvent casting. The conclusion of this study was that both high molecular weight and degree of crystallinity results in a decrease of the degradation rate. However, a better understanding of thermal histories of materials is required in order to provide better evidence for these findings, which is what this project intends to investigate.

Grizzi, Garreau, Li and Vert (1994) conducted a study comparing the degradation rates of different forms and sizes of PLA. PLA samples included 15 x 10 x 2 mm compression moulded plates, beads, microspheres and cast films. They found that larger size plates and beads degraded heterogeneously (i.e. The rate of degradation is greater inside the sample than on the surface) and faster than the smaller microspheres and films which degraded homogeneously (i.e. The same rate of degradation throughout the whole sample). This was

found to be the case in both in vitro and in vivo. The visual results found that the PLA initially displayed a transparent form. This gradually turned white on the surface with a yellowish core. After 11 weeks the outer layer became a thin shell (200 μm) with a viscous liquid core. This finding is similar to that found by Li, Garreau and Vert (1990). This viscous inner core was shown to disappear between weeks 11 and 13 leaving a hollow structure formed of a thin shell of degrading polymer. For plates, in terms of weight loss, there was no change in weight for the first 5 weeks. However, after 5 weeks the weight began to decrease and this loss became dramatic after 11 weeks. With films however, they rapidly lost 5-6% of their initial weight, but then no more weight loss was observed until 25 weeks. For water absorption in plates which was slight at the beginning, this then increased after 5 weeks but became dramatic after week 11. For films, the weight increased by about 50% in week 1, but was then constant up to week 30.

A study by Ali, Doherty and Williams (1993) investigated the polymer degradation of implantable devices using poly (DL - lactic acid). They used cylindrical PLA pellets and immersed them in $\text{Fe(II)/H}_2\text{O}_2$ and $\text{Co(II)/H}_2\text{O}_2$ solutions. These were stored in an incubator at 37°C for 30 weeks and then washed in distilled water and vacuum dried for a week. They used several techniques to study the degradation of the PLA including SEM, DSC and GPC. They found that OH^- radicals had an effect on the degradation of the PLA.

Vichaibun and Chulavatnatol (2003) conducted a study into the enzymatic degradation of polylactic acid. They degraded PLA hot pressed films and found that there was a linear relationship between fluorescence levels and enzyme concentration for Proteinase K, whereas at the same enzyme activity level the Proteinase K was most active aiding PLA degradation. They found Proteinase K to be a good catalyst and most effective for degrading PLA compared to other enzymes.

A study by Williams (1981) investigated PLA enzymatic degradation by various enzymes. For Proteinase K, he used a concentration of enzyme of 0.17 mg/ml at an incubation temperature of 37 °C for 2 weeks. He found that over the two week period, there was a weight loss of 66.9 %, although the PLA did not visibly change and the physical form remained similar. Upon comparison with various other enzymes including Pronase, Bromelain, Esterase, Ficin, Lactate dehydrogenase and Trypsin, Williams found Pronase, Proteinase K and Bromelain to have the most significant effects on the degradation process.

A study by Nam et al (2003) investigated the spherulite formation in PLA at varying crystallisation temperatures. They found that after isothermal crystallisation, as the crystallisation temperature increases, the size of the spherulites formed also increases. They found that at 140°C the spherulites formed were large, high ordered and ringed, whereas at 130°C and 120° the spherulites are smaller and less organised. They also found that introducing clay particles to the PLA causes smaller, less ordered spherulite formation at the same crystallisation temperatures. This finding was also supported by Park, S et al (2005). They found that in samples crystallised at 70°C, the spherulites formed had a smaller radius than the spherulites formed in the samples crystallised at 100°C. Yasuniwa et al (2007) studied the crystallisation behaviour of PLLA between 80°C and 160°C. They showed that the size of spherulites increased linearly with crystallisation temperature between 81°C and 126°C. They also found that between 96°C and 141°C as the temperature increased, the number of spherulites formed decreased.

Cam et al (1995) conducted a study investigating the degradation of high molecular weight poly (L-lactide) films under alkaline conditions. The study mainly focussed on the changes within the amorphous and crystalline areas and also the change in the density of the spherulites during degradation. They found that under alkaline conditions, the weight loss

of the samples occurred very quickly and linearly up until 100 days, after which the degradation rate then decreased. This was thought to be due to a two-phase microstructure of PLA. Degradation firstly occurs rapidly within the amorphous regions of the PLA, however as the amorphous areas become exhausted, degradation must then occur within the crystalline regions, which reduces the degradation rate significantly. They also found that under the alkaline conditions, weight loss occurred much faster in the samples with lower crystallinity than the samples with higher crystallinity levels. Another observation they made was that there was no water absorption found under the alkaline conditions, which differs from degradation in buffer solution at pH 7.4 when water is absorbed by samples. DSC results from the study showed an increase in crystallinity within the amorphous regions occurred during the degradation process. Cam et al also used light transmittance on the samples to check for variations in the transparency, they found that the transparency of the materials decreased much quicker for samples with lower crystallinity levels but found that after 80 days, all samples reached similar transparency levels. They attributed this decrease in transparency to an increase in spherulite formation within the amorphous areas of the sample. This study found mass loss to be much faster under alkaline conditions than buffered saline conditions, however they also found that the decrease in molecular weight was slower in alkaline conditions than buffered saline conditions. This can be attributed to the lack of auto-catalytic effect present in the alkaline conditions.

A study by Pantini et al (2007) looked into the FTIR analysis of hydrolysis in aliphatic polymers. The study included looking at the end group analysis by FTIR and the hydrolytic degradation of polymer films in an alkaline environment. The films included three aliphatic polyesters and one poly (ethylene dodecanedioate). The polymers all had around the same melting temperature and glass transition temperature and well as the same crystallinity but

all polymers had a different molecular weight. The degradation was conducted on polymer pellets compression moulded at 120°C to produce films (0.050-0.090mm). A 50 ml sodium hydroxide solution with a pH of 12.7 at 50°C was used. The solution was changed weekly to maintain concentration levels. After degradation, FTIR was conducted on the degraded films and a spectrum was obtained between 4000-400 cm^{-1} . A measurable increase in the absorbance of the infrared at 1570 cm^{-1} which isn't present in the dry non-degraded samples was attributed to the stretching of the carboxylate group. They also found that hydrolysis occurred linearly with time and that no significant weight loss (less than 10%) was observed in the films after degradation. A linear increase in acid end groups was also observed for all three polymers and they also all underwent bulk erosion.

A similar study was conducted by Vasantham et al (2009). They studied the effect of microstructure on the hydrolytic degradation of polylactic acid using FTIR and DSC. The material used in the study was PLLA that contained 6% D-lactide hot pressed at 200°C and then quenched in cold water to produce amorphous films (40-50 μm thick). The films were annealed at varying temperatures between 80°C and 120°C for 30 minutes. For the degradation process, 1 inch by 1 inch films were placed into 50 ml 0.1M NaOH solution at room temperature for 1, 2, 4, 6, 8 10 and 12 days. The films were then removed and washed with water and placed into a desiccator to dry. DSC was run on the films to determine the T_g , T_m and ΔH before and after degradation. 4-6 mg of film were placed in the DSC and held for 5 minutes at 25°C and then heated to 200°C at 10°C per minute. FTIR was also conducted between 4000-500 cm^{-1} for each annealed and hydrolysed sample. The results showed that both T_g and T_m increase with increasing annealing temperature. Double melting peaks were observed on the DSC traces and this was attributed to either recrystallisation of the unstable crystals during annealing or crystallisation of the amorphous region of the sample. However,

it could also be a combination of both these factors. The study found various differences in the FTIR spectra produced on amorphous and annealed samples. In the films annealed between the temperatures 100 and 120°C, differences in the spectra can be seen, at bands 697, 739, 921 and 1293 cm^{-1} . It was observed that the band absorbency increased with increasing annealing temperature whereas at 710, 757, 895, 956 and 1302 cm^{-1} band absorbency was seen to decrease with increasing annealing temperature. They attributed these differences in absorbency to the crystalline and amorphous phases of the PLA. Using the weights of the PLA films before and after degradation, the weight loss of the PLA was calculated. A film placed in standard distilled water for a week showed no sign of any weight change. Films with various degrees of crystallinity were also placed in distilled water for seven days and no change in the crystallinity of the samples was found, therefore showing that there was no crystallisation during the hydrolysis process. During degradation, it was seen that the weight loss increased as initial crystallinity increased. This conflicts with other reports of enzymatic degradation and was attributed to alkaline hydrolysis. The study also reported an increase in T_g after degradation but no change in both T_m and heat of fusion of the samples.

2.3 Aims and Objectives

From previous studies, it can clearly be seen that the degree of crystallinity and the molecular structure of Polylactic acid affects the physical and mechanical properties of the resulting polymer. So far, little work has been conducted investigating the changes in crystallisation temperatures, whilst maintaining the same degree of crystallinity and the effect this therefore has on the biodegradation process. This project will investigate two different parameters. Firstly, how controlling the microstructure of a PLA sample (3051D)

(i.e. Maintaining a single molecular weight but a controlled range of degree of crystallinity) will affect the degradation process. Secondly, how varying the lamella thickness (by altering the crystallisation temperature), whilst maintaining the same degree of crystallinity and molecular weight affects the degradation process. A better understanding of this biodegradation process would be useful in optimising the use of PLA as a biomaterial, by controlling the the rate of biodegradation by altering the microstructure being particularly beneficial.

3.0 Experimental Methods

3.1 Materials

PLA 3051D was supplied by Nature Works in pellet form. PLA 3051D is a co-polymer (poly(L-lactide-co-meso-lactide)) with a D-lactide content of around 3%. The glass transition temperature occurs at around 60°C and has a melting temperature of around 160°C.

The enzyme used is Proteinase K, also known as Protease K or Endopeptidase K, obtained from Sigma Aldrich Ltd. The enzyme works by causing cleavage of the peptide bond that is adjacent to the carboxyl group. Proteinase K has a molecular weight of around 28,900 Daltons and is functional across a wide range of pH (between 4 and 12), although the optimum pH is between 7.5 and 12. The proteinase K provided is a white lyophilised powder from tritirachium album and is soluble in water. The enzyme was used without further purification or preparation.

3.2 Sample Preparation

PLA 3051D pellets were placed into a Gallenkamp – OV 335 oven at 90°C for 24 hours in order to remove any moisture in the pellets. 35 mg of polymer pellets was pressed using a Moore Hydraulic Hot Press at 180°C with a 10 tonne pressure and held for 2 minutes to produce 155 x 180 x 1.2 mm sheets. The sheets were then quenched in order to ensure an amorphous structure and to erase any thermal history. Samples 4 x 4 x 1.2 mm squares were then cut from the sheets and were weighed to within +/- 0.2 of 5.6 mg.

3.3 Sample Conditioning

PLA samples were conditioned by isothermal crystallisation in a Gallenkamp OV 335 temperature controlled oven at five different temperatures: 90°C, 108°C, 118°C, 128°C and 140°C. These samples were held at each temperature for varying lengths of time from ten minutes up to nine hours. Three samples were conditioned at each time and temperature. Varying the temperatures and times allowed different degrees of crystallinity and microstructures to be developed for degradation testing.

3.4 Differential Scanning Calorimetry

3.4.1 DSC Theory

Differential scanning calorimetry is used to characterise thermal transitions in a sample of material. The DSC uses a sample cell and an empty aluminium reference cell and measures the heat flow between the two; this is recorded in milliwatts (mW). During heating, if the sample releases heat then an exothermic peak is visible on the DSC trace, if the sample takes in energy, an endothermic peak is visible on the DSC trace.

Differential scanning calorimetry can be used to measure the degree of crystallinity of a material. Firstly the DSC is calibrated using pure indium and tin, the polymer is heated then above the melting temperature of the material and the area under the melting peak is calculated from the initial onset of the curve to the last trace of crystallinity. This is known as the heat of fusion of the sample. Dividing the heat of fusion of a sample with the value for the heat of fusion of the fully crystalline material provides the degree of crystallinity of the sample (Equation 2) (Henton, 2005).

$$\text{Crystallinity (\%)} = \frac{\Delta H_m - \Delta H_c}{93.1} \times 100 \quad \text{Equation 2}$$

Where ΔH_m refers to the area under the endothermic peak, ΔH_c refers to the area under the exothermic peak and 93.1 J/g refers to the heat of fusion of a 100% crystalline sample.

3.4.2 Thermal Analysis

After isothermal crystallisation the samples underwent thermal analysis in the differential scanning calorimeter in order to determine the crystallinity levels of the samples. The samples were heated from 25°C through the melt to 180°C at a rate of 40°C per minute. An empty aluminium pan was used as a reference cell. The curves obtained were then used to establish the degree of crystallinity of each sample by using Equation 2 to work out the crystallinity percentage of the sample. The heat of fusion for 100% crystalline poly-lactic acid is assumed to be 93.1 J/g (Henton et al, 2005). The melting point and glass transition temperature were also recorded from the DSC curves for further degradation analysis.

The results obtained from the thermal analysis, established the time taken for PLA to reach 40% crystallinity at each temperature from 90 – 140 °C. Using these results, four samples were then crystallised to 40% at 90 °C, 108 °C and 118 °C to subject to the degradation process.

3.5 Hot Stage Microscopy

Thin samples (10 μ m) were taken from the amorphous sheets and then subjected to hot stage microscopy to determine the diameter of the spherulites at 40% crystallinity for each temperature. Previous thermal analysis results were used to establish the length of time at each temperature the sheets were to be held at to produce 40% crystalline samples.

3.6 Sample Biodegradation

3.6.1. Preliminary Biodegradation

Preliminary degradation was conducted on PLA 3051D pellets as received from NatureWorks. In the first preliminary degradation experiment PLA in the as received pellet form was subjected to a degradation process. Sample 1 (4 PLA pellets) was immersed in 10 ml Phosphate Buffered Saline (PBS) solution containing 0.5 mg of Proteinase K, Sample 2 (4 PLA pellets) was immersed in 10 ml of PBS solution without any enzyme present and Sample 3 (1 PLA pellet) was immersed in 10 ml PBS solution containing 0.5 mg of Proteinase K. These were placed in a dry seal desiccator within a water bath maintained at 37 °C and the desiccator was sealed using high vacuum grease. The samples were weighed after 24 hours, 48 hours, 72 hours, 96 hours and 169 hours. Prior to weighing, the samples were removed from the water bath and placed in a desiccator to dry.

Table 1. Original weights of the samples used in the first degradation experiment. Sample 1 and 2 represent the combined weights of 4 pellets and sample 3 the weight of a single pellet.

Sample	Weight \pm 0.02 (mg)
1	167.61
2	167.41
3	39.95

In the second degradation experiment PLA pellets were subjected to the degradation process. Sample 4 (1 PLA pellet) was immersed in 2.5 ml PBS solution containing 0.5 mg of Proteinase K and Sample 5 (1 PLA pellet) was immersed in 10 ml of PBS solution containing 0.5 mg of Proteinase K. The solution was changed every 24 hours to ensure the enzyme level was maintained. These were placed in a dry seal desiccator within a water bath maintained at 37 °C and the desiccator was sealed using high vacuum grease. The samples were weighed after 24 hours, 48 hours, 72 hours and 96 hours. Prior to weighing the samples were removed from the water bath and placed into a desiccator to dry.

Table 2. Original weights of samples used in degradation trial 2.

Sample	Weight \pm 0.02 (mg)
4	41.67
5	35.67

For the third degradation experiment PLA hot pressed films weighing 31mg (+/- 3 mg) were conditioned in the oven at 118 °C to 5% and 40% crystallinity. The crystallinity levels were determined by testing 6 mg (+/- 0.5 mg) of the conditioned sample in the DSC using the previously described method. These samples were then subjected to degradation using 0.5 mg of proteinase K enzyme in 10 ml PBS solution at 37 °C for 5 days. The solution was refreshed daily in order to maintain the enzyme activity throughout. After degradation, the samples were then removed from the water bath and placed into a desiccator to dry for 36 hours. Thermal analysis was then completed on 6mg (+/- 0.5) samples after biodegradation using the previously described DSC method.

3.6.2 Five Day Biodegradation

Five day biodegradation studies were conducted on PLA 3051D films with dimensions of 5 mm x 5 mm x 1 mm weighing 5.4 mg (+/- 0.2 mg). Sixteen samples were submitted to the degradation process; samples 8-11 (see table 3 for degradation conditions). The samples were placed in a 10 ml solution of phosphate buffered saline (pH 7.4) and 1.13 mg of Proteinase K enzyme and held in a water bath at 37 °C for five days. The sample solution was refreshed daily in order to maintain the enzyme activity. After five days the samples were removed from the water bath and placed into a desiccator to dry. The samples were then weighed daily until a constant weight was attained to ensure all water was removed from the samples.

3.6.3 Ten Week Biodegradation

Ten week biodegradation studies were conducted on PLA 3051D films with dimension of 5 mm x 5 mm x 1 mm weighing 5.6 mg (+/- 0.2 mg). Four samples were submitted to the ten week biodegradation procedure; samples 12-13 (see table 3 for degradation conditions). The samples were placed in a 10 ml solution of phosphate buffered saline (pH 7.4) and 1.13 mg of Proteinase K enzyme and held in a water bath at 37 °C for ten weeks. The sample solution was refreshed weekly in order to maintain the enzyme concentration. After ten weeks the samples were removed from the water bath and placed into a desiccator to dry. The samples were then weighed daily until a constant weight was attained to ensure all water was removed from the samples.

Table 3. Degradation conditions and reference codes for each sample.

