Enzyme Catalysed Modification of Polymers

Nigel Overton

Doctor of Philosophy

The University of Aston in Birmingham

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Summary

The aim of this project was to investigate the enzyme catalysed modification of synthetic polymers.

It was found that an immobilised lipase from *Candida antartica* (Novozyme 435) catalysed the selective epoxidation of poly(butadiene) in the presence of hydrogen peroxide and catalytic quantities of acetic acid. The *cis* and *trans* double bonds of the backbone were epoxidised in yields of up to 60 % whilst the pendent vinyl groups were untouched. The effect of varying a number of reaction parameters was investigated. These studies suggested that higher yields of epoxide could not be obtained because of the conformational properties of the partially epoxidised polymer.

Application of this process to the Baeyer-Villiger reaction of poly(vinyl phenyl ketone) and poly(vinyl methyl ketone) were unsuccessful. The lack of reactivity was found to be a property of the polymer rather than the enzymatic system employed.

Attempts to modify hydroxyl containing polymers and polymers bearing active esters close to the polymer backbone were unsuccessful. Steric factors appear to be the most important influence on the outcome of the reactions.

Keywords: Enzyme, Polymer modification, Lipase, Epoxidation and Poly(butadiene)

Dedication

To my mom and dad.

Acknowledgements

Firstly, I would like to thank my supervisors, Ann Jarvie, Chris St Pourçain and Eric Smith for their fine supervision, guidance and friendship over the past 3 years.

Next, I would like to thank my friends in labs 208 and 209 and the rest of the Chemical Engineering and Applied Chemistry department for my happy post-grad years with the many parties and beer drinking sessions.

My thanks also goes to Mike Perry for running all my "crappy" samples for me, Novo Nordisk for the enzyme and the EPSRC for funding of this work.

Finally, thanks must go to my special friend Kylie.

Quotations

If you think you're right, don't give up and don't let anybody down.

Don't try and be the best, try and do your best.

Personal quotations from Sir Harry Kroto, given to me at the June / July meeting of Nobel Laureates in Lindau 1998.

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List of Abreviations

Abbreviation	Description
AIBN	α,α′-Azobisisobutyronitrile
Asp	Aspartic acid
CLECs	Cross Linked Enzyme Crystals
CRL	*Candida Rugosa* Lipase
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribose Nucleic Acid
Enz	Enzyme
FT-IR	Fourier Transform Infrared
Glu	Glutamic acid
GPC	Gel Permeation Chromatography
His	Histidine
Mn	Number average molar mass
Mw	Weight average molar mass
NMR	Nuclear Magnetic Resonance
PPL	Porcine Pancreatic Lipase
RMM	Relative Molecular Mass
Ser	Serine

Chapter 1.

Introduction

1.0. Introduction

1.1. General Introduction

The use of enzymes in organic synthesis is becoming increasingly widespread. They have the advantage of being extremely active and environmentally benign catalysts which are able to catalyse a wide variety of reactions, under mild conditions, with a high degree of stereo-, chemo- and regioselectivity. These properties have allowed enzymes to be used in syntheses which were previously extremely difficult or impractical.[1]

Although there was occasional use of enzymes in organic synthesis from the beginning of this century, their application was not seriously explored until the early 1970s.[1, 2] One reason for this was that it was thought enzymes could only function in water, and were therefore very limited in their use, since many synthetic processes involve substrates which only dissolve in organic solvents and reactions which do not proceed in aqueous systems. However, the pioneering work of Klibanov and co-workers in the early 1980s demonstrated that enzymes were active in organic solvents and this has led to the development and much wider application of enzymes for organic synthesis.[3]

In contrast to the burgeoning literature describing the use of enzymes for the synthesis of small molecules, the use of enzymes in polymer synthesis has been less frequent.[4] Enzymes have been used for monomer synthesis,[5, 6] polymerisation (particularly for the synthesis of polyesters)[7, 8] and for the modification of synthetic polymers.[9]

1.2. Practical Uses of Enzymes

Enzymes have been exploited by industry in a variety of ways. The types of reactions that enzymes catalyse are varied and include, oxidation, reduction, inter- and intramolecular transfer of groups, hydrolysis, cleavage of covalent bonds by elimination, addition of groups to double bonds and isomerisation.[7]

Recombinant-DNA technology can now be used for the production of enzymes in large quantities, which makes them readily available for commercial use relatively cheaply.[10] Protein engineering allows redesigned forms of the enzyme with improved properties to be obtained.

Enzymes are used for the conversion of petrochemicals, transformations of oils and fats, selective removal of undesirable compounds, desulfurisation of oil, decomposition of peroxides in vegetable oils, flavourings and perfumes, and organic waste treatment. Enzymes are also used for removing unwanted compounds from blood and for analysis of blood. Enzymes have a variety of uses in analysis, including an alcohol oxidase based sensor for the analysis of ethanol in air (used as a breathalyser) and peroxide catalysts in temperature abuse sensors for refrigerated transport. The monitoring of temperature is important in the transport of frozen or chilled foods, pharmaceuticals and other temperature sensitive materials.

The ability of enzymes to perform reactions selectively under mild conditions is being exploited in the agrochemicals and fine chemicals industries. This is of particular importance in the synthesis of drugs and biomedical materials which are often needed in a stereochemically pure form.

1.3. Advantages and Disadvantages of Enzyme Catalysed Organic Synthesis

1.3.1. Advantages of Enzyme Catalysed Systems[11]

1. Enzymes are very efficient catalysts.
2. Enzymes are highly regio-, chemo-, and stereoselective catalysts.
3. Enzymes are able to function efficiently under very mild conditions.
4. Enzymes are able to catalyse a wide variety of reactions.
5. Enzymes are able to function in organic solvents.
6. Enzymes are more stable in anhydrous organic solvents than in water.
7. Enzymes are naturally occurring biodegradable proteins.
8. Enzyme catalysed reactions can be easily scaled up to a viable commercial scale.

1.3.2. Disadvantages of Enzyme Catalysed Systems

Despite the advantages of enzymatic transformations, there are some disadvantages which require consideration:

1. Enzymes remain active only under a limited range of conditions.

2. Enzymes can be less stable and less reactive in organic solvents than they are in aqueous systems.

3. Stereoselectivity may be altered.

4. Enzymes can suffer severe substrate or product inhibition.

5. The diffusion of products and substrates to and from the enzyme are frequently the rate-limiting steps.

6. Elimination of bulk water and use of organic solvents results in the rigidification of the enzyme structure, limiting the structural features of a suitable substrate.

1.4. What Are Enzymes?[12]

Enzymes are proteins which act as biological catalysts. They are able to increase reaction rates by a factor of 10^8 - 10^{10} although rates of up to 10^{14} are known.[11] As with any other catalyst, enzymes do not alter the reaction equilibrium but lower the free energy of activation of the reaction. Enzymes do this by providing a new pathway for the reaction in which the transition-state is lower in energy than in the absence of the catalyst.

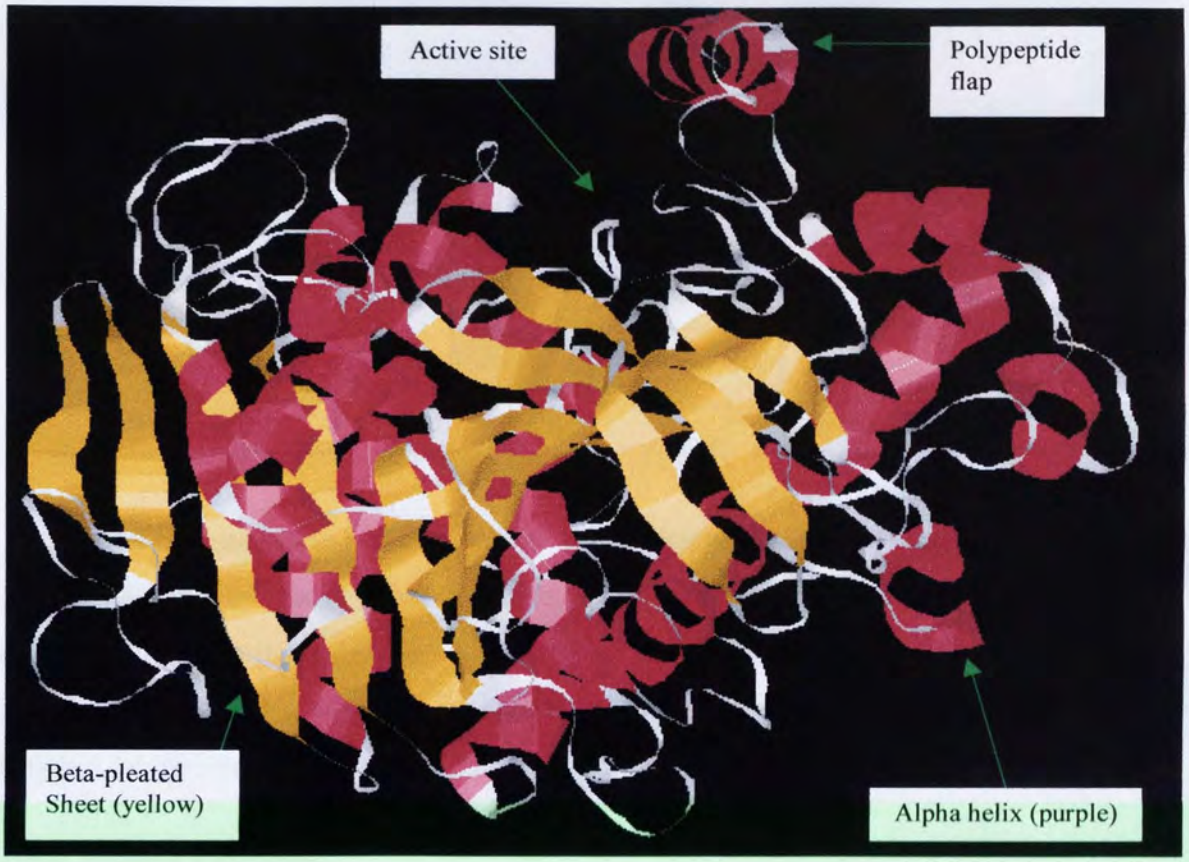
The first step in enzyme catalysis is the formation of an enzyme-substrate complex. Water is largely excluded from the active site by the binding of the substrate. The specificity of enzyme substrate interactions arises mainly from the three dimensional structure of the active site and a variety of intermolecular forces. These factors cause substrates to be denied access to the catalytic active site if they do not have a sufficiently complementary structure. It is for this reason that enzymes are able to control the stereo-, regio- and chemoselectivity of a reaction.