Degradation Condition	Sample Reference Code
Preliminary degradation	
<i>As received degraded in the following solution for a week and the solution was not refreshed throughout</i>	
1. 10ml PBS solution with 0.5mg Proteinase K (4)*	1
2. 10ml PBS solution without proteinase K (4)*	2
3. 10ml PBS solution with 0.5mg proteinase K (1)*	3
<i>As received degraded in the following solution for a week and the solution was refreshed every 24 hours</i>	
4. 2.5ml PBS solution with 0.5mg Proteinase K (1)*	4
5. 10ml PBS solution with 0.5mg Proteinase K (1)*	5
<i>As received, hot pressed into an amorphous sheet, oven conditioned at 118°C to varying degrees of crystallinity</i>	
6. 5% crystallinity, degraded in 10ml PBS solution with 0.5mg Proteinase K (2)*	6
7. 40% crystallinity, degraded in 10ml PBS solution with 0.5mg Proteinase K (2)*	7
Five day degradation	
<i>As received, hot pressed into an amorphous sheet, oven conditioned at varying temperatures (each sample weighed 5.6mg +/- 0.2). Degraded in the following solution for five days and the solution was refreshed every 24 hours</i>	
8. Amorphous samples (4)*, degraded in 10 ml PBS solution with 1.13mg proteinase K	8
9. Crystallised to 40% at 90°C (4)*, degraded in 10 ml PBS solution with 1.13mg proteinase K	9
10. Crystallised to 40% at 108°C (4)*, degraded in 10 ml PBS solution with 1.13mg proteinase K	10
11. Crystallised to 40% at 118°C (4)*, degraded in 10 ml PBS solution with 1.13mg proteinase K	11
Ten week biodegradation	
<i>As received, hot pressed into an amorphous sheet, oven conditioned at varying temperatures (each sample weighed 5.6mg +/- 0.2). Degraded in the following solution for ten weeks and the solution was refreshed weekly</i>	
12. Crystallised to 40% at 90°C (2)*, degraded in 10 ml PSB solution with 1.13mg proteinase K	12
13. Crystallised to 40% at 118°C (2)*, degraded in 10 ml PSB solution with 1.13mg proteinase K	13

* The number in brackets indicates how many samples were used per condition; individual samples are denoted as a, b, c, d etc. For example when referring to the third sample in condition 1 the code 1c will be used.

3.7 FTIR

Fourier Transform Infrared Spectroscopy (FT-IR) was conducted using a Nicolet 8700 FT-IR spectrometer. The attenuated total reflection technique was used and a 'Golden Gate' (Specac) accessory was recorded the spectra. The 'Golden Gate' diamond ATR accessory is a versatile sampling system that provides high quality spectra data for a wide range of different materials. In order to produce a high quality spectra the sample must have good contact with the diamond. The infrared beam passes diamond and reflects off the sample surface forming an evanescent wave which then enters the sample. The beam is collected by a detector as it exits the sample and the spectrum is recorded.

Initially, the background spectrum was recorded to eliminate this from the sample results.

Spectra were obtained for samples before and after being submitted to the degradation process on samples crystallised to 40% crystallinity at 90 °C, 108 °C and 118 °C. The FT-IR spectra were obtained for the wavelengths ranging from 4000-600 cm^{-1} at 2cm^{-1} resolution and 200 scans.

4.0 Results and Discussion

4.1 PLA

A DSC scan of the hot pressed PLA quenched to produce a 155 x 180 x 1.2 mm plaque is shown in Figure 5. The graph shows that the hot pressed PLA has a T_g of around 60 °C but there is no endothermic melting peak indicating that the hot pressed sheets are amorphous.

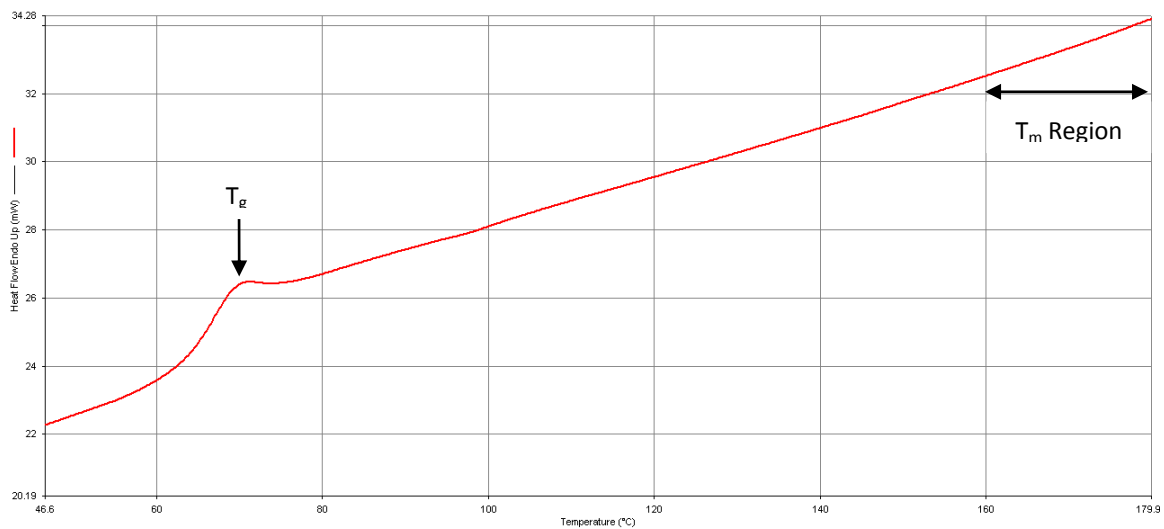


Figure 5. DSC scan of the hot pressed amorphous PLA plaque.

4.2 The Effect of Crystallisation Time and Temperature on PLA

Samples that are exposed to varying crystallisation times and temperatures are shown to display a range of different levels of crystallinity (Figures 6-10). The graphs show that as the time at which the samples were held increases, the melting temperature increases indicating an increase in the degree of crystallinity of the samples.

Figure 6A shows the endotherms produced during DSC analysis on samples that were isothermally crystallised for a range of times between 10 minutes and 180 minutes at 108 °C. It can be clearly seen that as the hold time increases, so does the melting endotherm and therefore the degree of crystallinity of the PLA. This trend is also seen in the other samples crystallized at 90 °C, 118 °C, 128 °C and 140 °C as illustrated in Figures 7A, 8A, 9A and 10A, respectively.

Figure 6B shows the average degree of crystallinity and ΔH_f values obtained against crystallisation times at 108 °C. It can be seen that as crystallisation time increases there is an increase in the degree of crystallinity and ΔH_f of the samples. This trend is also seen in the other samples crystallised at 90 °C, 118 °C, 128 °C and 140 °C as illustrated in figures 7B – 10B respectively.

DSC analysis at temperatures 90 °C (Figure 7A) shows the melting endotherm to display double melting peaks as it is heated in the DSC to the melt at 40 °C per minute. This can be attributed to the melt re-crystallisation mechanism where small, imperfect crystals (first peak) gradually become more stable and perfectly formed (second peak). This is in good agreement with the findings reported by He et al (2007), Yasuniwa et al (2002) and Di Lorenzo (2005). He et al (2007) found that double melting peaks were observed at 105 °C, 110 °C and 115 °C, whereas single melting peaks were observed at 120 °C and 125 °C.

The double melting peaks are visible best at the heat treatment conducted at 90 °C (Figure 7A). When a heating rate of 10 °C per minute is used (figure 11), a clear double melting peak is visible at each crystallisation time. This result suggests that at 90 °C, the thermal stability of the crystals is not affected by the crystallisation time used. However, the endothermic peaks produced at a heating rate of 40 °C per minute show slightly different results. The

double melting peaks observed at a heating rate of 40 °C per minute are less clearly defined than at 10 °C per minute. This could be attributed to an increase in thermal lag at the higher crystallisation rate and the rate being too fast to allow melting and re-crystallisation of the less stable crystals.

At 108 °C the double melting peaks are only observed in the samples held at the crystallisation temperature for 10 minutes. The double melting peaks observed in the samples crystallised at 108 °C are also not as prominent as those seen in the samples crystallised at 90 °C. For the samples held at 108 °C for 20 -180 minutes a single melting peak is observed. He et al (2007) attributed this transition to a single melting peak to an increase in the thermal stability of the crystals resulting in a reduction in the melt re-crystallisation mechanism.

The DSC scans at 118 °C, 128 °C and 140 °C (Figures 8A, 9A, 10A) all display single melting peaks which can also be attributed to an increase in the lamellar thickness and thermal stability of the crystals at higher temperatures preventing the melt re-crystallisation mechanism from occurring.

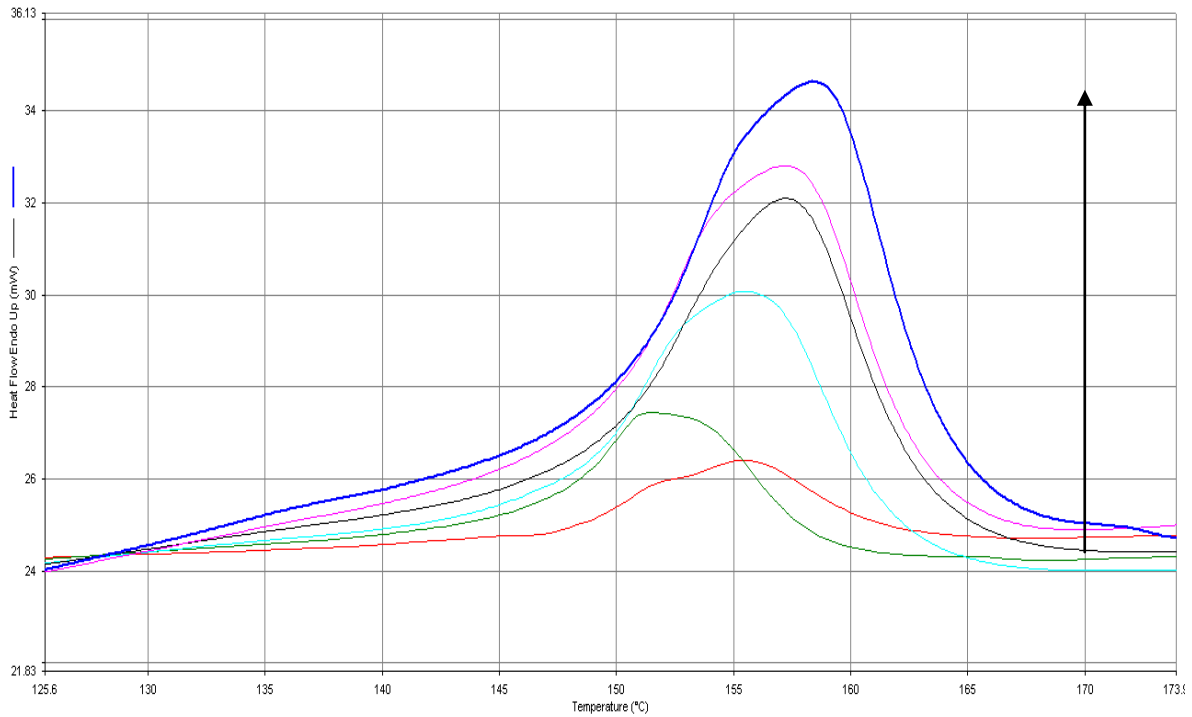


Figure 6A. DSC traces showing the melting endotherms for samples crystallised at 108 °C for various lengths of time between 10 mins and 180 mins. The arrow indicates the increase in melting endotherm by increased crystallisation time.

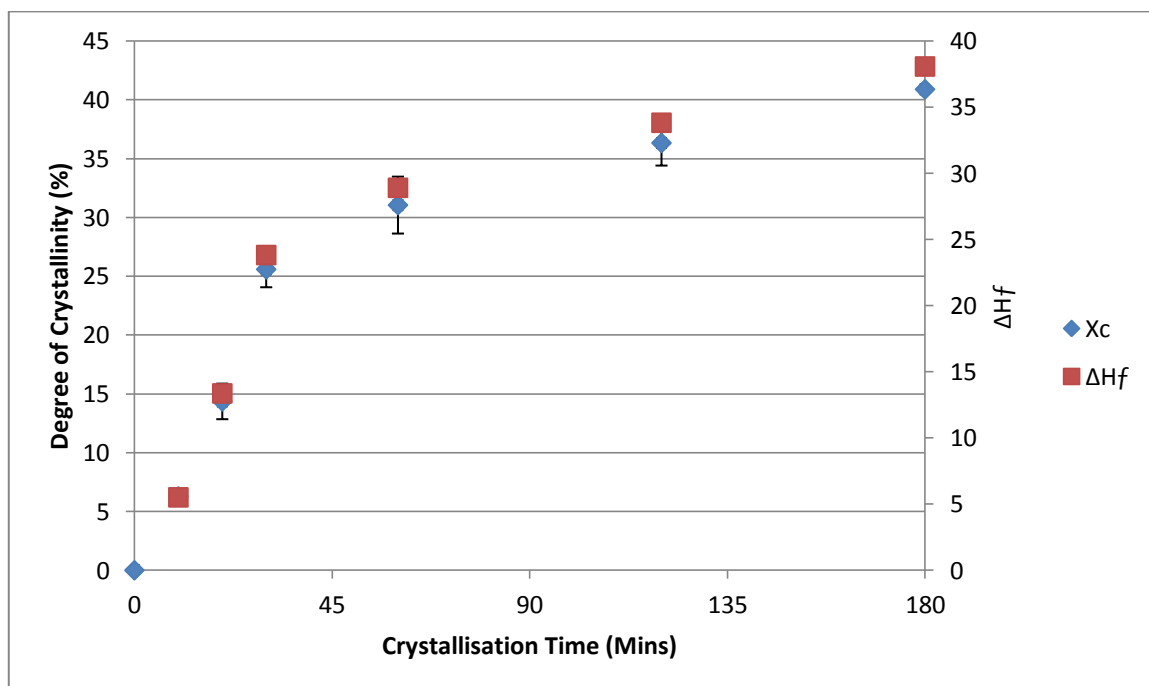


Figure 6B. Degree of crystallinity and ΔH_f against crystallisation time at 108 °C. Error bars display the standard deviation between the samples.

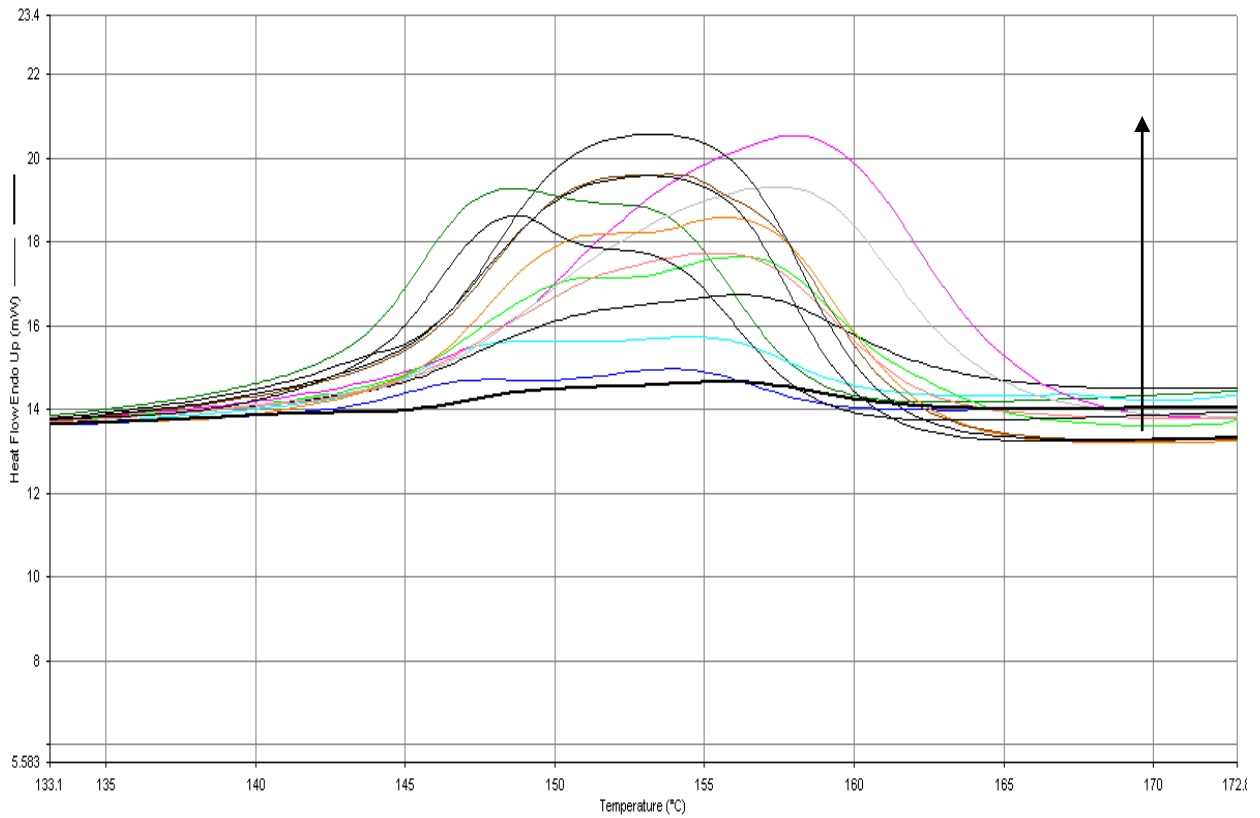


Figure 7A. DSC traces showing the melting endotherms for samples crystallised at 90 °C for various lengths of time between 10 mins and 180 mins. The arrow indicates the increase in melting endotherm by increased crystallisation time.