The shape of the enzyme and its catalytic site is governed by the primary, secondary and tertiary structures of the protein molecule. The primary structure defines the amino acid sequence along the protein chain, whilst the secondary structure arises from folding of the protein chain into specific structures. Secondary structures include the beta pleated sheet and alpha helix, in conjunction with other less specific twists and turns of the protein chain. The tertiary structure refers to the overall three dimensional shape of the protein molecule and defines where each part of the chain is located in relation to other parts of the chain. The overall structure is held together by a variety of intermolecular forces including hydrogen bonding , electrostatic interactions and disulphide bridges. Quaternary structure refers to the larger aggregate structures formed by the association of several protein molecules.

1.5. Mechanism of Enzyme Substrate Interaction

The structures of enzymes can be determined by X-ray crystallography of the pure crystallised enzyme.[13] Information gained from this and other sources such as NMR analysis enables the position and the action of the active site to be ascertained. One approach to gain such information is to crystallise an enzyme with the substrate bound into its active site. X-ray crystallographic studies on the enzyme-substrate complex highlights interactions between the enzyme and the substrate.[13] An example of the structural information provided by X-ray crystallographic analysis is given in Figure 1.

Figure 1. *Candida antarctica* Lipase Determined by X-ray Crystallography[13]

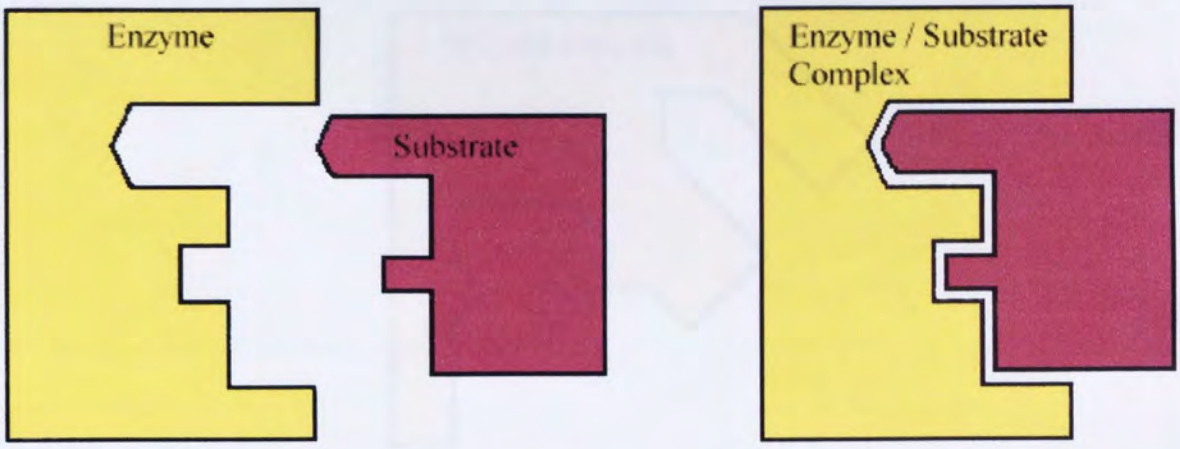


1.5.1. Lock and Key Model

It was first suggested by Fischer in 1894 that enzymes operated by what has been termed the "Lock and Key Model".[14] This model accounts for many of the properties exhibited by enzyme catalysed systems. According to this model, an enzyme (Enz) first combines with a substrate (S) to form an enzyme-substrate (Enz-S) complex (shown in Figure 2), which can then form a product (P). Alternatively, the enzyme-substrate complex can dissociate back into enzyme (Enz) and substrate (S).

$$\text{Enz} + \text{S} \rightleftharpoons \text{Enz-S} \longrightarrow \text{Enz} + \text{P}$$

Figure 2. Lock and Key Mechanism Redrawn from Reference 15

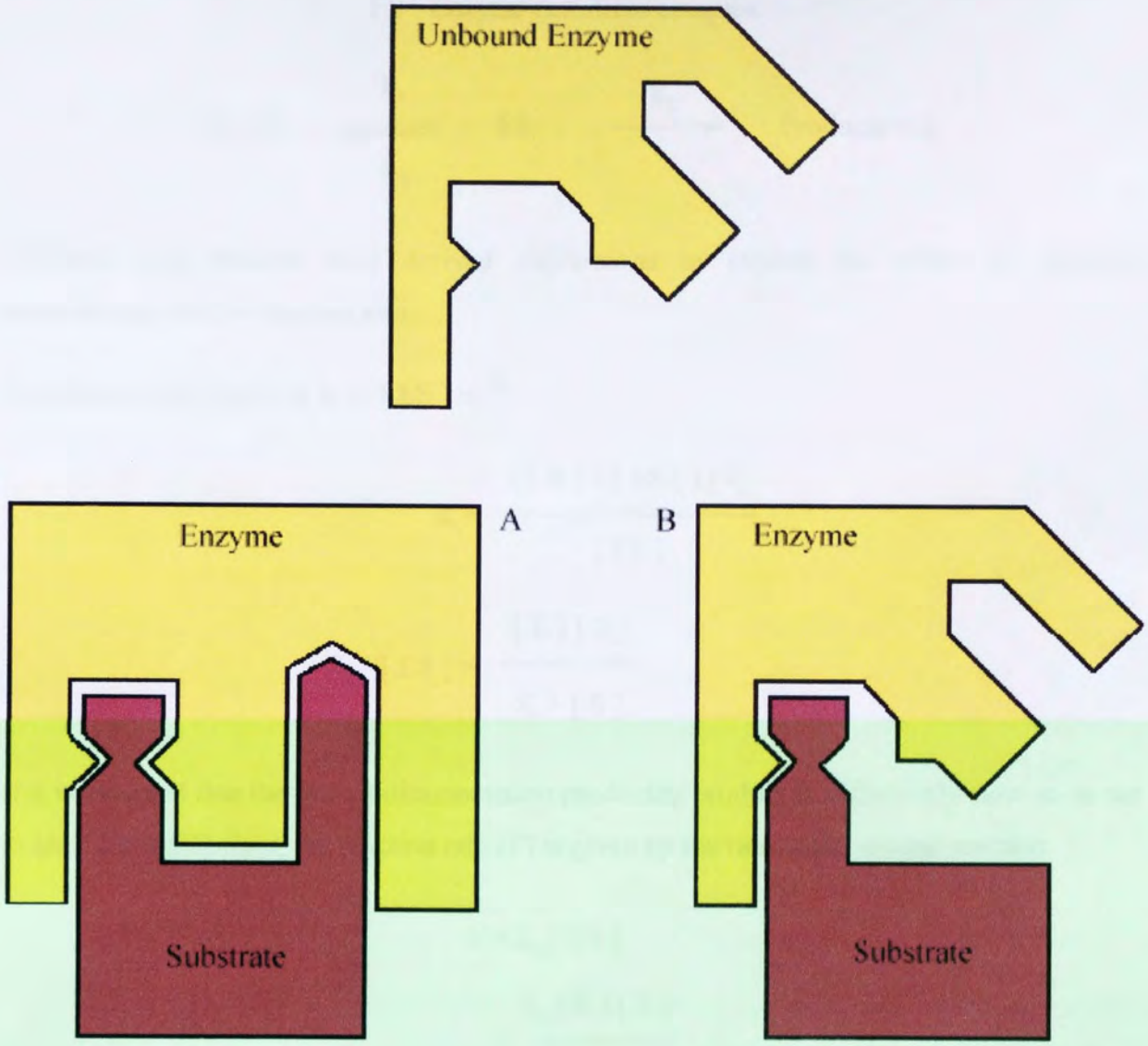


This model is quite crude and has several limitations as it assumes a rigid enzyme structure. The "Lock and Key" model does not explain why an enzyme is able to act on large substrates whilst smaller, similar substrates are inactive. Further to this, the simple "Lock and Key" model does not explain why enzymes are able to accept many different sized molecules which may not be their natural substrates.

1.5.2. Induced Fit Model

The limitations of the "Lock and Key" model in adequately explaining the properties of enzyme catalysis prompted a more sophisticated model to be developed. The induced fit model, shown in Figure 3, assumes that the enzyme is able to deform *via* slight conformational changes whilst the substrate is binding.[16] This model accounts for the ability of many enzymes to accept a wide variety of substrates.

Figure 3. Induced Fit Model Redrawn from Reference 15



It can be seen that in the first instance (A) that the substrate fits the active site correctly. In the second instance (B) the substrate does not fit the active site correctly. This is explained by noting that in the first case (A) the substrate causes the enzyme to alter its shape into such a conformation whereby a suitable enzyme-substrate interaction is now possible.

1.5.3 Michaelis-Menten Kinetics

The mechanism of enzyme catalysed reactions often follow Michaelis-Menten Kinetics.[17]

E = Enzyme
S = Substrate
ES= Enzyme-Substrate complex

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} Products + E$$

Michaelis and Menten have derived expressions to explain the effect of substrate concentration on the reaction rate.

The dissociation constant K of [ES] is:[18]

$$K = \frac{([E] - [ES])[S]}{[ES]}$$

$$[ES] = \frac{[E][S]}{K + [S]}$$

If it is assumed that the rate of disassociation producing product is sufficiently slow so as not to alter the equilibrium, the reaction rate (V) is given by the rate of the second reaction.

$$V = k_2 [ES]$$

$$= \frac{k_2 [E][S]}{K + [S]}$$

The maximum reaction rate (V_{max}) is attained when all of the enzyme is complexed, therefore [ES] = [E] under these conditions.

$$V_{max} = k_2 [E]$$

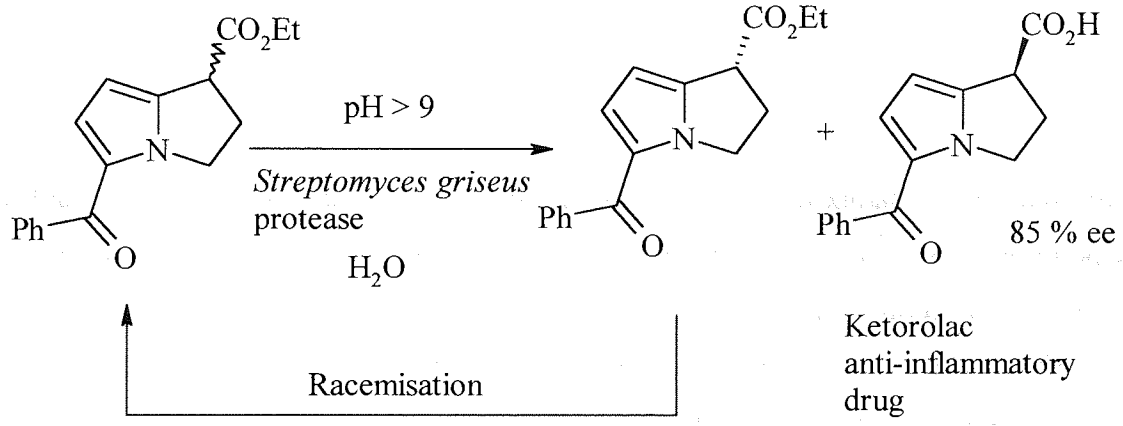
Substitution of V_{max} gives the Michaelis-Menten equation with K being known as the Michaelis-Menten constant.

$$V = \frac{V_{max}[S]}{K + [S]}$$

1.6. The Role of Water and Solvent Systems

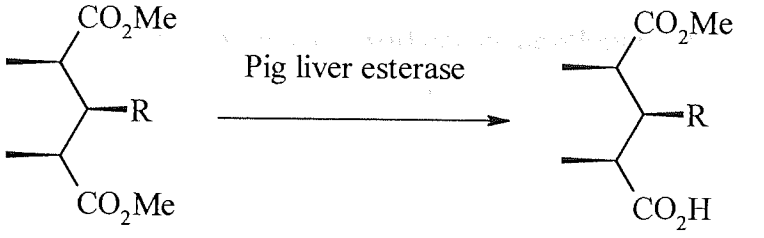
In nature enzymes act in aqueous fluids or emulsions. Enzymes have been used widely in aqueous media for organic synthesis, the most commonly used being the hydrolase enzymes for selective hydrolyses which allow resolution of racemic substrates.[19, 20, 21] An example of such a reaction is the use of a protease from *streptomyces griseus* in the synthesis of the anti-inflammatory drug Ketorolac shown in Figure 4.

Figure 4. Resolution by Hydrolysis[22]



Although the enzymatic resolution of racemic substrates is an extremely important process, one disadvantage is that only 50 % of the starting material can be converted to the desired enantiomer unless a racemisation reaction is carried out; unfortunately this is not always possible. One very useful method of producing an entiomerically pure product in a theoretical yield of 100 % is to make use of the so called "meso trick".[23] The "meso trick" involves the reaction of a prochiral meso compound in which only one prochiral group or atom reacts. An example of the "meso trick" is shown in Figure 5.

Figure 5. The "Meso Trick"



Meso compound

Quantitative yields
98 % ee

When enzymes are used in water miscible organic solvents, water immiscible organic solvents or reverse micelles, they are generally surrounded by a pool of water molecules.[24] In anhydrous organic solvents, the question arises of how many water molecules are necessary for the retention of catalytic activity. A systematic study to investigate the effect of water on enzyme stability and catalytic activity examined the hydration of dry lysozyme.[25] It was found that water added to the enzyme, initially interacted with the charged functional groups of the protein, and subsequently with the uncharged polar and non-polar regions. It was found that the structure of the lysozyme, in the presence of approximately 500 molecules of water per molecule of lysozyme was nearly identical to that found for lysozyme in aqueous media. This study suggests that less than a monolayer of water is required for lysozyme to remain catalytically active. Similar studies on a variety of enzymes show large differences in the minimum number of water molecules required for catalytic activity.[26, 15] For example, α-chymotrypsin was found to require only 50 molecules of water per enzyme molecule to remain catalytically active, whereas, each molecule of polyphenol oxidase needs the presence of approximately 3.5×10^7 molecules of water.[27] The addition of water to solid enzyme preparations in organic solvents often increases activity *via* enhancement of the polar environment and flexibility of the enzyme's active site.[7] Hydration of enzymes is a necessary and absolute prerequisite for their catalytic activity. It is thought that in the anhydrous state, charged and polar groups on the enzyme interact producing a rigid and inflexible, inactive conformation.[28, 29] It has been suggested that one of the roles played by water is to form hydrogen bonds with these polar groups, thus shielding them from each other. Not only does water participate in all ionic interactions, maintaining proteins in their active conformations, but it also plays a crucial role in enzyme dynamics, conferring flexibility on the protein chain, enabling a wide variety of substrates to enter the active site.[29]

1.6.1. Enzyme Stability in Organic Solvents

Since the initial work by Klibanov and co-workers in developing the use of enzy organic solvents, the subject of enzyme stability has become of primary importanc stability of enzymes in organic solvents depends on the hydrophobicity of the sol Studies by Klibanov examined the role of water in enzyme catalysed reactions in a nu anhydrous, undried, polar, and non-polar organic media.[3] It was found that enzym need a thin layer of water on the surface of the protein to retain their catalytically conformation. In general, enzymes are found to be more active in non-polar organic s than in polar solvents. Polar solvents can replace water molecules associated w enzyme, thereby altering its conformation.[31] This change in three-dimensional struc severe enough, can result in the enzyme becoming deactivated. Water immiscible s containing water below the solubility limit of the solvent (about 0.02 to 10% depending solvent) permit certain lyophilised enzymes to be catalytically active. Within this ra water content, the enzyme activity in an appropriate organic solvent can be optimised, some cases, is comparable to that in aqueous solution.[32] It is usually found that enzyr much less active in these systems, but are still of sufficiently high activity to permit th as catalysts.

In support of these ideas, investigations carried out by Dordick utilising solid state NM ray diffraction, linear free energy correlation, and kinetic isotope effects suggest tl transition state structure in non-polar organic solvents is nearly the same as that in a solution for most enzymes.[33, 34] This indicates that the microenvironment of the e active site in non-polar organic solvents is virtually the same as that in water.

Some enzymes have been reported to have higher thermostabilities in organic solvents water.[35] In general this is because enzymes are conformationally less flexible in non-a media and resist the changes of structure which accompany thermal denaturing. Cha stereoselectivity on changing from water to an organic solvent have also been observe These changes in stereoselectivity are thought to arise mainly from the alteration release of water during the binding of the substrate to the enzyme.[38, 39]

The polar water molecules surrounding the enzyme, through which substrates must c may prevent the product and substrate molecules from diffusing to and from the en This can result in inhibition phenomena and side reactions. Product and substrate mo which are able to partition into the non-aqueous phase are less likely to cause inhibition

In an effort to establish the solvent properties that affect enzyme activity, Klibanov con a study into water-immiscibility and the polarity of the solvent and how this affected e efficiency.[40] It was concluded from this work that the often cited polarity and immis of solvent with water cannot be individually used to predict enzyme activity. In these it was reasoned that if the enzyme exhibited an increase in activity upon transition from miscible to water immiscible solvents, then immiscibility with water is indeed a releva defining solvent characteristic. It was found that enzyme activity does vary consid between solvents, but there was no consistent rise in activity when water miscible sc were replaced by water immiscible solvents. These studies suggest that the so hydrophobicity (expressed by the log *P* value) is the only useful, albeit rather crude pr of the level of enzyme activity in non-aqueous media.