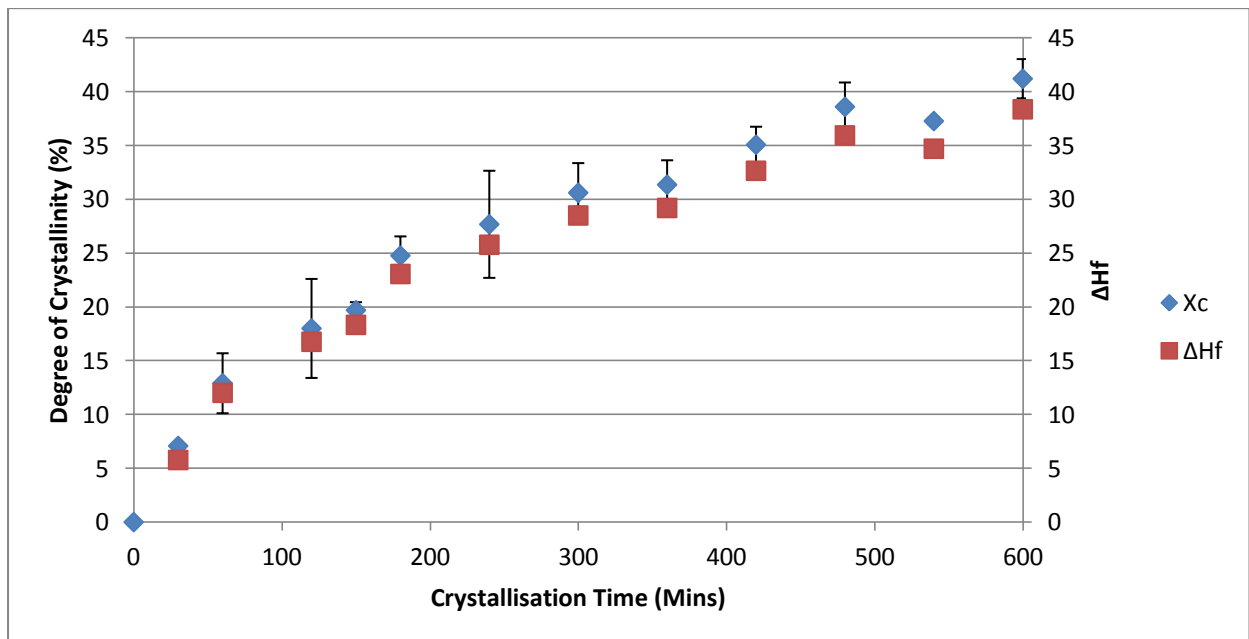


Figure 7B. Degree of crystallinity and ΔH_f against crystallisation time at 90 °C. Error bars display the standard deviation between the samples.

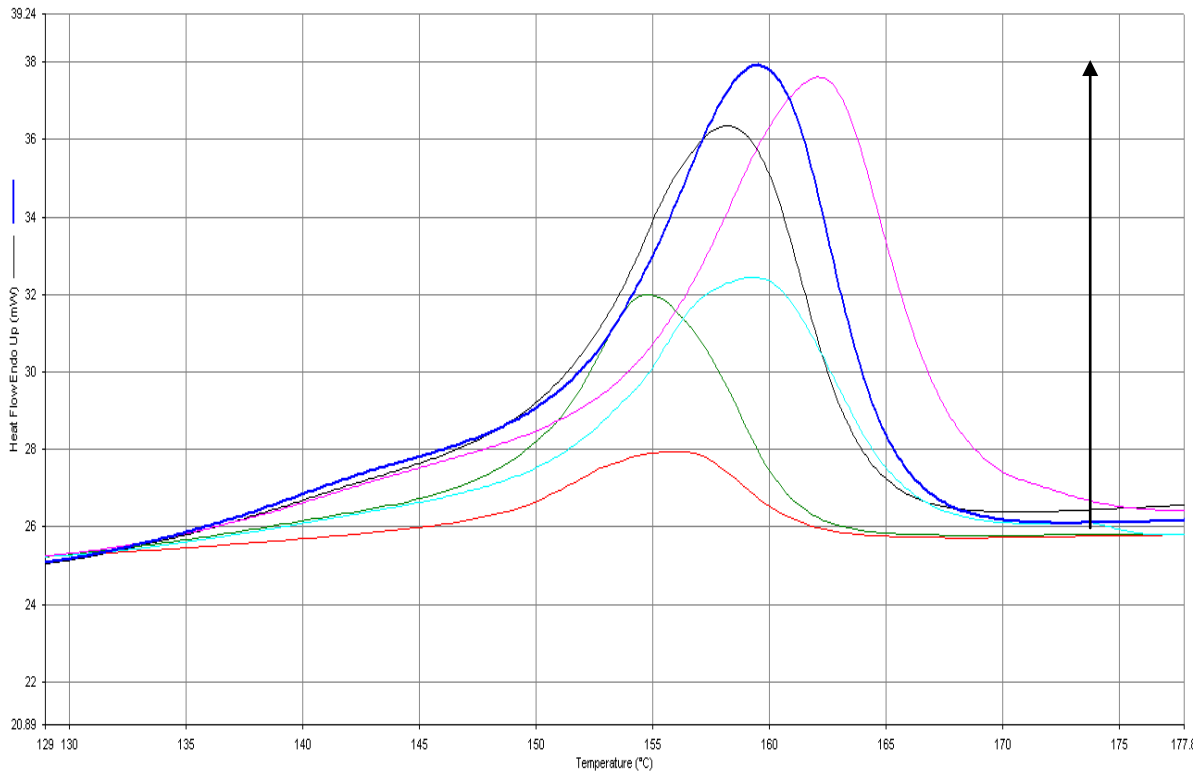


Figure 8A. DSC traces showing the melting endotherms for samples crystallised at 118 °C for various lengths of time between 10 mins and 180 mins. The arrow indicates the increase in melting endotherm by increased crystallisation time.

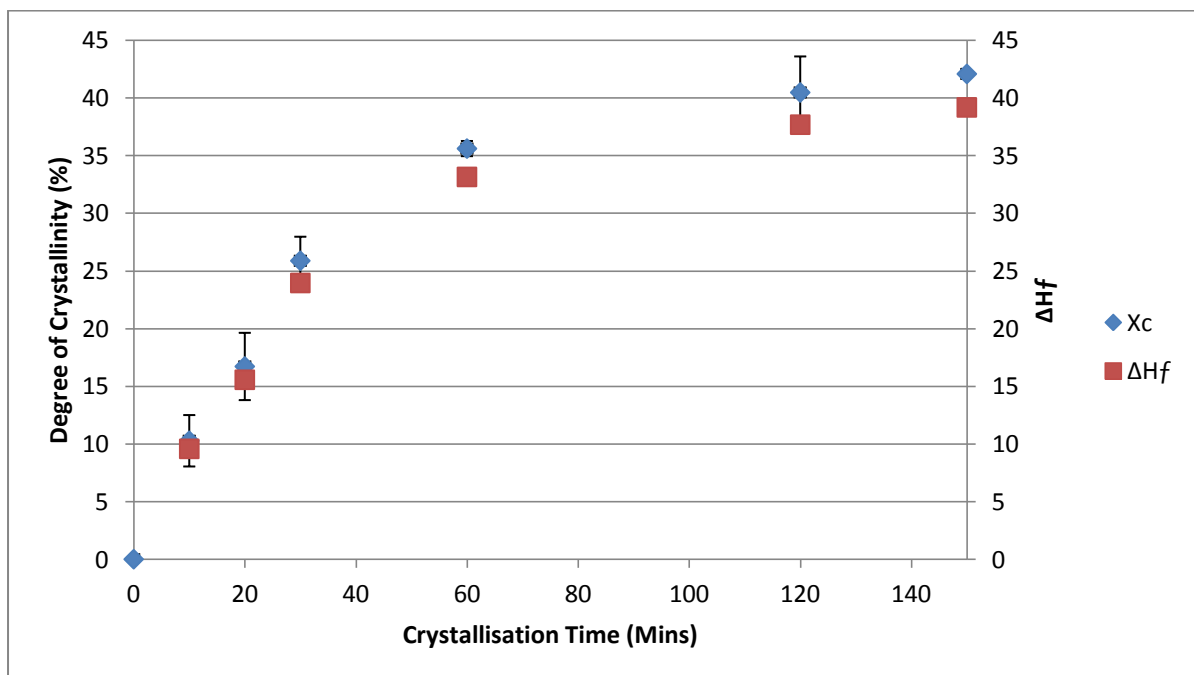


Figure 8B. Degree of crystallinity ΔH_f against crystallisation time at 118 °C. Error bars display the standard deviation between the samples.

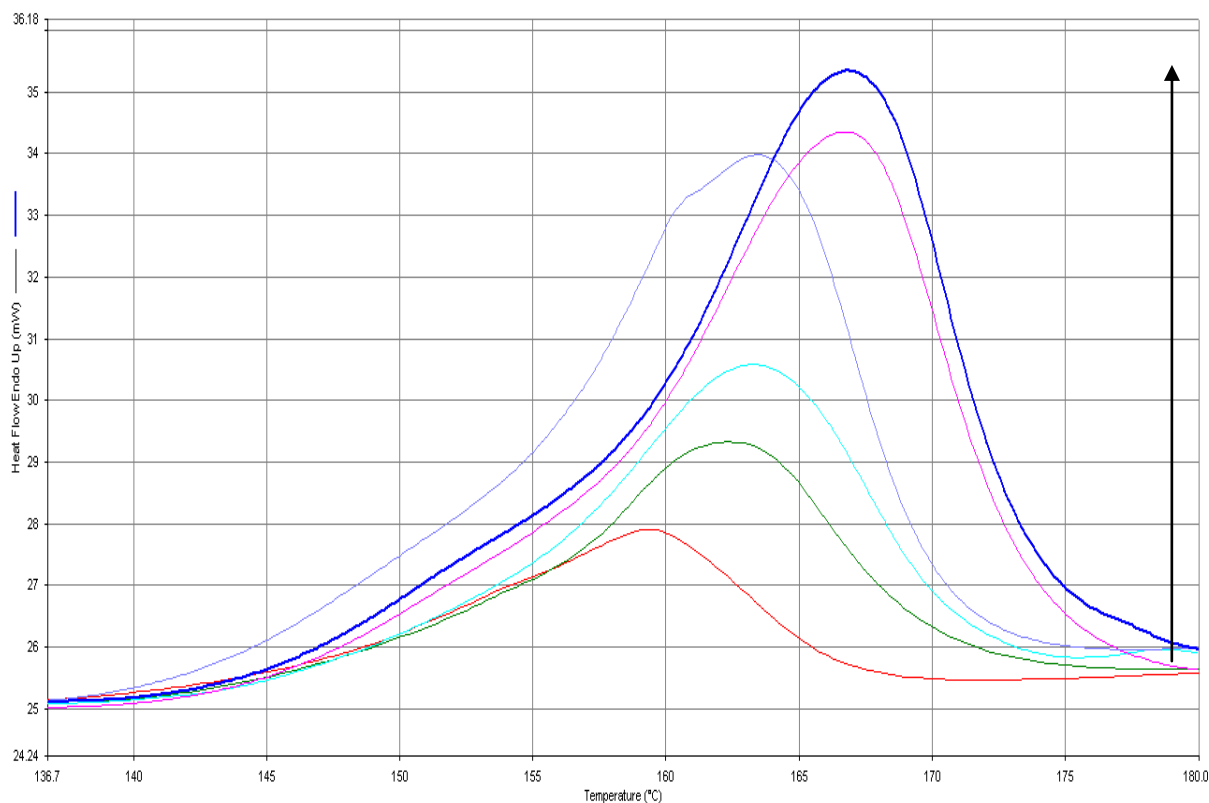


Figure 9A. DSC traces showing the melting endotherms for samples crystallised at 128 °C for various lengths of time between 10 mins and 180 mins. The arrow indicates the increase in melting endotherm by increased crystallisation time.

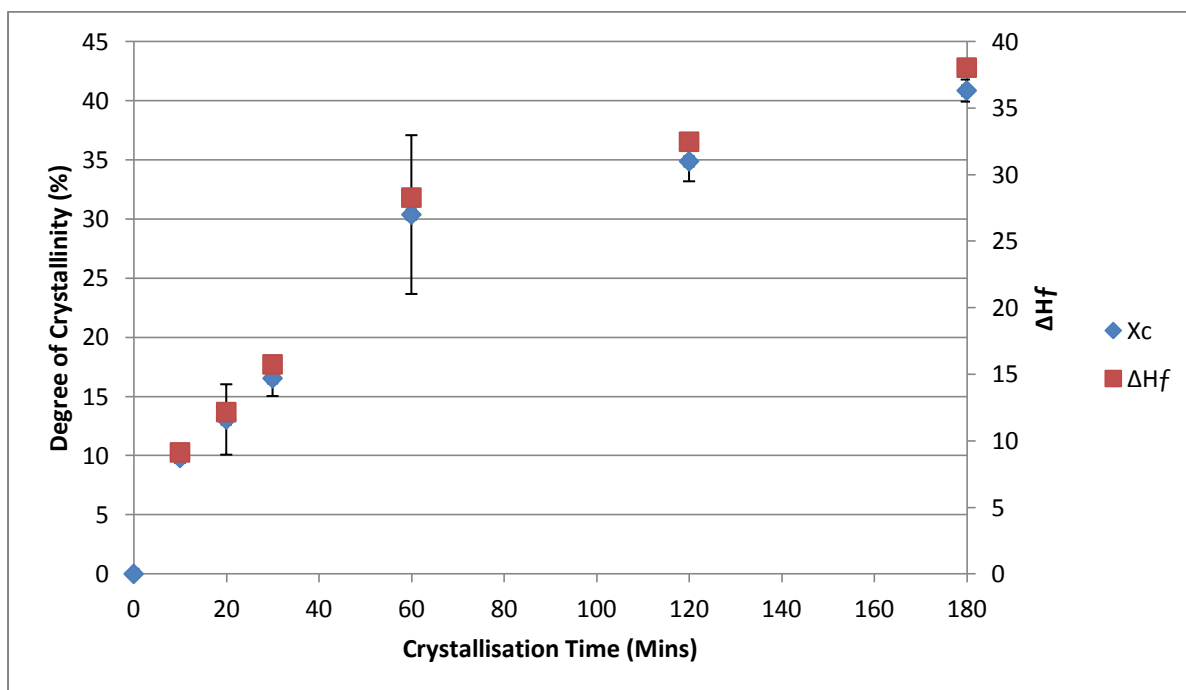


Figure 9B. Degree of crystallinity and ΔH_f against crystallisation time at 128 °C. Error bars display the standard deviation between the samples.

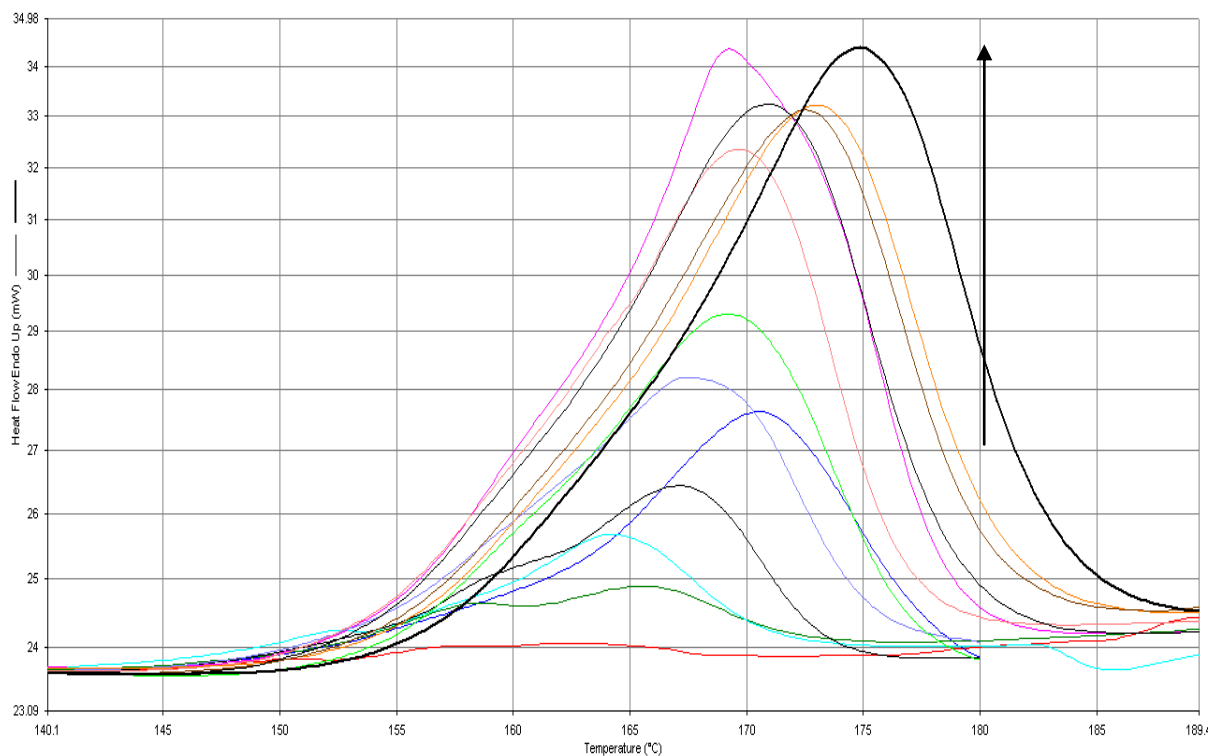


Figure 10A. DSC traces showing the melting endotherms for samples crystallised at 140 °C for various lengths of time between 10 mins and 180 mins. The arrow indicates the increase in melting endotherm by increased crystallisation time.