Solvent index log *P* has become the most commonly used index to predict enzyme acti organic solvents, where log *P* is the solubility ratio of a solvent partitioned between c and water:[41]

$$P = [\text{Solvent}]_{\text{octanol}} / [\text{Solvent}]_{\text{water}}$$

Log *P* is an index of solvent hydrophobicity, and therefore a guide to compatibility of o solvents with high enzyme activity. Only solvents with log $P > 3.5$ are considered as immiscible and these show minimal detrimental effects on enzyme activity. Solven have low log *P* values are more likely to interact with the water layer on the surface enzyme and in the active site, with the possibility of adversely affecting the enzyme's ac

1.7. Temperature Considerations

It is widely known that enzymes are sensitive to heat.[42] Klibanov showed that in ultr reaction systems, containing less than 0.015% water, enzymes remained active for 10 h even at 100 °C.[42] In contrast, the same reaction carried out using solvents that had not rigorously dried, resulted in the enzyme remaining catalytically active for less than 2 min Under the ultra-dry conditions, the enzyme retained high catalytic activity but its subs specificity was noticeably altered. This difference in specificity results from the enz backbone being more rigid in anhydrous systems which inhibits conformational change prevents the acceptance of bulky substrates. This places a limitation on the use of ultra high temperature systems. Water is essential if the enzyme is to accept a variety of subst as the water behaves as a lubricant, allowing the protein chains to move across each other

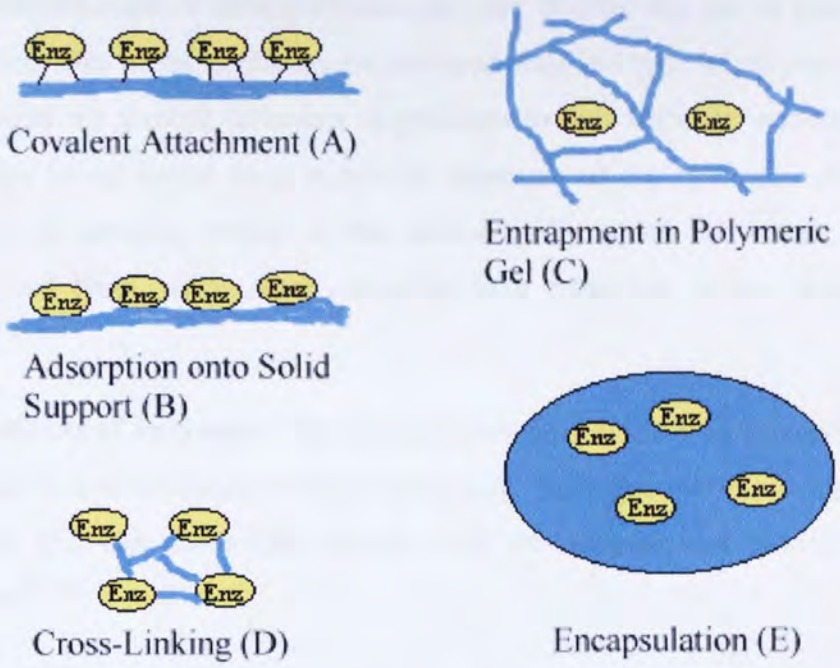
1.8. Enzyme Preparations

1.8.1. Immobilisation of Enzyme Catalysts

The folded conformations of enzymes are relatively fragile and prone to unfolding v subjected to strongly acidic or basic environments, high concentrations of salt, polar solv heat or radiation.[28] This process is termed denaturing and can be defined as a major ch from the original native structure without alteration of the amino acid sequence. If enz are to be used under relatively harsh conditions, they need to be protected from denaturir stabilisation.

There are various mechanisms by which enzymes can become denatured and these inc simple irreversible unfolding of the protein chains, S-S bridging groups being destroye way of beta elimination, hydrolysis and deamidation reactions. A number of technique the stabilisation of enzymes have been developed, allowing enzymes to be used under a r wider variety of experimental conditions,[10] see Figure 6.

Figure 6. Methods for Immobilising Enzymes



A. **Covalent attachment of enzymes to solid supports.** Enzymes are usually immobilised through their amino or carboxyl groups. Some loss in enzyme activity is to be expected with this technique as conformational changes are induced by the formation of covalent bonds. A variety of supports have been investigated including, porous glass, ceramics, stainless steel, sand, charcoal, cellulose, synthetic polymers and metallic oxides.[43, 44]

B. **Adsorption of enzymes onto solid supports.** Ion exchange resins readily adsorb most proteins *via* electrostatic interactions. This is a simple technique in that a pH buffered solution of the enzyme is mixed with the resin, and the resultant supported enzymes are then lyophilised. In contrast to covalent attachment, the weak binding forces used in this approach reduce the loss in enzyme activity compared to that of non-supported enzyme systems, but the added problem of desorption must now be considered.[43, 44]

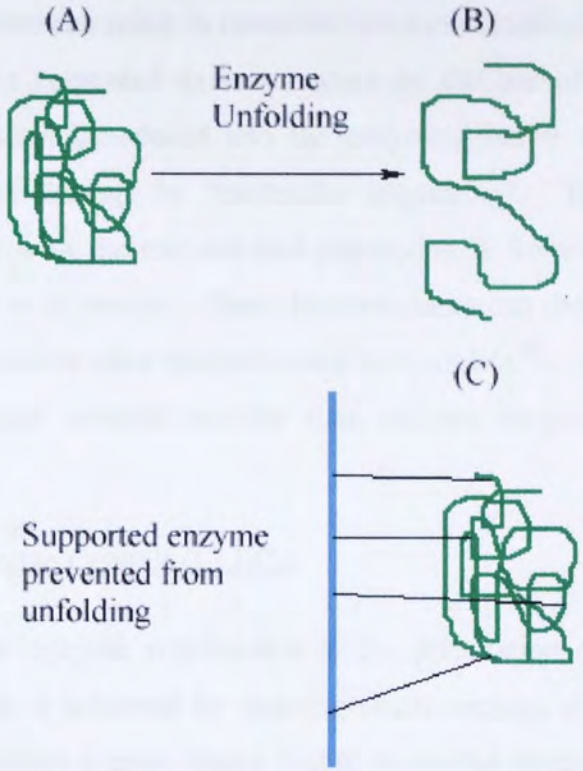
C. **Entrapment of enzymes in polymeric gels.** In this technique the enzyme is trapped within a polymeric matrix. The supported enzyme is produced by adding the enzyme to a suitable monomer and then polymerising it to form a gel. Using this approach monomers have to be carefully selected as the product gel must allow both substrates and products to diffuse through its structure unhindered.[43, 44]

D. **Cross-linking of enzymes with bifunctional reagents.** The cross-linking may be either intermolecular or intramolecular and may involve the use of filler proteins such as albumin. One of the problems encountered with this type of enzyme modification is the potential for limited diffusion of products to and from the enzyme due to some active sites being buried deep within the structure of the material. Additionally, the formation of covalent bonds in this method of support can cause conformational changes and block active sites, resulting in a reduction of the enzyme's catalytic activity.[43, 44]

E. **Encapsulation of enzymes.** The enzyme is encapsulated by a membrane that is only permeable to low molecular weight substrates. This system allows for the control of molecules that can come into contact with the enzyme and provides a degree of selectivity.[43, 44]

The methods A and D create strong covalent bonds between the enzyme and support but can lead to deactivation of the enzyme due to attachment through their active centres. The other methods are simple but create no strong bond between the enzyme and support and hence detachment of the enzyme from the support can occur, this is not usually a problem in organic solvents. Immobilisation techniques such as those described above can increase the tolerance of the enzyme to many of the potential denaturing conditions. In addition to enzyme supported systems, attempts have also been made to increase the internal binding forces (e. g. by increasing H-bond interactions, metal binding, and conformational restrictions) and minimising the surface charge of the enzyme so that the enzymes will be more stable in polar solvents.[7] As with the other immobilisation techniques, the problem arises that on altering the enzyme in this way the catalytic activity may be altered or even destroyed. An example of how supports protect enzymes from denaturing is shown in Figure 7.

Figure 7. Enzyme Denaturing



1.8.2. Modification of Enzymes

1.8.2.1. Lyophilisation of Enzymes

Enzymes such as lipases and proteases are commonly available as lyophilised powders.[33] In this method the enzyme is lyophilised in a pH buffer. Selecting the correct pH buffer is critical as pH alters the overall conformation of the enzyme and also the ionisation of groups within the active site. Lipases are an excellent example of the importance of pH on enzyme conformation. All lipases for which X-ray crystallographic structures have been determined possess a polypeptide flap or lid covering the active site[13] as shown in Figure 1. This flap can be either closed over the active site or open exposing the active site. Selection of the correct pH buffer solution causes the flap to attain the open conformation, giving the most active form of the enzyme. Once the appropriate pH buffering system has been determined, the lipase is then freeze dried, locking the flap into the open, active conformation.

1.8.2.2. Lyoprotectants

The process of lyophilisation can result in reversible conformational changes which deactivate the enzyme. This can be prevented to some extent by the use of lyoprotectants such as sugars.[45] Lyoprotectants are introduced into the enzyme / buffer solution mixture before lyophilisation and prevent damage by "molecular imprinting". This is achieved by the lyoprotectants interacting with the enzyme and preventing it from distorting by inhibiting enzyme chain movement as it freezes. These lyoprotectants can then be removed from the enzyme by pyridine extraction after lyophilisation is complete.[45] Enzymes treated in this manner often exhibit higher catalytic activity than enzyme preparations not treated with lyoprotectants.

1.8.2.3. Cross Linked Enzyme Crystals (CLECs)

A promising approach to enzyme stabilisation is the preparation of cross-linked enzyme crystals (CLECs).[46] This is achieved by reacting small enzyme crystals (~ 10^{-4} m) with glutaraldehyde. This produces a cross-linked highly organised structure containing channels where reasonably sized substrates and products can diffuse to and from active sites. CLECs are more robust and active than conventional immobilised enzymes. This remarkable stability is a consequence of the enzyme crystal packing being reinforced by covalent cross-links. In general CLECs show a higher or equal activity and are more stable than conventional enzyme preparations produced by other methods. For example, thermolysin CLECs have been shown to be remarkably active and stable under a broad range of conditions.[47, 48]

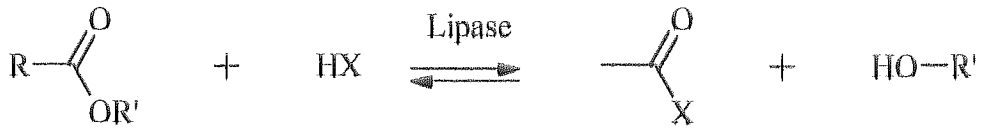
1.9. Enzyme Catalysed Organic Reactions

Enzymes are able to catalyse a wide variety of reactions, for example, halogenation and oxidation (haloperoxidases[49]), epoxidation (mono-oxygenase[50]), nitrile hydrolysis (nitrilases[51]), Diels-Alder additions (Diels-Alderase[52]), Michael addition[53] (bakers yeast[54, 55, 56]), glycosidation and transglycosidation (carbohydrase[57] and glycosidase[10]), transcyanation (oxynitrilase[58]), esterifications, transesterifications, aminations and hydrolysis (hydrolases[59]).

1.9.1. Hydrolytic Enzymes

About two thirds of the research reported on the biotransformations of non-natural organic compounds makes use of enzymes from the group of hydrolases.[58, 60] The popularity of hydrolases arises from their ready availability and their ability to function without expensive cofactors. The most common enzymes in this category are lipases, which have been examined extensively and are commercially available at very affordable prices. Besides their natural function in the hydrolysis of lipids, lipases catalyse a large number of other reactions including esterification, transesterification, amination, transamination, thiolation, transthiolation and oxidation,[61, 62] see Figure 8.

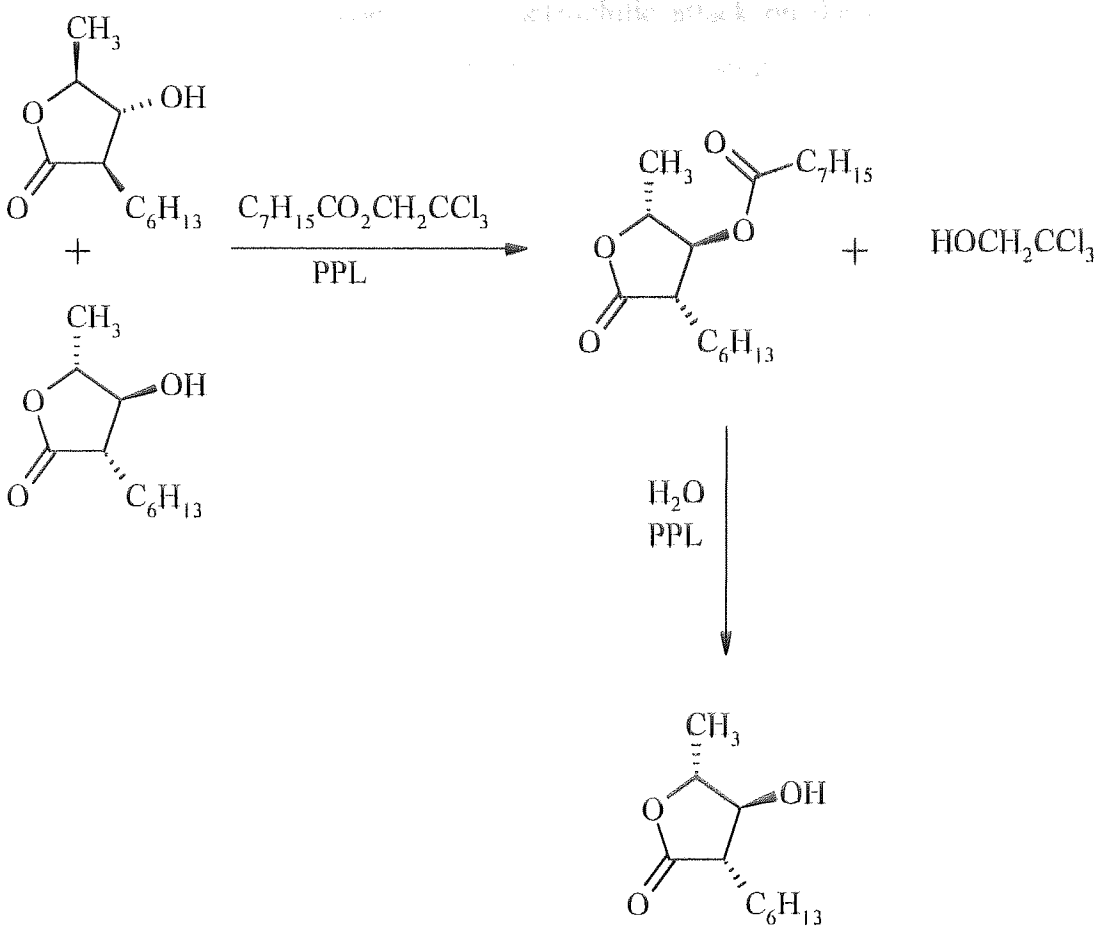
Figure 8. Some Lipase Catalysed Reactions



X = R"O-, R"NH-, R"S-, $-NH_2$ or HO_2-

An example of a transesterification reactions is shown[63] in Figure 9.

Figure 9. Example of Transesterification



>98 % ee, 20 % yield

1.9.1.1. Mechanism of the Lipase Enzyme-Substrate Interaction

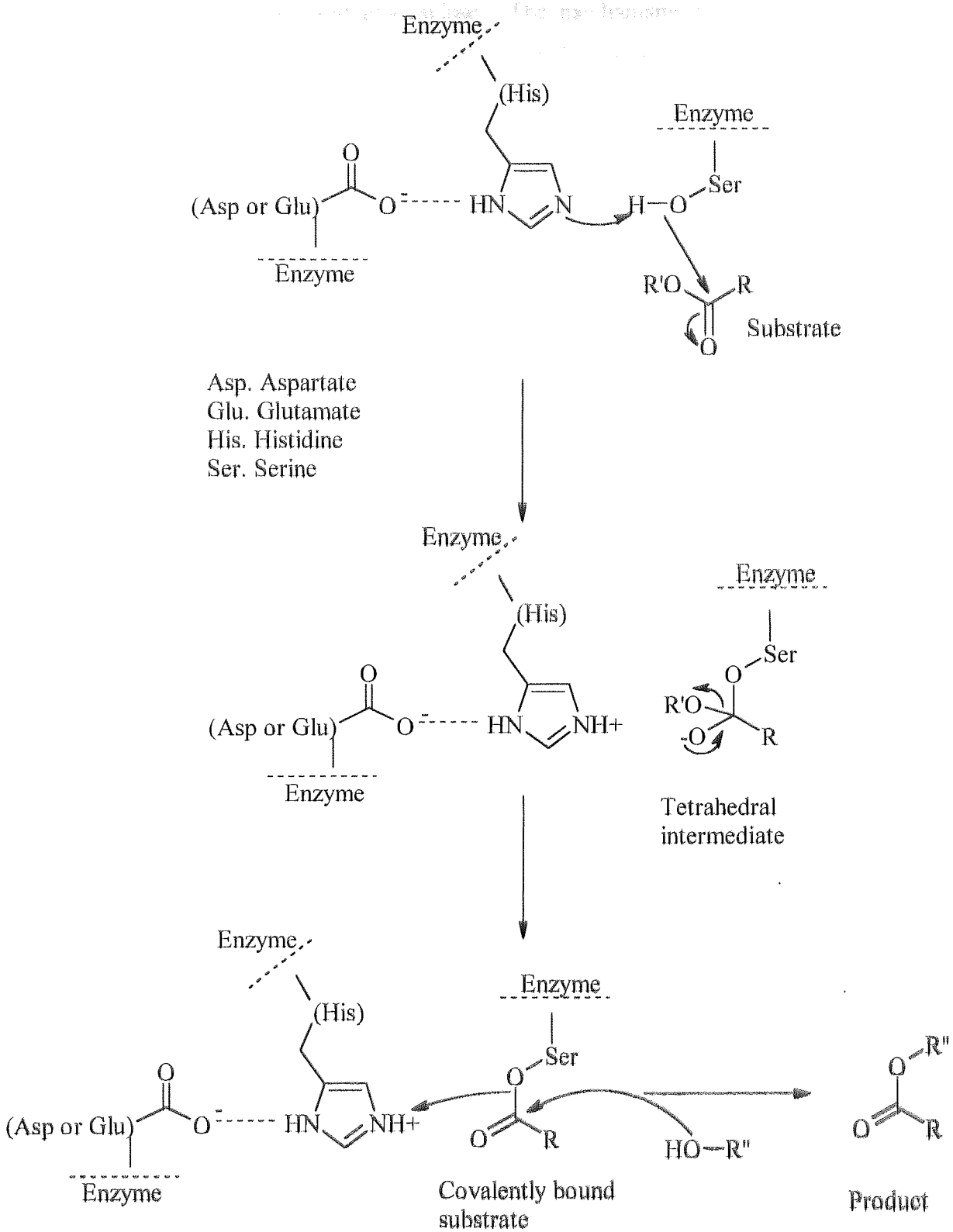
The lipases for which structures have been determined have a groove or cleft on the surface, with the active site at the bottom of the cleft and a lid or polypeptide flap covering the opening of the cleft.[64, 65]

Lipases belong to the serine hydrolase group of enzymes which all act *via* a similar mechanism. Three amino acid residues, serine, histidine and aspartic / glutamic acid (the catalytic triad) are responsible for the actual catalytic process.[66] This process is represented diagrammatically in Figure 10.

Histidine acts as a general base catalyst and enhances the nucleophilicity of the oxygen of serine. This oxygen atom carries out a nucleophilic attack on the carboxyl group of the enzyme bound (not covalently bound at present) substrate. An unstable tetrahedral intermediate is formed whose negatively charged oxygen atom may be stabilised by hydrogen bonding with –NH of a glycine residue in the backbone. The carbonyl substrate is now covalently bonded to the serine, and undergoes a nucleophilic attack by water (or other nucleophile), reforming serine hydroxyl group and producing product. The role of the carboxylate group is to stabilise the protonated imidazole ring.

Lipases are able to accommodate a variety of different structures in the acyl donor and the acyl acceptor. With chiral substrates, many of these reactions proceed with a high degree of enantioselectivity.[67, 68]

Figure 10. The Catalytic Mechanism of Serine Hydrolases



Other enzymes used in this work are chloroperoxidase, an oxynitrilase from bitter almonds and the glycosidases, lactase and glucosidase. The mechanisms by which these enzymes operate are much less well understood than those of the serine hydrolase enzymes. A brief discussion of these mechanisms will be given in the relevant discussion chapters.