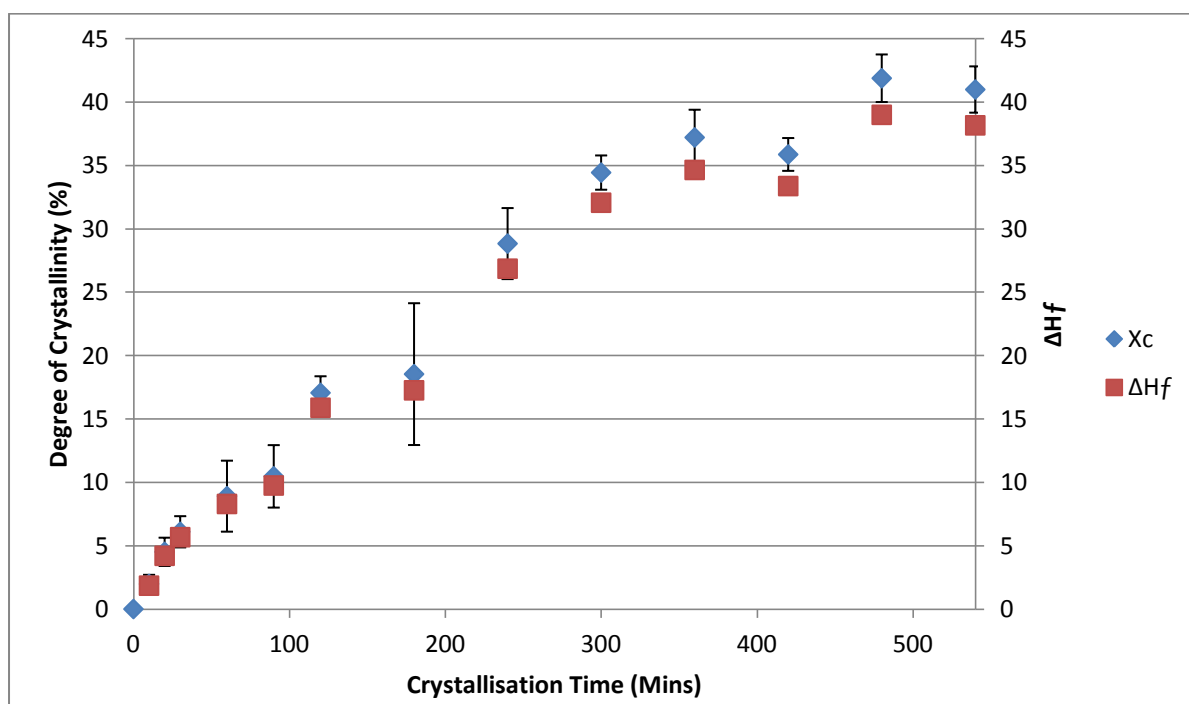


Figure 10B. Degree of crystallinity and ΔH_f against crystallisation time at 140 °C. Error bars display the standard deviation between the samples.

DSC analysis at temperatures 90 °C (figure 11) show the melting endotherm to display double melting peaks as it is heated in the DSC to the melt at 40 °C per minute. This can be attributed to the melt re-crystallisation mechanism where small, imperfect crystals (first peak) gradually become more stable and perfectly formed (second peak). This finding was also reported by He et al (2007), Yasuniwa et al (2002) and Di Lorenzo (2005). The study by He et al (2007) provides similar results found in this work. They found that double melting peaks were observed at 105 °C, 110 °C and 115 °C, whereas single melting peaks were observed at 120 °C and 125 °C.

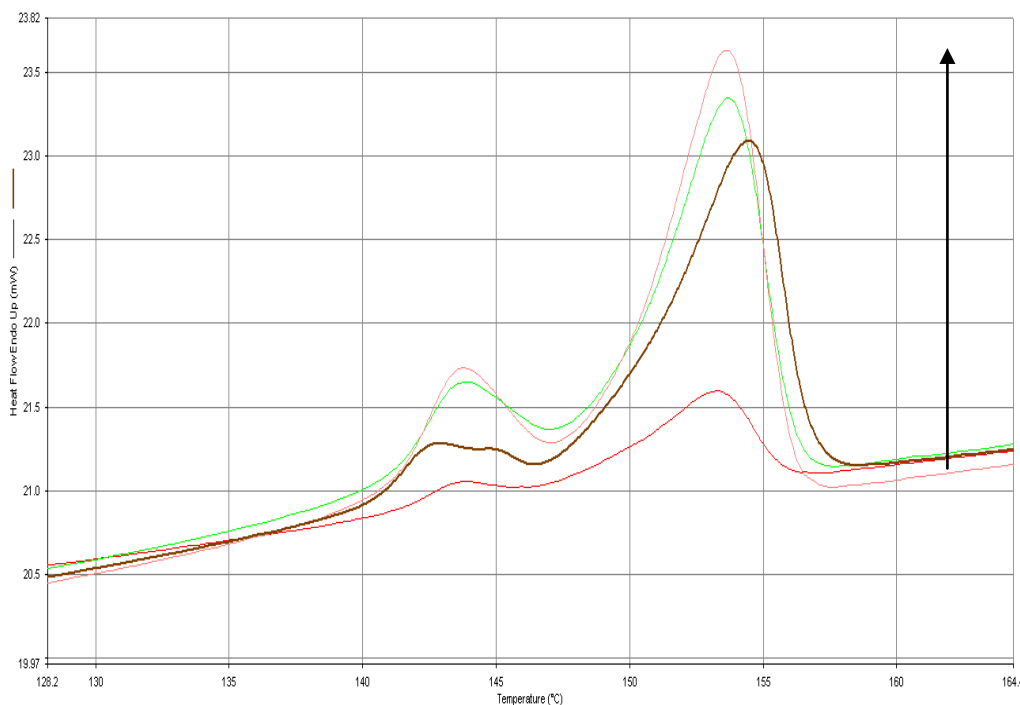


Figure 11. DSC traces displaying the double melting peaks obtained on crystallisation at 90 °C for various times between 45 mins and 420 mins at a rate of 10 °C per minute. The arrow indicates the increase in melting endotherm with increased crystallisation time.

Figure 12 shows the crystallisation times taken to reach 40% crystallinity, after this point there is very little increase in the degree of crystallinity and so it is thought that this is the maximum level achievable in this grade of PLA.

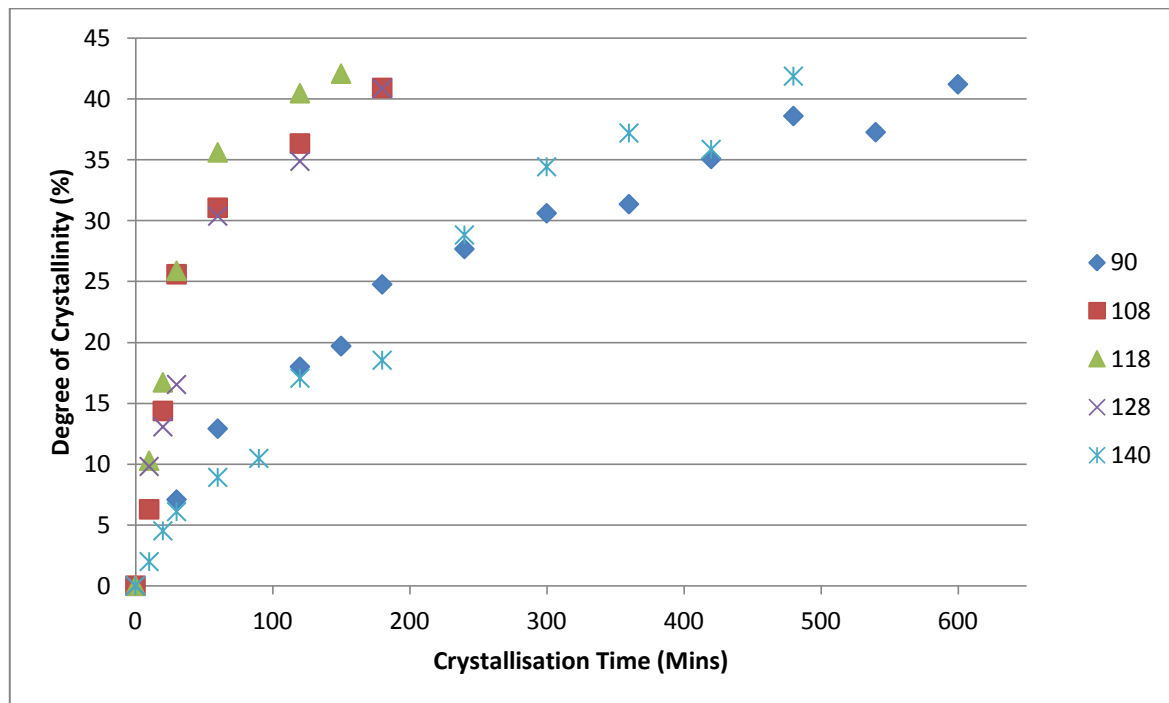


Figure 12. Degree of crystallinity against crystallisation time between 90 and 140 °C

It can be seen that crystallisation to 40% occurs fastest at 118 °C taking just 150 minutes. At temperatures below and above 118 °C, the time taken to reach 40% crystallinity increases with 90 °C and 140 °C taking the longest at 480 and 600 minutes respectively.

Figure 13 shows the direct relationship between crystallisation temperature and the amount of time taken to reach 40% crystallinity. These differences in crystallisation rates can be attributed to the differences in spherulite formation within the polymer. At lower temperatures, nucleation rate is high resulting in a large number of spherulites present

whereas at higher temperatures the nucleation rate is lower but the growth rate is increased resulting in fewer but larger spherulite formation. These differences in spherulites formation affect the crystallisation rates with 118 °C being the optimum temperature, resulting in the fastest crystallisation rate. Di Lorenzo (2005) supported these results and found a very high crystallisation rate in PLA between 110 and 118 °C and this was attributed to a sudden acceleration in spherulite growth as a result of fewer nucleation sites, but not necessarily as a direct result of the difference in spherulite appearance. Di Lorenzo (2005) also reported that the fastest rate of crystallisation occurs at 118 °C and above and below this point crystallisation rate decreases. This therefore supports the results found in this work.

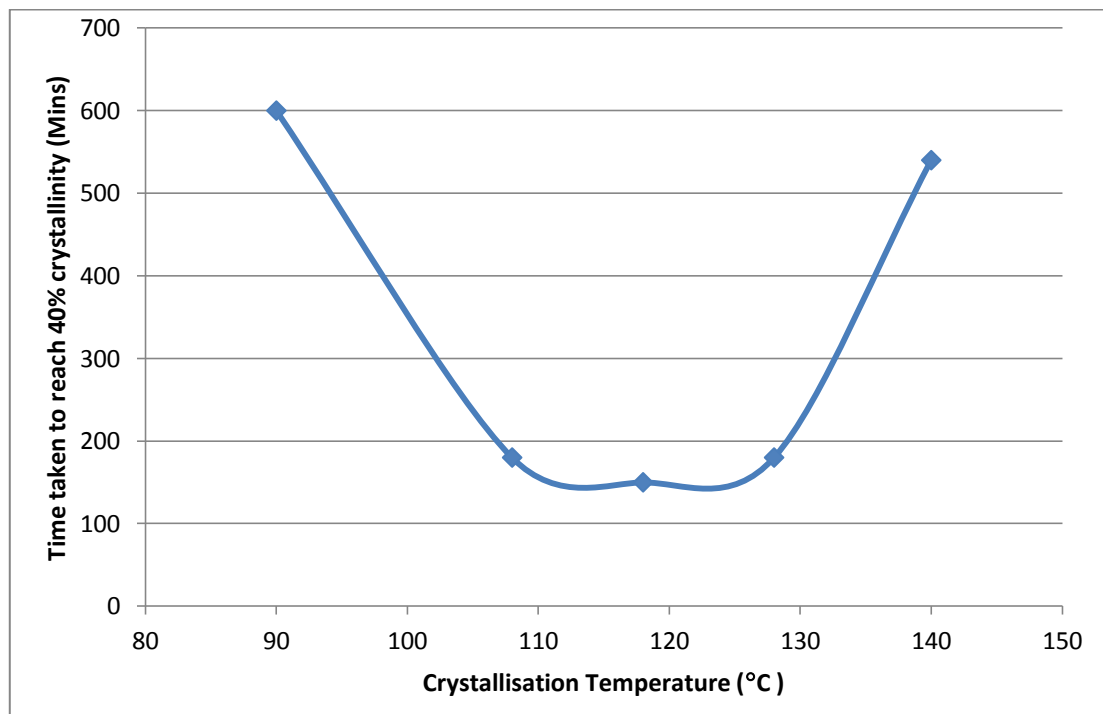


Figure 13. Time taken to reach 40% crystallinity against crystallisation temperature.

4.3 The Effect of Crystallisation Temperature on Spherulite Formation

Figure 14 shows images taken using hot stage microscopy of samples crystallised to 40% crystallinity between 90 °C and 140 °C. A clear increase in spherulite size and decrease in the number of spherulites can be seen as the crystallisation temperature increases.

Spherulites are structured and ordered lamellae formed during crystallisation from the melt of the polymer and represent the semi-crystalline regions of the polymers. The spherulite formation is affected by various factors such as the number of nucleation sites available, the polymer structure and the crystallisation temperature used. Amorphous regions can be seen between the spherulites, this being more obvious at the higher crystallisation temperatures, 118-140 °C (Figures 14C – 14E).

Spherulite size is greatly influenced by the crystallisation temperature. After nucleation, spherulites will continue to grow linearly with time until they begin to touch each other after a small amount of growth. At lower temperatures more nucleation sites are available resulting in a large number of spherulites being formed. Due to the large number of spherulites being formed, this results in growth being inhibited. However, at higher temperatures there is a reduction in the nucleation sites available, this increase in space favours growth meaning that larger spherulites are formed. A study by Yasuniwa et al (2007), supports these findings. They found that at lower temperatures (80-126 °C) spherulite nucleation increased linearly with time and at higher temperatures (96-141 °C) spherulite nucleation decreased but growth increased.

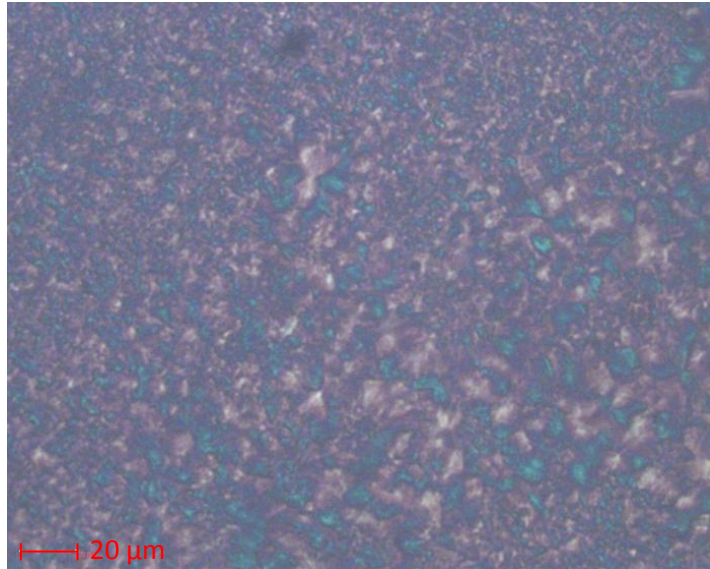


Figure 14A. Spherulite formation in a PLA sample crystallised at 90 °C for 600 minutes to reach 40% crystallinity.

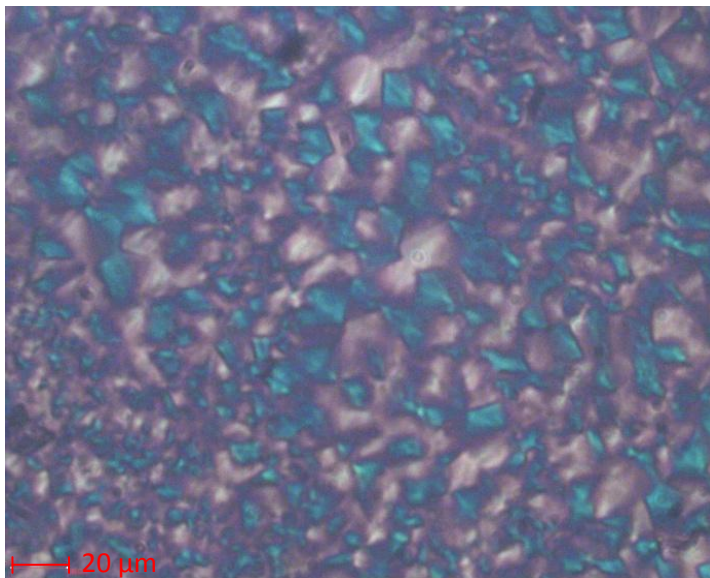


Figure 14B. Spherulite formation in a PLA sample crystallised at 108 °C for 180 minutes to reach 40% crystallinity.

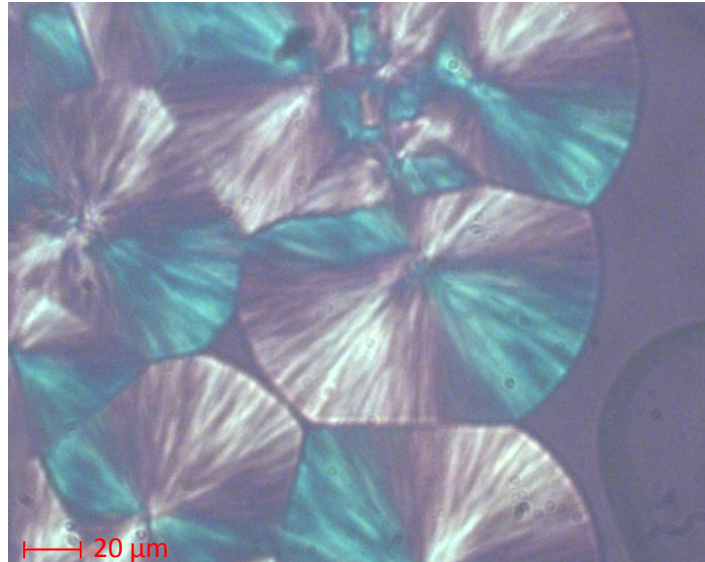


Figure 14C. Spherulite formation in a PLA sample crystallised at 118 °C for 150 minutes to reach 40% crystallinity.