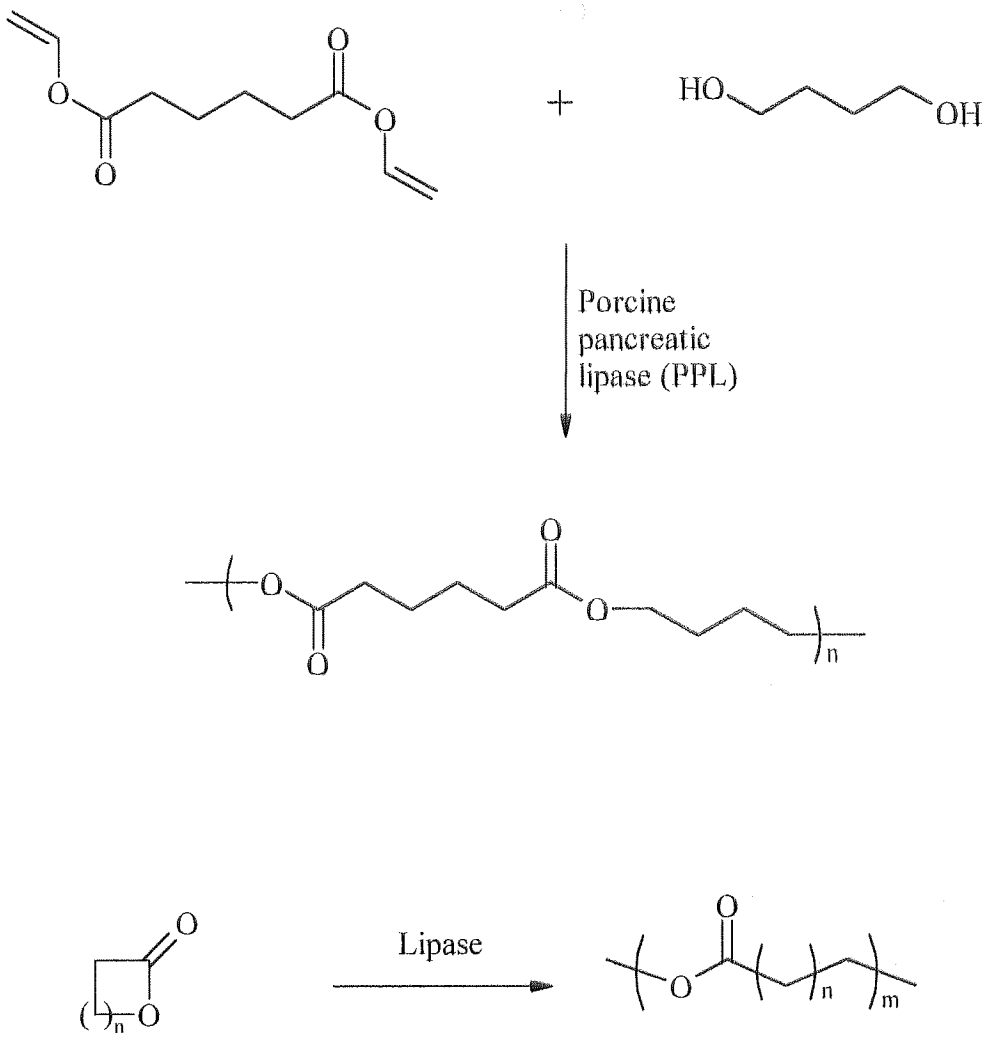
1.10. Enzymes in Polymer Synthesis

1.10.1. Enzymatic Polymerisation

In contrast to the use of enzymes in organic synthesis, the number of reports of enzymes being used in polymer synthesis is much smaller.[4, 69] There are three main areas of polymer synthesis in which enzymes have been applied. By far the largest of these, is the use of hydrolases in organic solvents as catalysts for polyester synthesis.[70]

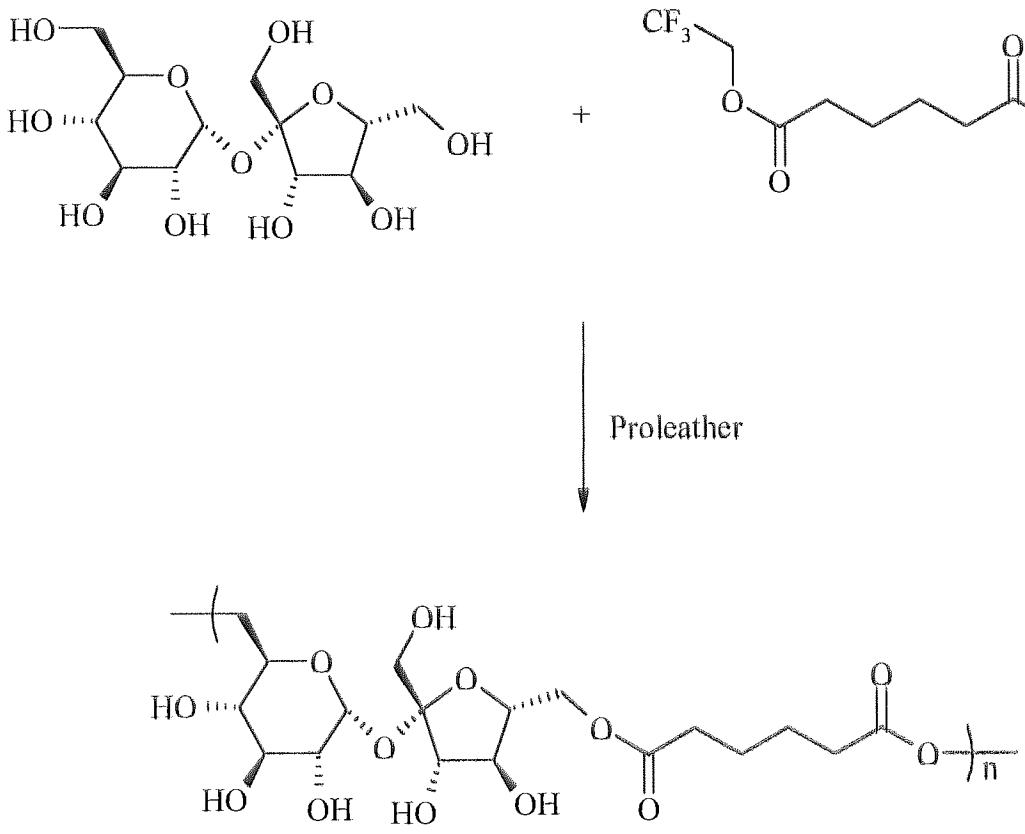
Lipases have been shown to catalyse polyesterification, polytransesterification and ring opening polymerisation in anhydrous organic solvents[4, 70, 71] see Figure 11.

Figure 11. Polyester Synthesis



These enzymatic processes often require reaction times of several days to achieve high molecular weight polymers. The stereoselectivity of enzyme catalysed reactions has been used to prepare optically active polyesters from racemic starting materials.[72, 73] The regioselectivity of enzymes allowed a one step synthesis of a sucrose containing polyester, without the need for any protecting groups.[74, 75] The reaction of bis(2,2,2-trifluoroethyl) adipate with sucrose in the presence of the protease proleather resulted in the incorporation of sucrose into the polymer backbone,[75] see Figure 12.

Figure 12. Enzymatic Synthesis of a Sucrose Polyester



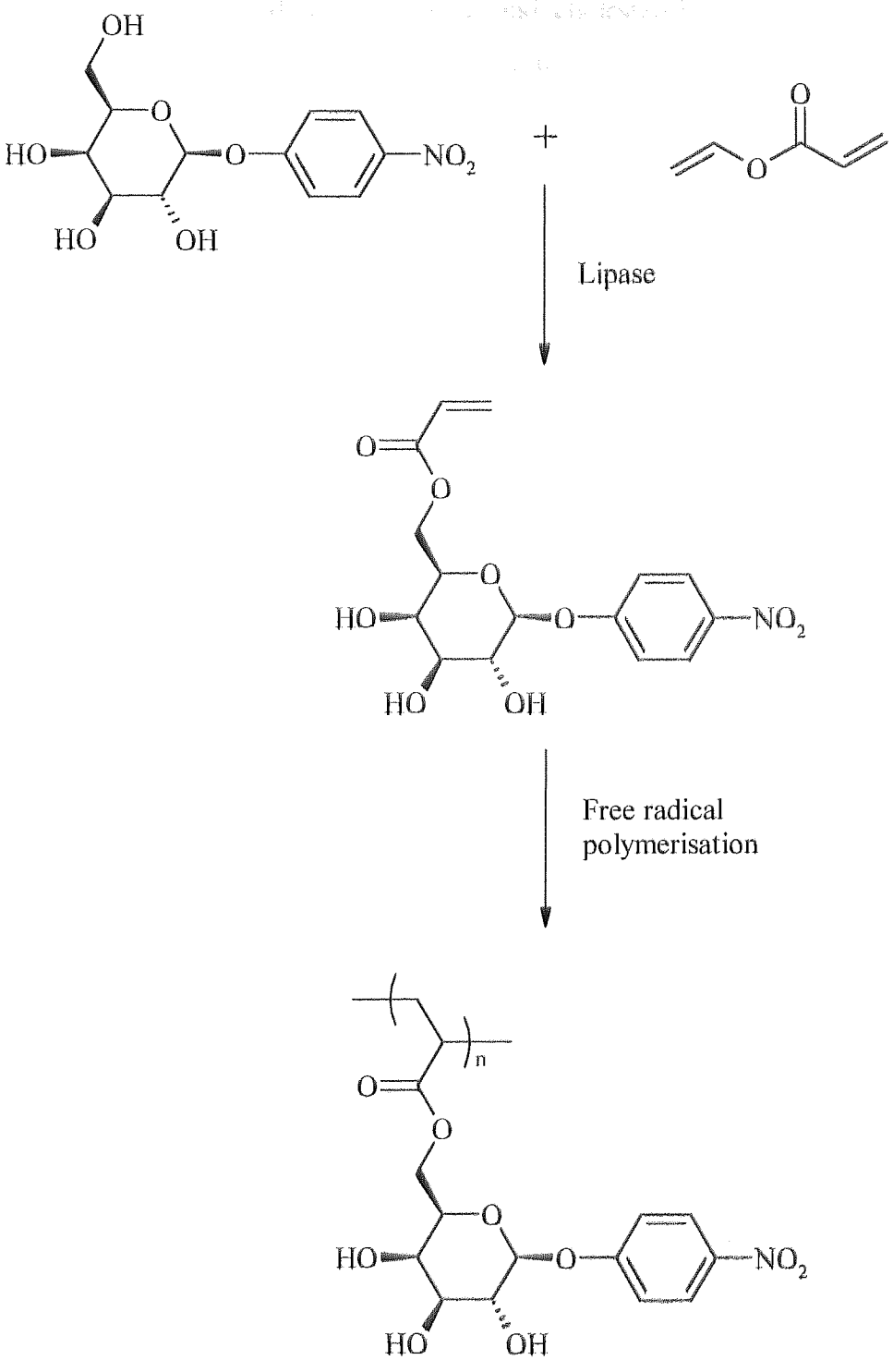
Proleather was able to achieve this remarkable selective synthesis by its ability to catalyse the reaction of the primary hydroxyl groups on sucrose, leaving the secondary hydroxyl groups intact.

1.10.2. Monomer Synthesis

In addition to enzymes being used to synthesise polymers, they have also been used to synthesise novel monomers that have subsequently been polymerised.[5] Dordick esterified carbohydrates such as β-methyl galactoside with acrylates. Polymerisation of these monomers gave polymers with β-methyl galactoside pendent groups.[76, 77]

In another study Dordick and co-workers used lipases to carry out a selective transesterification reaction between vinyl acrylate and 4-nitrophenyl-β-D-galactopyranoside.[78, 79] This monomer was radically polymerised giving a polymer containing pendent sugar groups, see Figure 13.

Figure 13. Sugar Containing Monomers



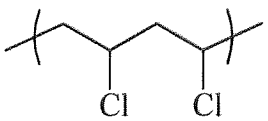
Ritter has used enzymes to prepare a variety of acrylate based monomers containing either terpenes or steroids, e.g., menthol, testosterone and cholesterol.[80] These optically active monomers, were then polymerised with a free radical initiator to give functionalised, optically active polymers. This is a valuable route to optically active polymers that would be difficult to synthesise with conventional chemical synthetic techniques.

1.11. Modification of Synthetic Polymers

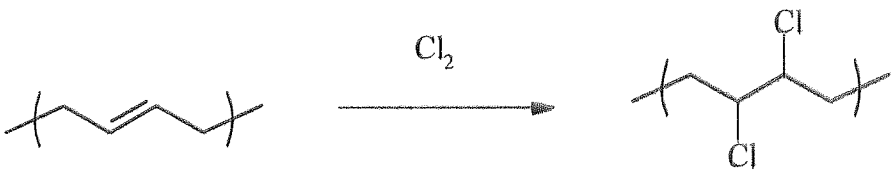
Although there are now many examples of enzymes being used as polymerisation catalysts and for monomer synthesis, relatively little work has been carried out using enzymes to modify synthetic polymers.

Chemical modification of synthetic polymers is well known and is especially attractive when the resultant polymer cannot be easily manufactured from readily available monomers, indeed, some polymers can only be synthesised by modifying a preformed polymer.[81, 82] Figure 14 shows how poly(vinyl chloride) is predominantly head to tail when produced by free radical polymerisation methods. Head to head architecture can only be obtained to a high degree if *cis* / *trans* poly(butadiene) is halogenated.[83]

Figure 14. Modification of Synthetic Polymers



Head to Tail
Poly (vinyl chloride)



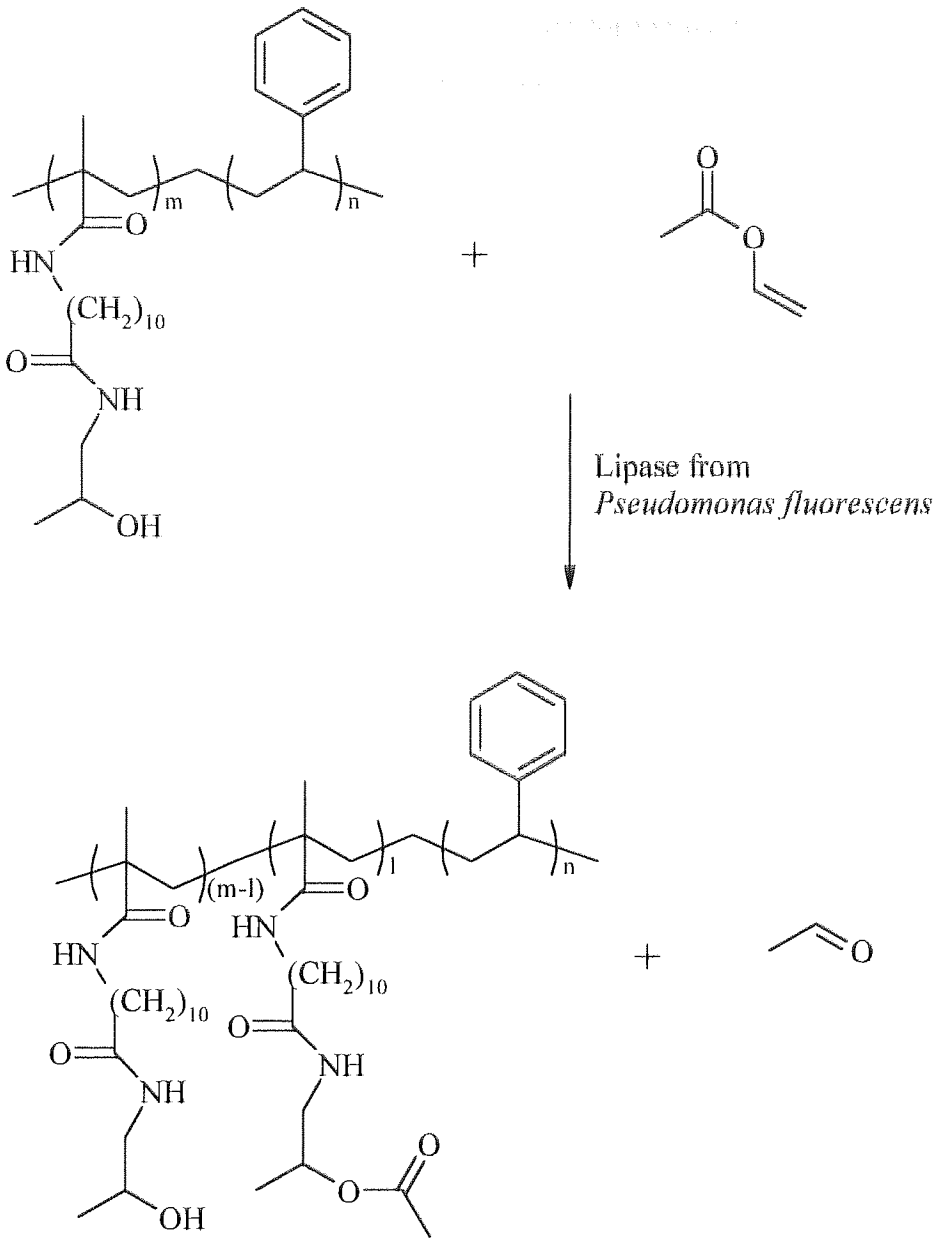
Poly(butadiene)

Head to Head
Poly(vinyl chloride)

Enzymes have been used for the modification of the naturally occurring polymers such as chitosan and alginate.[84, 85] However, the only examples of enzymes being used to modify a synthetic polymer were reported by Ritter and by Wallace.[86, 87]

Ritter utilised a polymer with hydroxyl groups on the penultimate carbon of a long side chain ("comb-like" polymers) to transesterify activated esters such as vinyl acetate, see Figure 15. Polymers containing racemic hydroxyl groups were selectively transesterified, producing chiral polymers. The study also showed that the rate of reaction was dependent on the polymer's structure. Polymers containing longer spacer groups, either in the polymer backbone, or the pendent group, promoted the rate of reaction by increasing interactions between the enzyme and side chain. In conclusion Ritter suggested that the catalytic activity of a lipase is not limited to the modification of low molecular weight compounds, but can also be used to acylate larger comb-like polymers. In other experiments Ritter was able to esterify comb-like polymers containing a carboxylic acid functionality at the end of the side chain.[86] Similar conclusions were also drawn from these experiments.

Figure 15. Modification of "Comb-like" Polymers



1.12. Aims and Scope of Project

The work discussed above involves the use of enzymes for the modification of groups distant from the polymer backbone. There has however been no research into the enzyme catalysed modification of the backbone or groups close to the polymer chain.

With this in mind it was decided to investigate the application of enzymes to the modification of the following systems:

1. The epoxidation and halogenation of polyalkenes.
2. The Baeyer-Villiger and transcyanation reactions of polymers containing ketones.
3. The transesterification, esterification and transglycosidation reactions of polymers containing hydroxyl or ester functionalities.

Chapter 2.