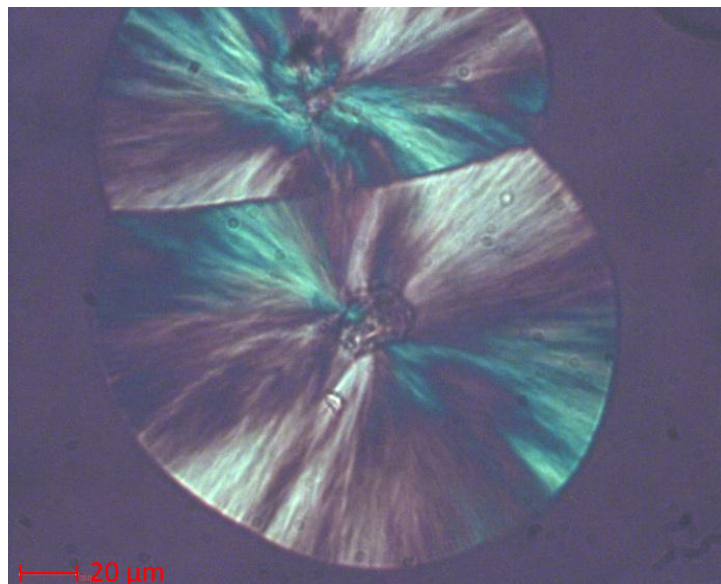


Figure 14D. Spherulite formation in a PLA sample crystallised at 128 °C for 180 minutes to reach 40% crystallinity.

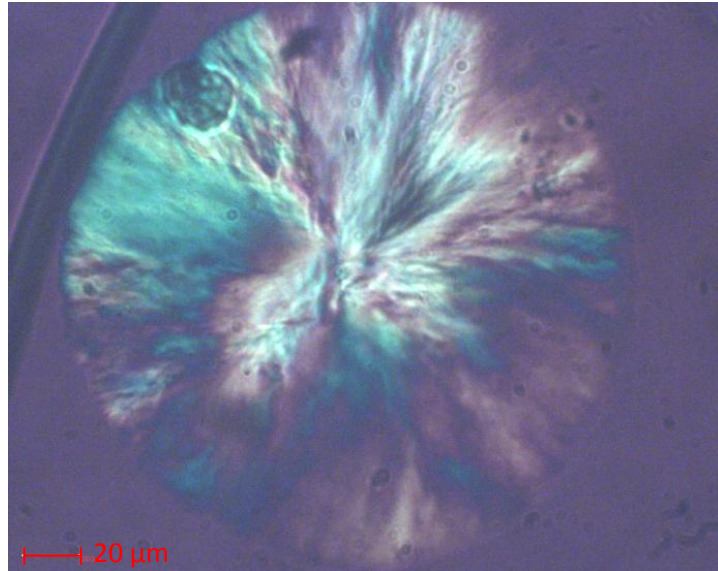


Figure 14E. Spherulite formation in a PLA crystallised at 140 °C for 480 minutes to reach 40% crystallinity.

4.4 The Effect of Crystallisation Temperature on Peak Melting Point

The melting peak of each sample was obtained from the DSC traces; these can be seen in Figure 15. In Figure 15, the relationship between the crystallisation time and peak melting temperature can be clearly observed. It can be seen that generally, as crystallisation time increases, there is an increase in the melting peak observed. Figure 15 also shows that an increase in crystallisation temperature results in an increase in the melting temperature.

Figure 16 displays the relationship between peak melting temperature on samples crystallised to 40% and crystallisation temperature. It can be seen that the average peak melting temperature increases as crystallisation temperature increases. Farrow (2003) reported similar findings in polypropylene and attributed the increase in the melting temperature to an increase in the perfect formation and size of crystallites as crystallisation temperature increases. An increase in spherulite size with increasing crystallisation temperature can be seen in Figure 14.

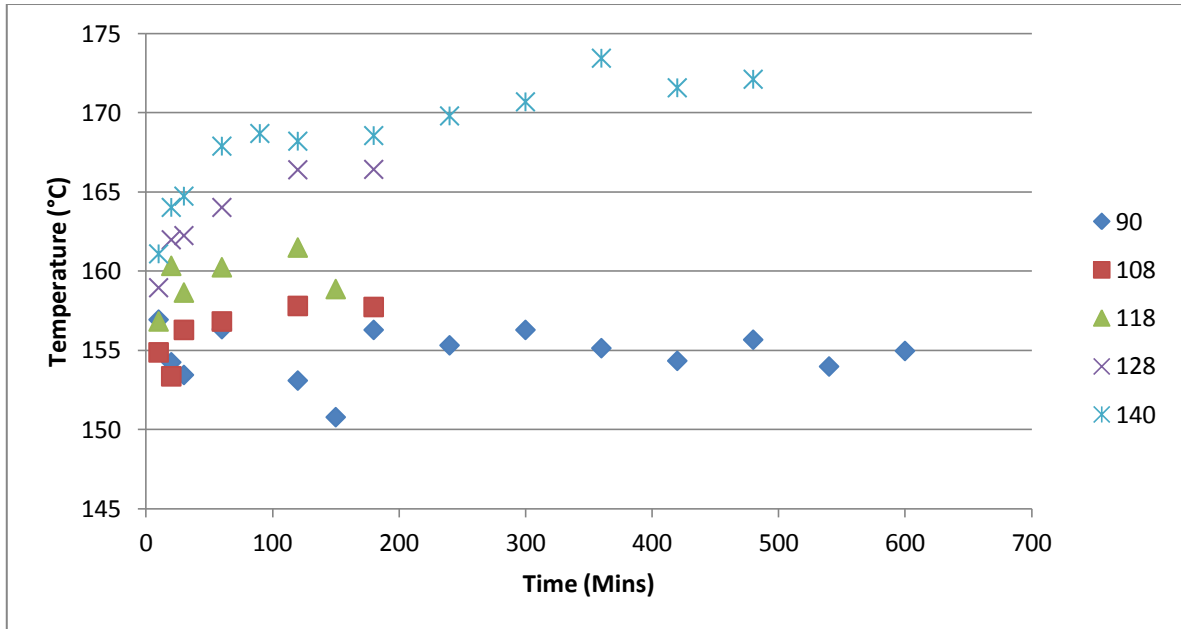


Figure 15. Average peak melting temperatures between 10 and 600 minutes of heat treatment at each crystallisation temperature.

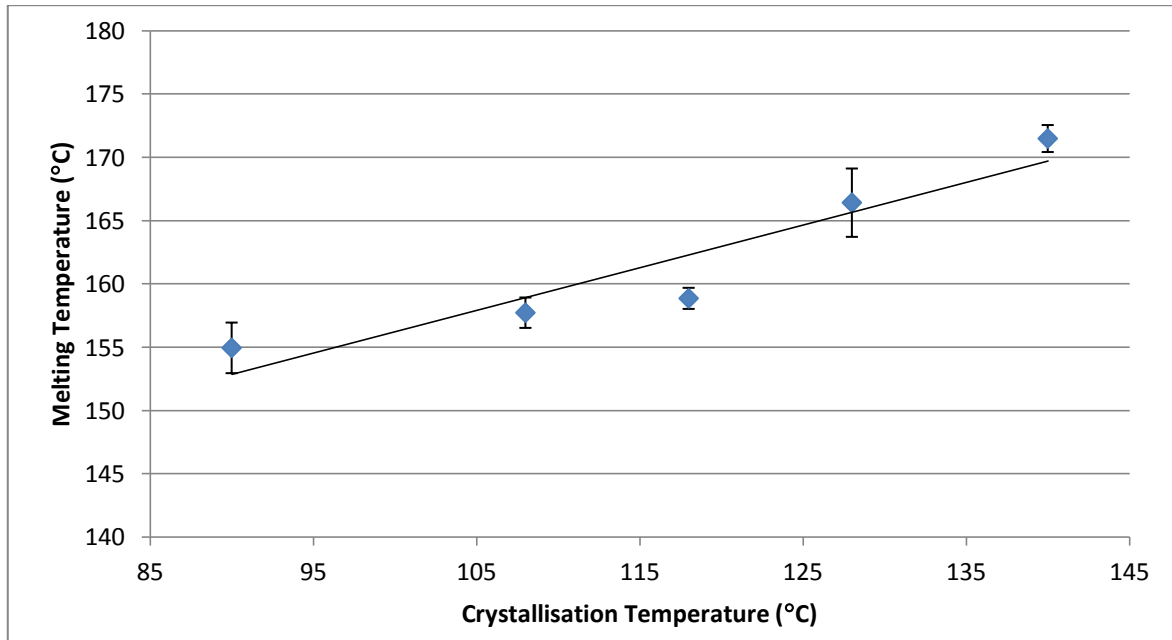


Figure 16. The relationship between the average peak melting temperature and the crystallisation temperature. The error bars represent the standard deviation at each temperature.

Figure 17 shows the equilibrium melting temperature, T_m^0 as described by the Hoffman-Weeks principle, and it is determined plotting the melting temperature, T_m , against varying crystallisation temperatures, T_c . A $T_m=T_c$ line is then plotted on the same graph and the point at which these two lines intercept gives the T_m^0 . The equilibrium melting point is found to be around 186 °C.

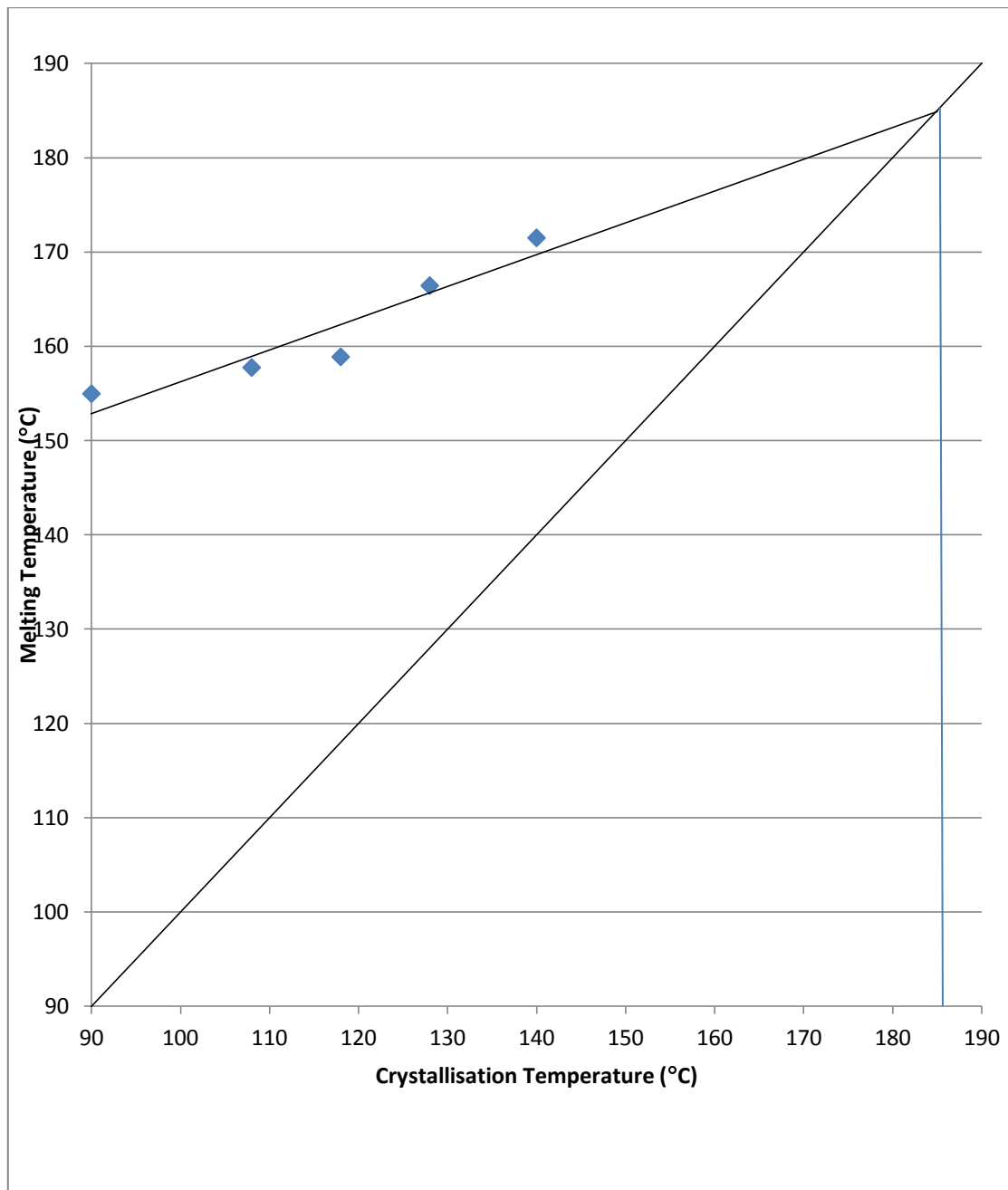


Figure 17. Hoffman-Weeks Extrapolation.

4.5 The Effect of Degree of Crystallinity on the Melting Temperature

Figure 18 shows the relationship between melting temperature with ΔH_f , and therefore the degree of crystallinity. It can be seen that generally, as the degree of crystallinity increases, there is an increase in the melting temperature of the PLA. However, this dependence is not really seen in samples crystallized at 90 °C (Figure 18A), this could be attributed to the high levels of nucleation taking place during crystallisation and low levels of growth of the spherulites. It can be seen that a fairly linear increase is seen in samples crystallised between 108 °C and 140 °C (18B – 18E). This suggests that as crystallisation time increases, so does the lamellae thickness resulting in a higher melting point.

At 108 °C, 118 °C, 128 °C and 140 °C increases of 2.86 °C, 2.04 °C, 7.50 °C and 10.40 °C respectively were observed. Similar findings were observed by He et al (2006), they reported that as crystallisation time increased, there was a corresponding increase in the melting peak observed and attributed this to an increase in the lamellar thickness and changes in nucleation and growth rates.

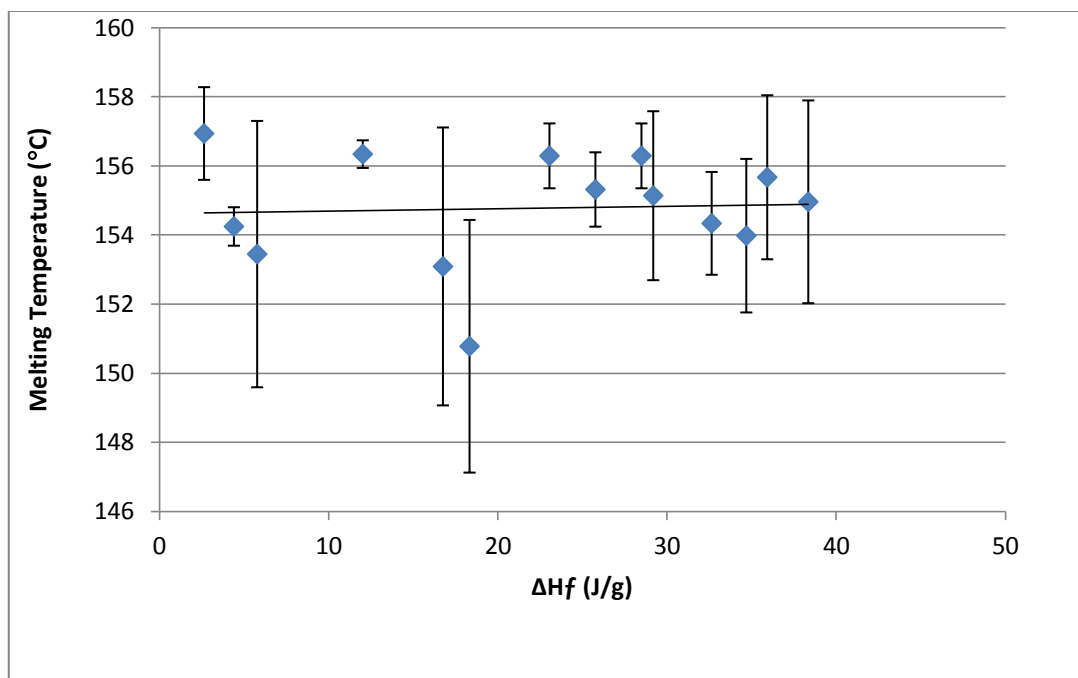


Figure 18A. Melting temperature as a function of ΔH_f in samples crystallized at 90 °C. Error bars represent standard deviation within the samples.

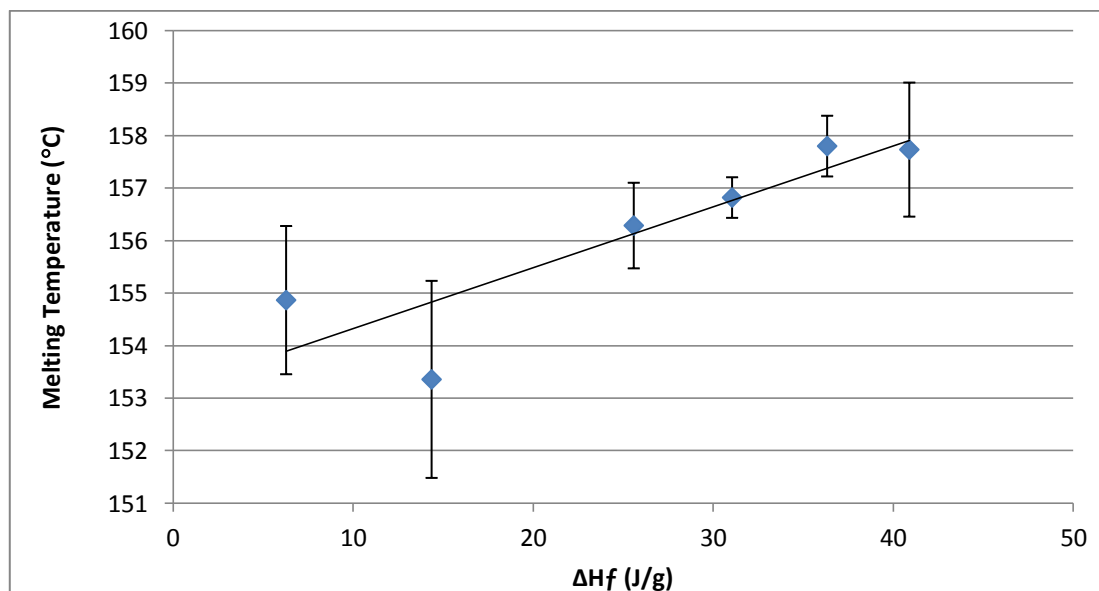


Figure 18B. Melting temperature as a function of ΔH_f in samples crystallized at 108 °C. Error bars represent standard deviation within the samples.

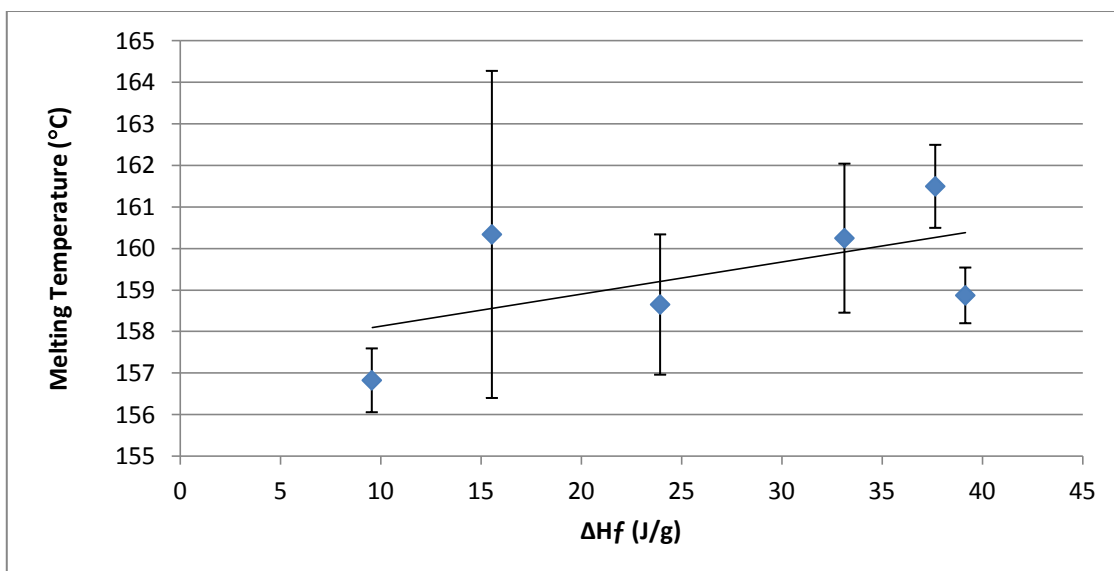


Figure 18C. Melting temperature as a function of ΔH_f in samples crystallized at 118 °C. Error bars represent standard deviation within the samples.

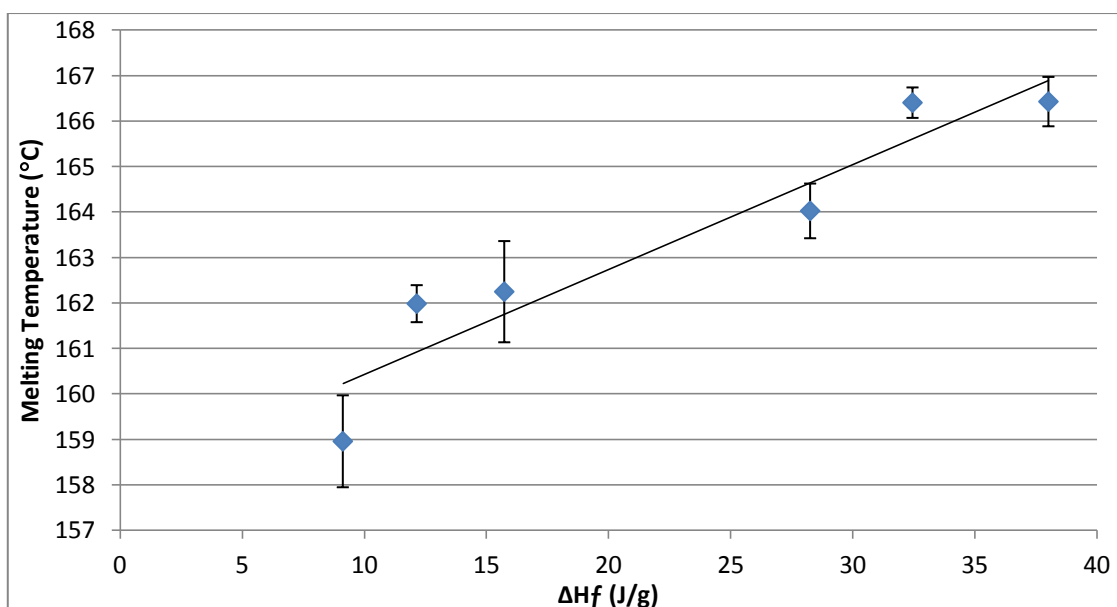


Figure 18D. Melting temperature as a function of ΔH_f in samples crystallized at 128 °C. Error bars represent standard deviation within the samples.

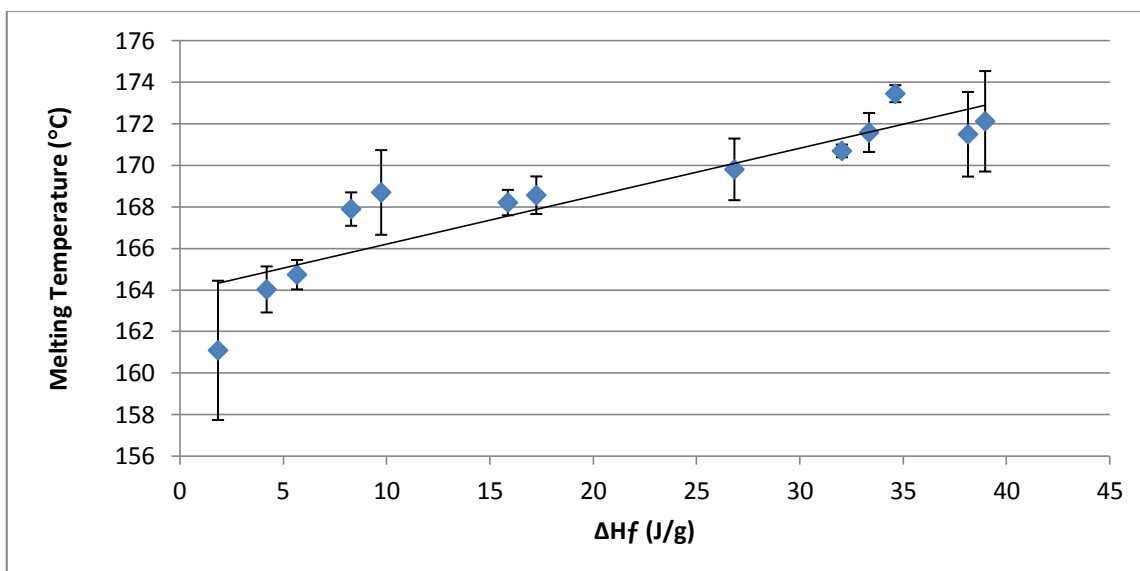


Figure 18E. Melting temperature as a function of ΔH_f in samples crystallized at 140 °C. Error bars represent standard deviation within the samples.

4.6 Biodegradation

4.6.1. Preliminary Biodegradation

The results of the first trial degradation showed no signs that any weight has been lost over a two week period suggesting that degradation is unlikely to have occurred. This may be due to a number of reasons. The concentration of the enzyme may not have been enough to bring about the degradation process in the 3051D grade PLA. As the solution was not refreshed throughout the experiment, it may be that the enzyme activity was not maintained due to a change in pH and so therefore there was no enzyme acting on the PLA pellets. It could be that this grade of PLA is not susceptible to degradation or requires a different enzyme to initiate the process. In the second trial experiment it was attempted to eliminate some of these suggestions by increasing the concentration of the enzyme and also refreshing the solution every 24 hours in order to maintain the enzyme activity levels.

Table 4. Weight loss observed in trial degradation experiments. See table 3 (page 28) for sample conditions.

	Original Weight \pm 0.02 (mg)	End Weight \pm 0.02 (mg)	Percentage Change (%)
Sample 1	41.67	41.6	-0.17
Sample 2	35.67	22.28	-37.54
Sample 3	39.95	39.96	+0.03

These results show that increasing the enzyme concentration level had no effect on the weight loss of the PLA pellets. However, there is a big increase in weight loss when the

enzyme is refreshed every 24 hours. This therefore suggests that in trial degradation one the enzyme activity was reduced. These results therefore suggest that the 10ml/0.5 mg concentration is suitable for biodegrading the PLA, but the solution must be refreshed regularly to maintain adequate enzyme activity.

Table 5 shows the results of the biodegradation process for the preliminary degradation trials on PLA 3051D thin films. After 7 days biodegradation in a 10 ml phosphate buffer saline solution with 0.5 mg Proteinase K enzyme, it can be seen that there was an increase in weight of the samples crystallised to 5% and 40% crystallinity at 118°C as opposed to weight loss. This therefore suggests that no biodegradation occurred in the samples at both 40% crystallinity and 5% crystallinity. This result could be due to a number of reasons including that the degradation time may have been too short, the enzyme concentration may not have been high enough or the 3051D grade PLA may not be susceptible to the biodegradation process.

Table 5. Weight change in biodegraded samples degraded in 10 ml PBS solution with 0.5 mg Proteinase K for 7 days.

Sample Number	Degree of Crystallinity (%)	Initial Weight ± 0.02 (mg)	End Weight ± 0.02 (mg)	Percentage Change (%)
6a	5	28.08	28.17	+0.32
6b	5	23.90	23.98	+0.34
7a	40	23.79	23.97	+0.76
7b	40	27.03	27.26	+0.851

Figure 19 shows DSC traces of the samples from Table 5 both before and after biodegradation. Although samples 6a and 6b (5% degree of crystallinity) displayed an increase in weight after degradation, the DSC traces show a big increase in crystallinity after biodegradation (Figures 19A and 19B). A crystallisation exotherm is clearly visible at 110°C which suggests that crystallisation has occurred during the DSC heating run. After degradation, samples 6a and 6b clearly show double melting peaks with a prominent peak and a lower second peak.

Samples 6a and 6b (Figures 19A and 19B), 5% degree of crystallinity, display a large increase in crystallinity after the degradation process. A crystallisation exotherm is clearly visible at 110 °C which suggests that crystallisation has occurred during heating in the DSC. Double melting peaks are also visible in the 5% crystallinity samples after degradation suggesting that the melt recrystallisation mechanism is taking place causing melting and recrystallisation of thermally unstable crystals.

Samples 7a and 7b, Figures 19C and 19D, (40% degree of crystallinity) also displayed an increase in weight after degradation. The DSC traces show a slight increase in crystallinity. The samples also show a slight decrease in T_m from 153.47 °C to 150.97 °C for sample 7a and 153.47 °C to 150.27 °C for sample 7b. Similar findings were reported by Tsuji and Ikada (1996), they found a drop in T_m after degradation and stated that this was most likely due to a decrease in the molecular weight of the samples after degradation has taken place.

In Figures 19A-19D, it can be seen that there is larger peak on the T_g after degradation which can be attributed to physical aging of the samples. Aging refers to the changes in chain length over time, degradation of long chain polymers to shorter chain molecules. This results in the properties of the polymer also changing. Aging is an indication of a drop in molecular weight of a polymer, and so the increase in the peak on T_g after degradation suggests that a drop in molecular weight has occurred during the degradation process.

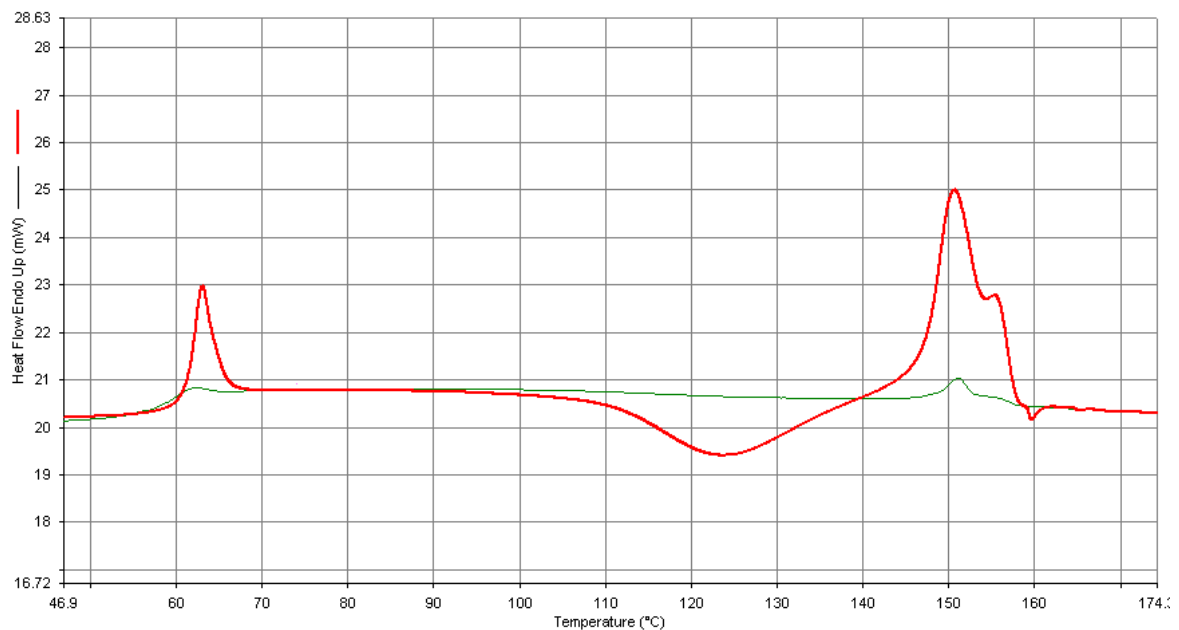


Figure 19A. Sample 6a, 5% crystallinity. DSC traces before (green) and after (red) one week degradation.

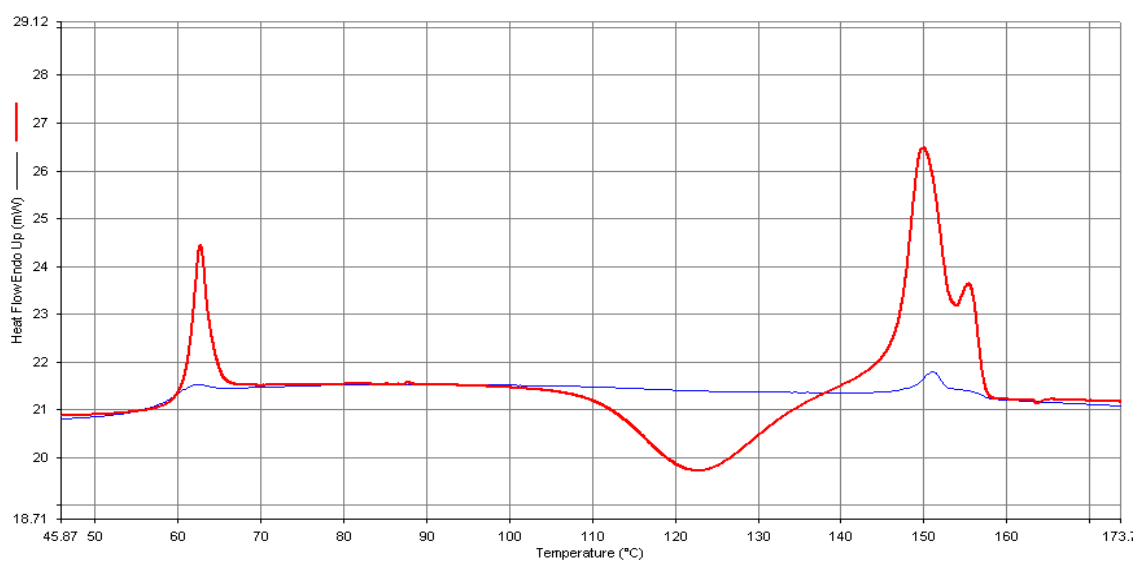


Figure 19B. Sample 6b, 5% crystallinity. DSC traces before (blue) and after (red) one week degradation.

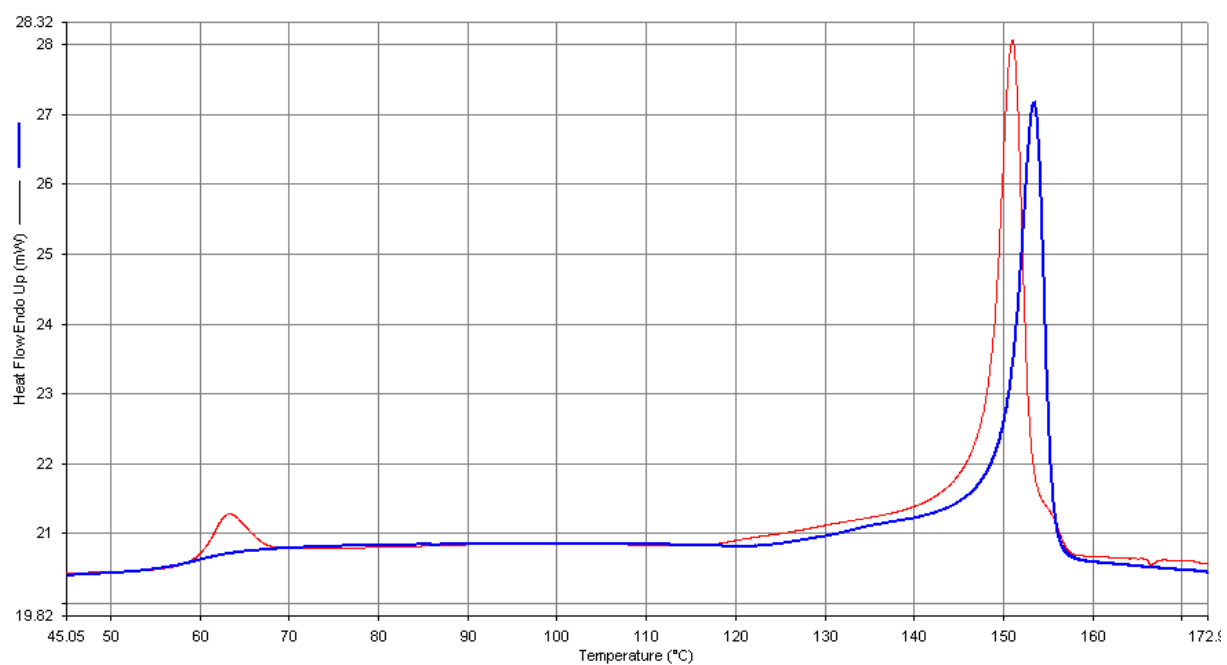


Figure 19C. Sample 7a, 40% crystallinity. DSC traces before (blue) and after (red) one week degradation.

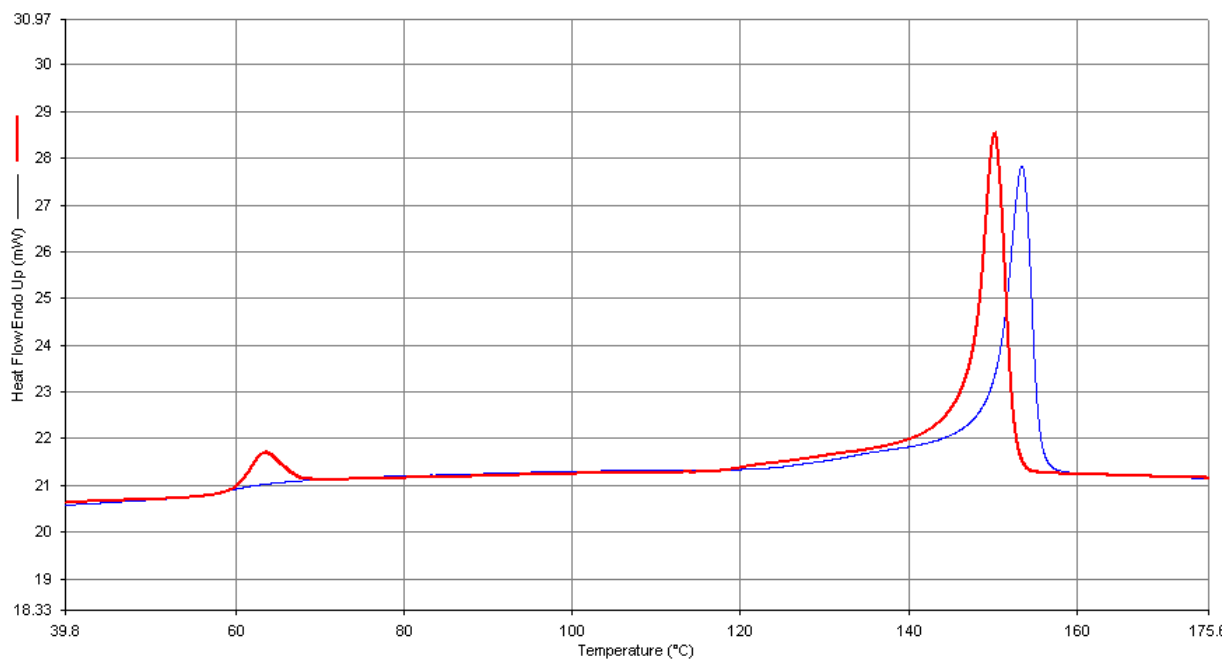


Figure 19D. Sample 7b, 40% crystallinity. DSC traces before (blue) and after (red) one week degradation.

Figure 19. DSC traces before and after degradation.

4.6.2. One Week Biodegradation

Table 6 shows the weights of 16 samples (samples 8-11) before and after degradation. It can be seen that the biggest weight loss occurred in the amorphous polymer samples with an average percentage mass loss of 3.47%, this is to be expected as it is widely reported that amorphous regions are more easily degraded than crystalline regions. Figure 20 shows the average percentage loss of the samples as a function of their crystallisation time in the samples crystallised to 40%. It can be seen that as the crystallisation temperature increases, so does the percentage loss observed in the samples. This could be attributed to the differences observed in the microstructure of samples formed at different temperatures (figure 14). At 90 °C (sample 9), nucleation of spherulites is high and growth of spherulites is limited, whereas at 118 °C (sample 11) the nucleation is limited and the growth of spherulites is high. At 118 °C, there are greater amorphous regions between spherulites due

to the lower nucleation rate. It is widely reported that amorphous polymers degrade more easily and faster than semi-crystalline polymers, and so it is possible that the increase in the size of amorphous regions in the PLA samples as crystallisation temperature increases results in the samples degrading more readily causing a greater weight loss as crystallisation temperature increases. However, the weight loss observed in the degraded samples is only slight in the crystallised samples and so the significance of the weight loss results is questionable. In order to determine the significance of these results, further degradation work is required.

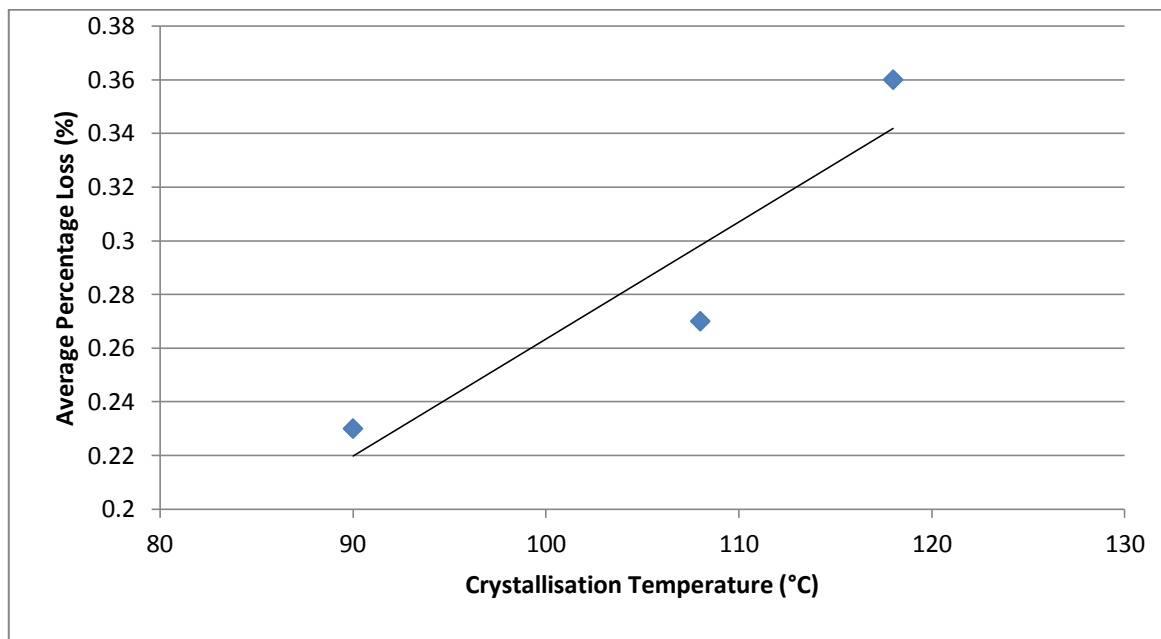


Figure 20. The relationship between the samples crystallisation temperature (°C) and the average percentage loss observed (%).

Table 6. Weight loss measurements of 16 samples after five days degradation.

	Sample	Initial Weight ± 0.02 (mg)	Final Weight ± 0.02 (mg)	Percentage Change (%)	Average Percentage Change (%)
Amorphous	8a	5.45	5.26	-3.49	-3.47
	8b	5.49	5.26	-4.19	
	8c	5.28	5.15	-2.46	
	8d	5.37	5.17	-3.72	
90°C	9a	5.54	5.53	-0.18	-0.23
	9b	5.65	5.64	-0.18	
	9c	5.60	5.59	-0.18	
	9d	5.61	5.59	-0.36	
108°C	10a	5.57	5.56	-0.18	-0.27
	10b	5.58	5.56	-0.36	
	10c	5.53	5.52	-0.18	
	10d	5.53	5.51	-0.36	
118°C	11a	5.61	5.59	-0.36	-0.36
	11b	5.69	5.67	-0.35	
	11c	5.54	5.52	-0.36	
	11d	5.58	5.56	-0.36	

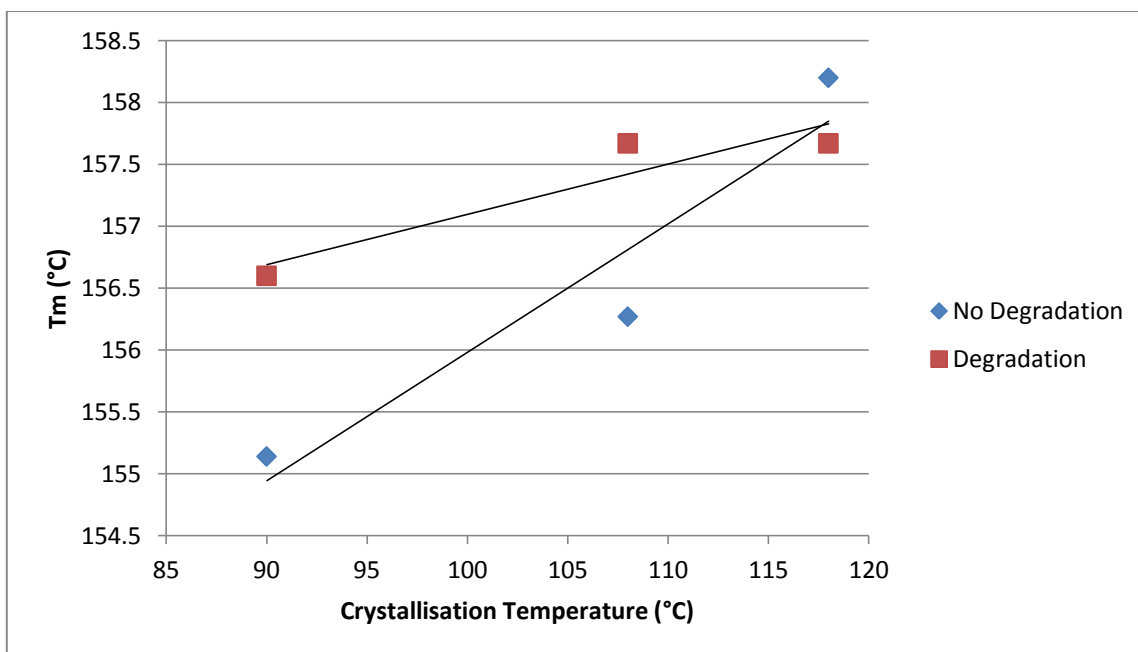


Figure 21. Melting point values in samples crystallised to 40% at 90 °C, 108 °C and 118 °C before and after biodegradation.

Figure 21 shows the average melting peak observed in samples before and after degradation. It can be seen that after degradation at lower temperatures an increase in T_m was observed, however at 118 °C a decrease in T_m is observed after degradation.

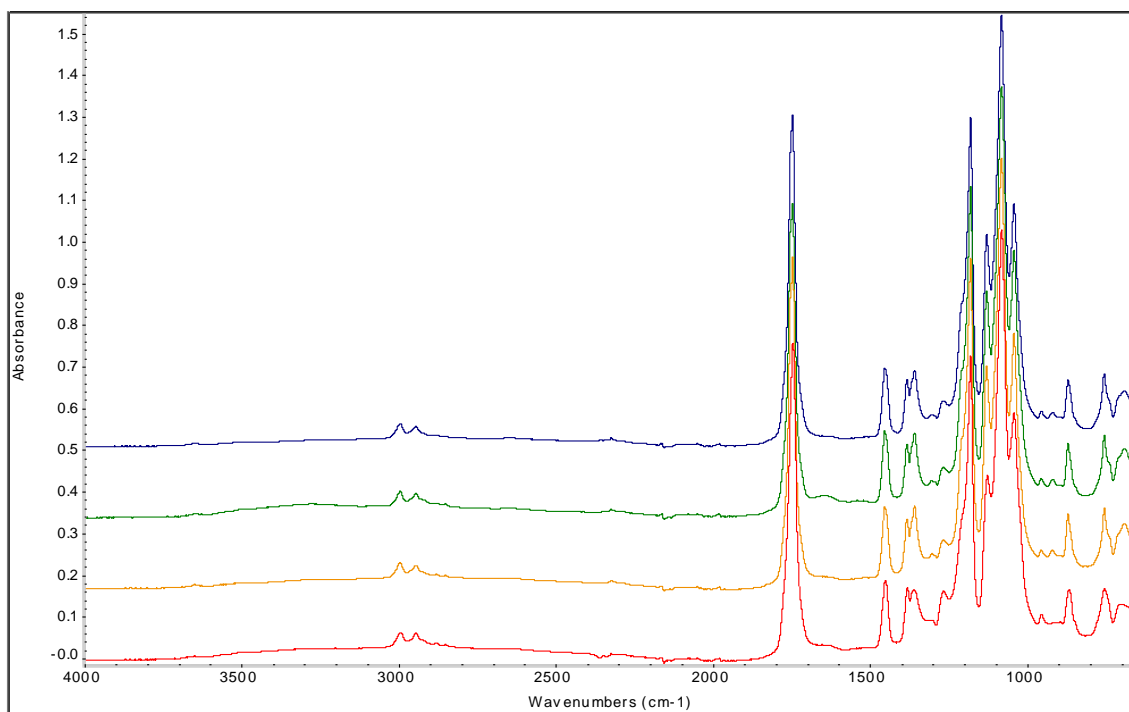


Figure 22. FT-IR spectra obtained after biodegradation in four samples, amorphous (red), crystallised to 40% at 90 °C (orange), 108 °C (green) and 118 °C (blue).

Figure 22 shows the FT-IR spectra obtained in four samples (amorphous and crystallised to 40% at 90 °C, 108 °C and 118 °C) after degradation. FT-IR showed there to be no difference between the samples after degradation. This could be because the weight loss between samples was not a significant amount to cause differences at a molecular level. It could also be because the degradation time was not long enough and further studies need to be conducted to investigate this, this however was not possible in this project due to time constraints.

4.6.3. Ten Week Biodegradation

Preliminary long term degradation work was conducted on four samples, two crystallised to 40% at 90 °C and two crystallised to 40% at 118 °C. Figure 23 shows the weight measurements of each sample taken each week throughout. Weeks 1-9 represent the wet weight of the sample, week 0 the initial dry sample weight and week 10 the final dry weight.

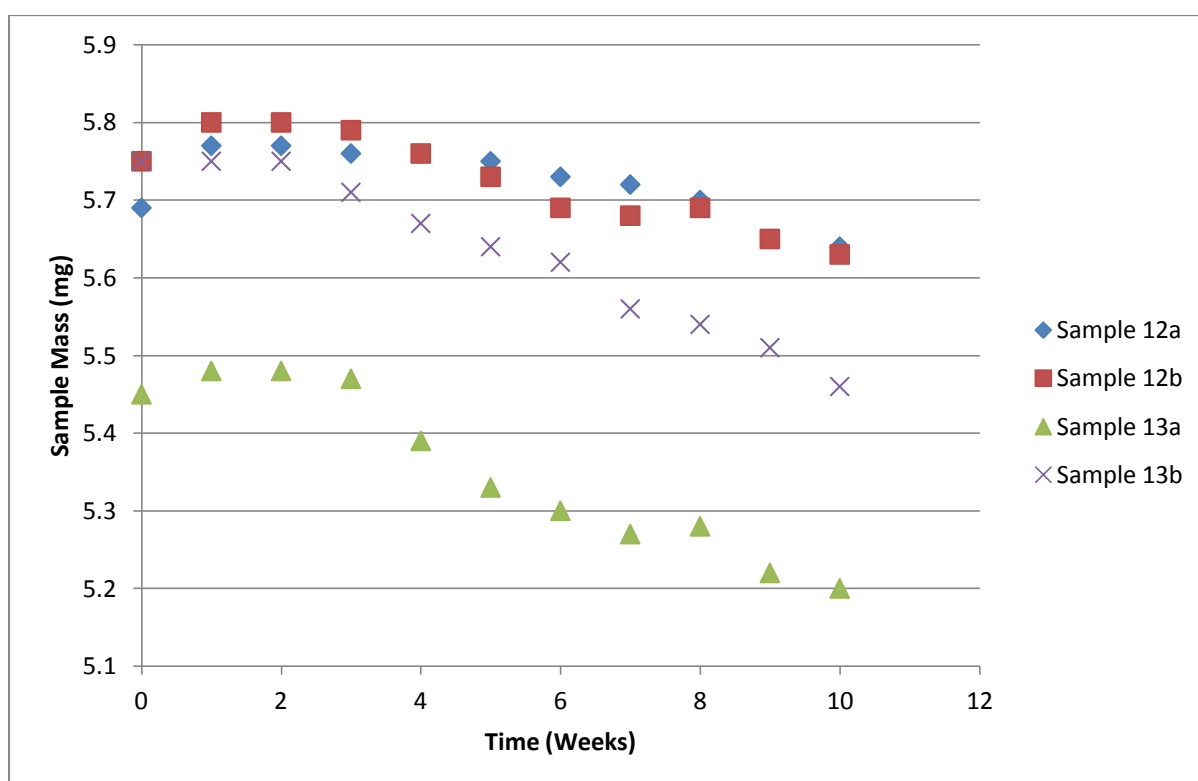


Figure 23. Sample masses during ten week degradation. Samples 12a and 12b crystallised to 40% at 90 °C and samples 13a and 13b crystallised to 40% at 118 °C.

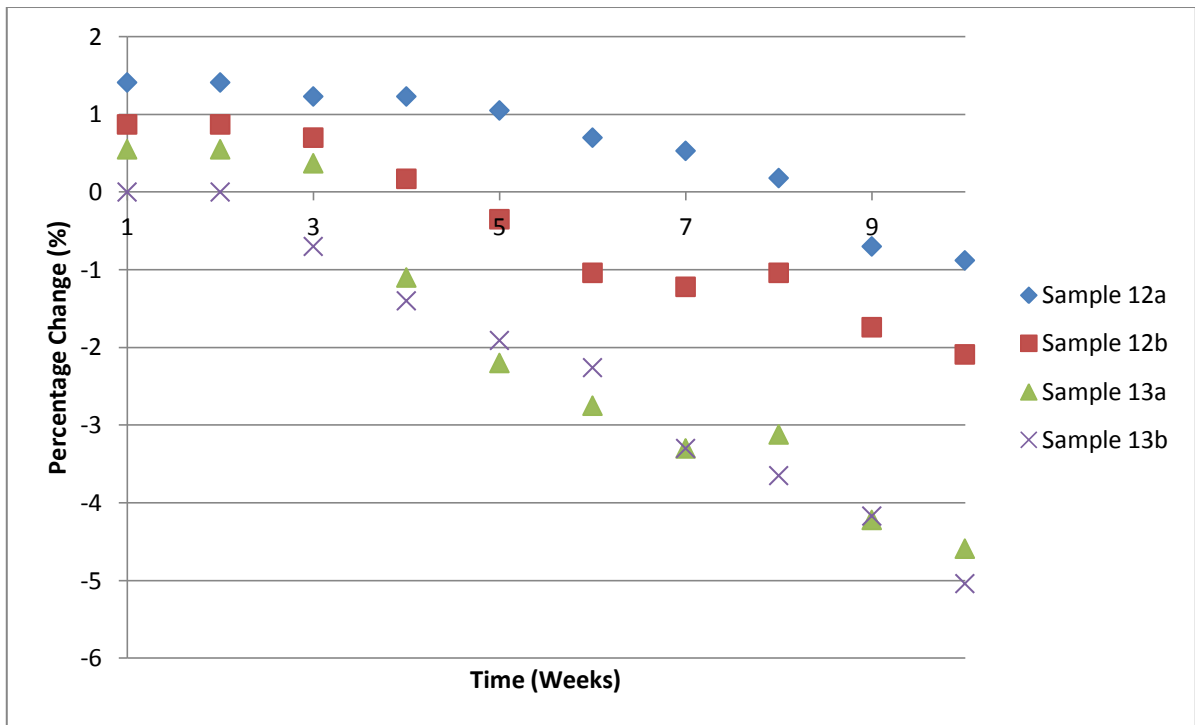


Figure 24. Percentage change observed in samples each week. Samples 12a and 12b crystallised to 40% at 90 °C and samples 13a and 13b crystallised to 40% at 118 °C.

It can be seen that initially there is an increase in mass of the polymer due to water absorption as the samples take in water; this is seen in all samples for the first 2 weeks. At week 3 a decrease in mass is then observed in all samples, which continues until week 10 suggesting that degradation begins to take place after three weeks. Figure 24 shows the percentage change of weight loss observed in samples each week, it shows that the biggest percentage change in weight loss occurred in the samples crystallised to 40% at 118 °C, suggesting that the lamellar thickness and spherulite formation has an influence on the degradation in PLA. However, further work is required in order to confirm this.

Table 7. The percentage decreases observed in samples after 10 weeks degradation.

Sample	Initial Weight ± 0.02 (mg)	End Weight ± 0.02 (mg)	Percentage Decrease (%)	Average Percentage Decrease (%)
12a	5.69	5.64	0.88	1.49
12b	5.75	5.63	2.09	
13a	5.45	5.2	4.59	4.82
13b	5.75	5.46	5.04	

Table 7 shows the percentage decreases in mass loss observed after biodegradation. It can be seen that in the samples conditioned to 40% crystallinity at 118 °C the mass loss observed is almost double the mass loss observed in samples crystallised to 40% at 90 °C. These results support the results found in the one week degradation experiment where the mass loss was found to be highest in those samples crystallised a 118 °C. The ten week degradation experiment also explains the small mass loss observed in the one week study, as degradation is seen to greatly increase after three weeks, suggesting that longer degradation trials are required in order to study the effect of crystallisation temperatures on the degradation process.

Figures 25 shows the T_g values in samples crystallised to 40% at 90 °C and 118 °C. It can be seen that at 90 °C there is a decrease in T_g after degradation has taken place, whereas in the samples crystallised to 40% at 118 °C there is an increase in T_g after degradation.

Figure 26 shows the T_m values in samples crystallised to 40% at 90 °C and 118 °C. It can be seen in samples submitted to degradation there was an increase in the T_m of the samples

crystallised at both 90 °C and 118 °C. Due to the small number of samples used in the preliminary long term degradation tests, it is unclear how significant the results in T_g and T_m changes are. Further long term degradation work is required in order to support the findings, this was not able to be conducted due to time restraints.

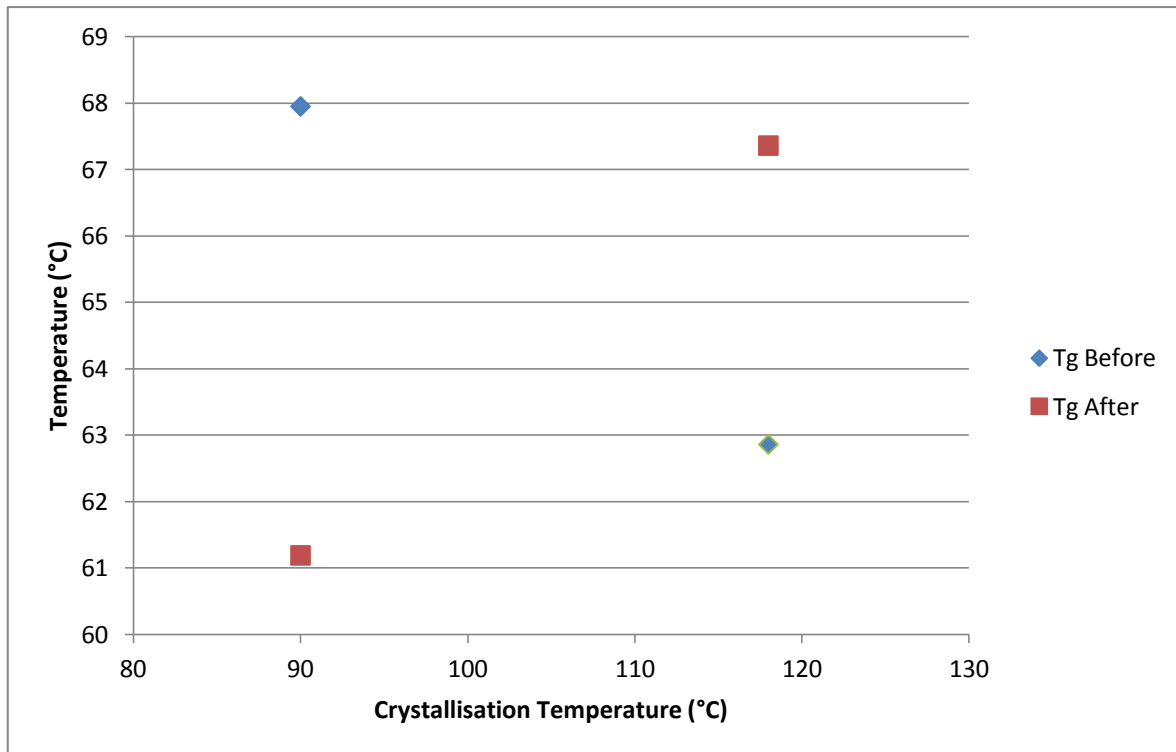


Figure 25. Average glass transition temperatures of the samples before and after degradation in samples crystallised to 40% at 90 °C and 118 °C.

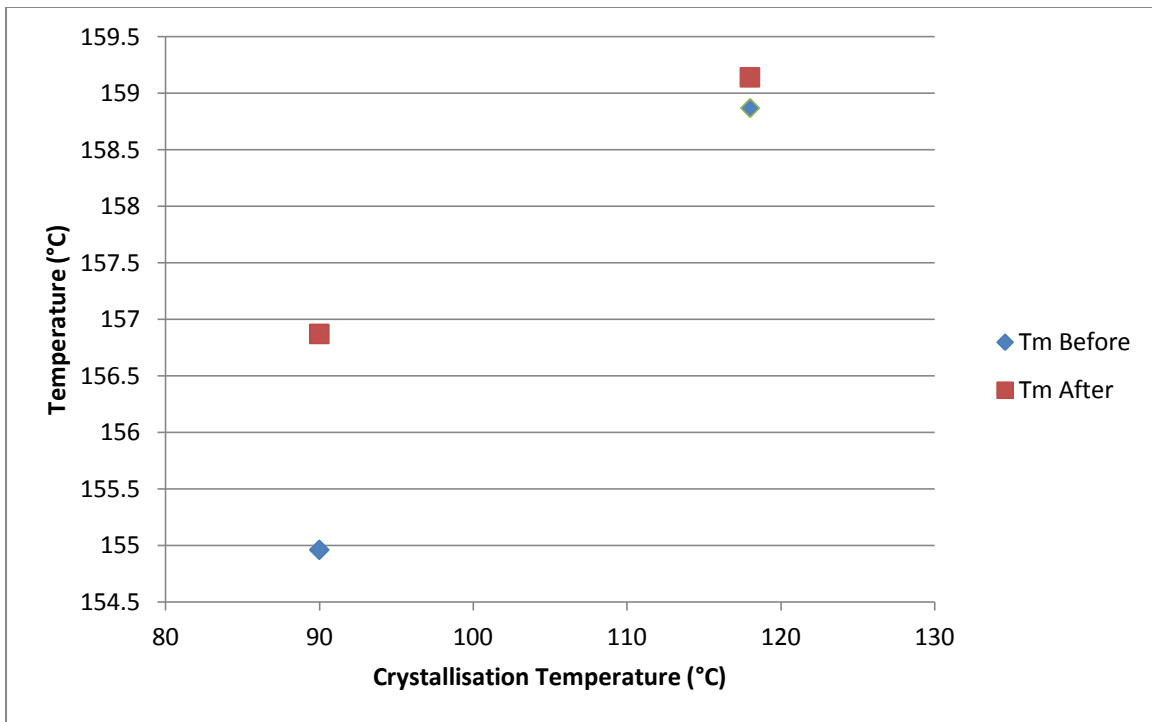


Figure 26. Average peak melting temperatures of the samples before and after degradation in samples crystallised to 40% at 90 °C and 118 °C.

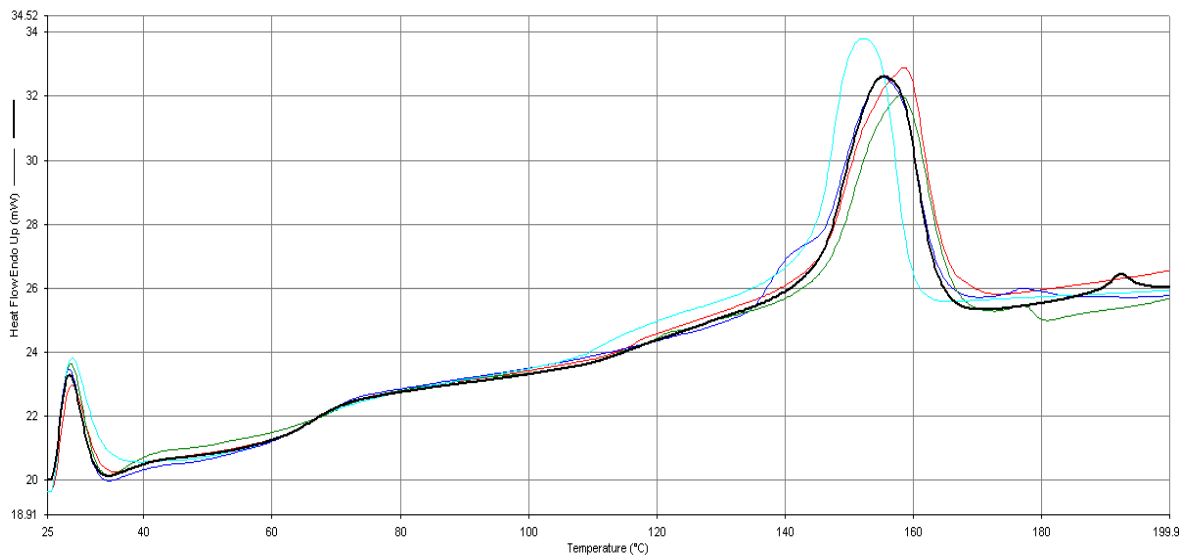


Figure 27. DSC traces of samples crystallised to 40% at 90 °C. Red and black traces represent samples submitted to the degradation process.

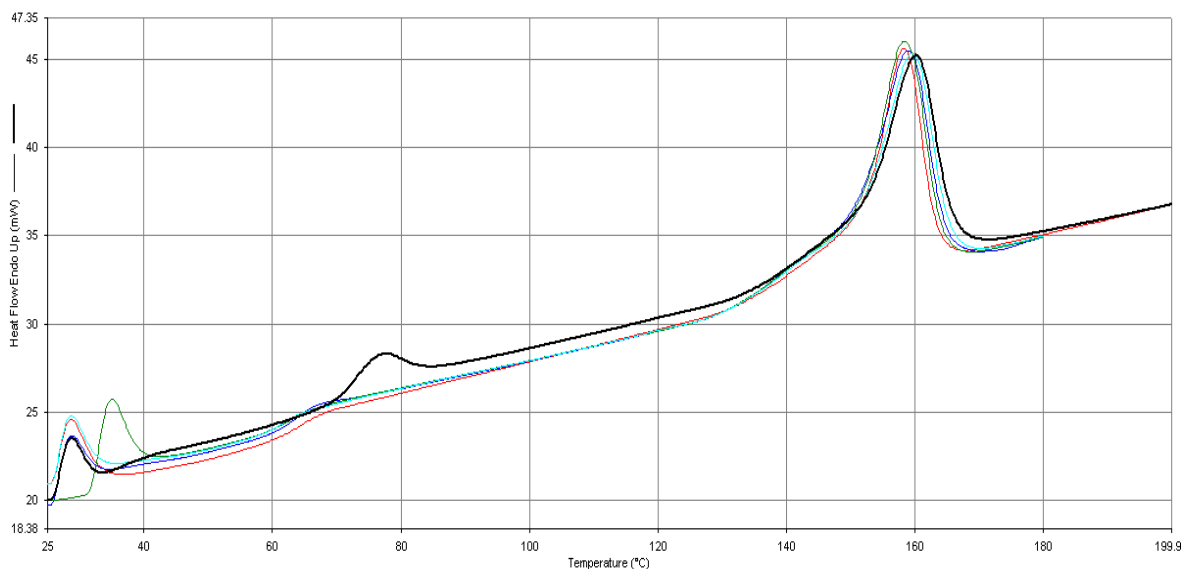


Figure 28. DSC traces of samples crystallised to 40% at 118 °C. Red and black traces represent samples submitted to the degradation process.

Figures 27 and 28 display the DSC traces obtained in samples crystallised to 40% at 90 °C and 118 °C respectively both with and without degradation. It can be seen in all samples that there is no significant difference between the traces obtained prior to and after degradation has taken place.

5.0 Conclusions and Further Work

The behaviour of PLA 3051 D during crystallisation and degradation has been studied.

From DSC analysis, it appears that crystallisation time has an influence on the degree of crystallinity of the material. 118 °C is the optimum crystallisation temperature and these samples reach 40% crystallinity in 150 minutes. Above and below this temperature, longer crystallisation times are required to reach 40% crystallinity.

The holding temperature greatly influences the microstructure and the spherulite formation within PLA. Through hot stage microscopy it was shown that as crystallisation temperature increases, spherulite growth also increases but spherulite nucleation decreases.

Preliminary biodegradation studies suggest that there is a link between the microstructure in PLA and degradation rate. A higher crystallisation temperature lead to an increase in lamellar thickness and as a result the larger the spherulite size the higher the degradation rate. Degradation was also shown to occur more readily in thin PLA films than the thicker PLA plaques.

For future research, it would be beneficial to study a wider range of crystallisation temperatures for shorter lengths of time to gain further knowledge of the spherulite formation as a function of crystallisation times and temperatures. Furthermore, a wide range of engineered microstructures could be used for biodegradation to investigate the effect of spherulite formation on degradation rate. Finally, longer term degradation tests should be conducted on samples crystallised at a wide range of temperatures to test and enhance the results provided in this work.

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