Oxidation of Polymers

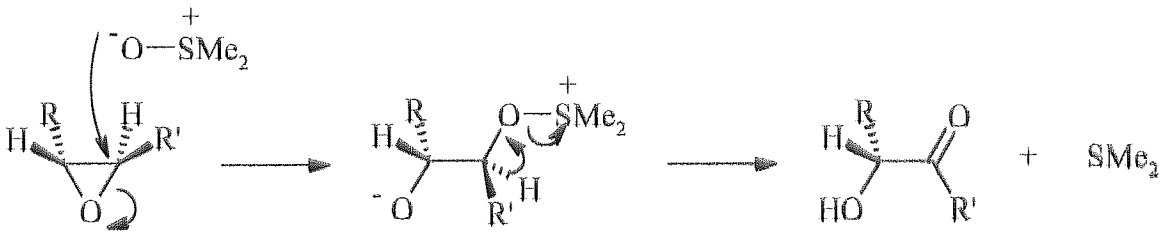
2.0. Oxidation of Polymers

2.1. Epoxidation

2.1.1. The Importance of Epoxides

Epoxides are one of the most important and versatile groups used in organic synthesis. This is due to the ability of the epoxide ring to be opened stereoselectively by a variety of nucleophiles. Alcohols, amines, HX (X=halogen), sulfoxides and Grignard reagents are amongst the many nucleophiles used for opening the epoxide ring,[88] see Figure 16.

Figure 16. Ring Opening with Dimethyl Sulfoxide to Form an α-Ketol

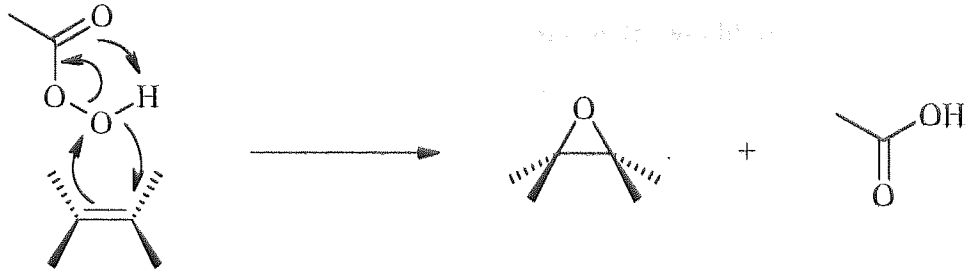


The direction in which an unsymmetrical epoxide ring is opened depends on the conditions used. If a basic nucleophile is used, attack takes place at the less hindered epoxide carbon. If acid conditions are used, however, a different reaction course is followed, and attack of the nucleophile occurs primarily at the more highly substituted carbon atom.[89]

2.1.2. Epoxide Synthesis

Chemical epoxidation is usually achieved by use of peroxyacids or by the use of complex metal based catalysts such as in the Sharpless epoxidation.[90] Epoxidation *via* peroxyacids follows the mechanism[89, 91] shown in Figure 17.

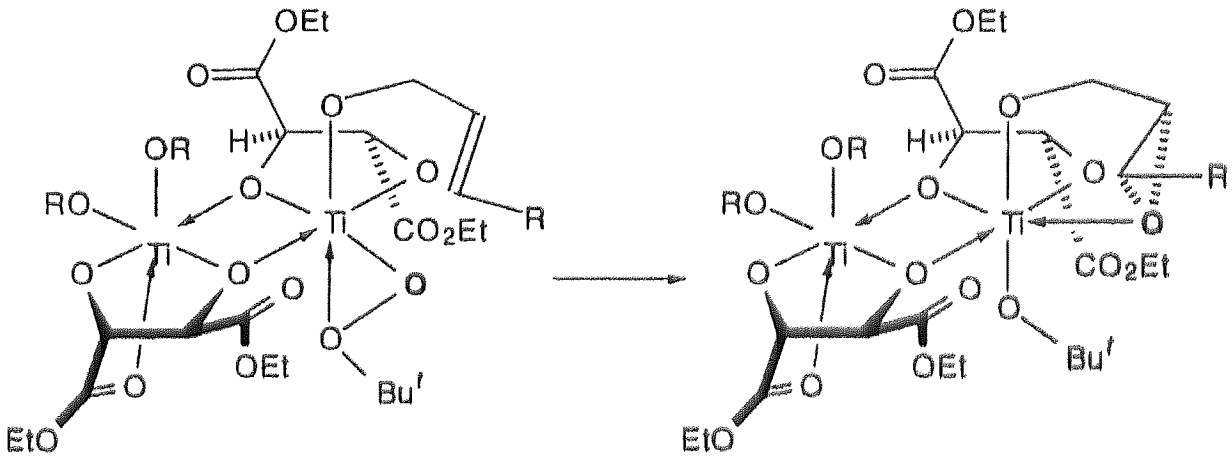
Figure 17. Mechanism of Peroxyacid Epoxidation



Alkenes epoxidised with this method show increased activity if the alkene contains electron releasing groups or if the peroxyacid contains electron attracting groups.

Unlike standard epoxidation techniques, the Sharpless epoxidation converts allylic alcohols to optically active epoxides with enantiomeric excesses typically greater than 90 %.[90] The Sharpless epoxidation involves the use of *tert*-butyl hydroperoxide in the presence of Ti(O*i*-Pr)$_4$ and a chiral dialkyl ester of tartaric acid. It is thought that oxygen is transferred from the hydroperoxide to the alkene within a complex in which the configuration of tartaric diester used determines which face of the alkene the oxygen is delivered to. This complex[92] is shown in Figure 18.

Figure 18. Structure of the Sharpless Epoxidation Catalyst Redrawn from Reference 92



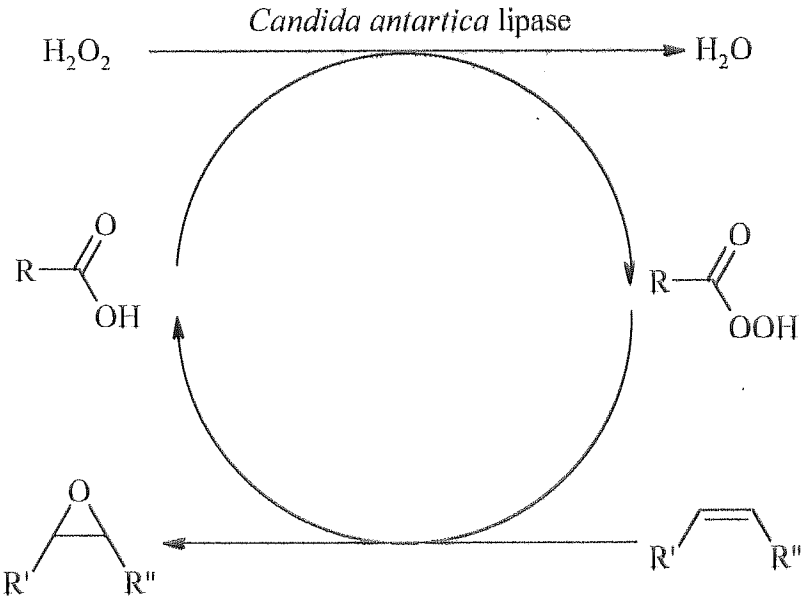
2.1.3. Chemical Epoxidation of Poly(butadiene)

Poly(butadiene) has been epoxidised by Akcelrud with *m*-chloroperoxybenzoic acid.[93] Poly(butadiene) often contains three types of double bond, *cis* and *trans* double bonds of the backbone and pendent vinyl groups. Akcelrud investigated the different reactivity of the three types of double bond and found that *trans* double bonds were more reactive than *cis* double bonds, which in turn were far more reactive than the vinyl double bonds. Yields of epoxide were found to be as high as 77 %. Cole-Hamilton utilised a molybdenum based catalyst to achieve 100 % epoxidation of *cis* and *trans* backbone alkenes leaving vinyl groups untouched.[94] It was reported that most of the backbone double bonds had reacted before the vinyl double bonds began to react. Attempts to achieve 100 % conversion of all double bonds using conditions that were more vigorous resulted in a complex mixture of different products. Cole-Hamilton also reported that *cis* double bonds were more reactive than *trans* double bonds which is contrary to Akcelruds' conclusions. One possible explanation put forward by Zuchowska which would explain these apparently contradictory results is that the reactivity of the *cis* and *trans* double bonds depends on the polymer's microstructure.[95]

2.1.4. Enzymatic Epoxidation of Poly(butadiene)

Alkenes can be epoxidised by mono-oxygenase enzymes. However, there are a number of disadvantages to the use of these systems. At present the instability of the enzymes, the importance of cofactors, and the need for several proteins to function together, rule out the use of isolated enzyme systems. The alternative is to rely on whole cells but this would also be impractical as these biotransformations would require the use of specialist microbiological techniques. In addition, the ability of these organisms to assimilate the large molecules of synthetic polymers would be doubtful. Another disadvantage is that expensive cofactors such as NADPH need to be recycled. Also, whole cells cannot be used in organic solvents thereby severely limiting the number of suitable synthetic polymers. The separation of the modified polymer would probably be problematic as it would need to be separated from the cell debris, much of which is also polymeric. The only notable isolated enzyme which can be used for the epoxidation of alkenes is chloroperoxidase from *Caldariomyces fumago*. Although this enzyme does not require cofactors, it operates in aqueous buffer thereby limiting the number of suitable polymeric substrates. The enzyme catalysed epoxidation procedure developed by Björkling shows more promise as it involves the reaction of an alkene in the presence of a lipase, catalytic quantities of a long chain carboxylic acid and hydrogen peroxide as an oxidant.[96, 97] The enzyme catalyses the synthesis of the peracid which then reacts with the alkene in a conventional chemical reaction. The enzyme does not interact with the alkene substrate during the actual epoxidation step. The reaction developed by Björkling is shown in Figure 19.

Figure 19. *Candida antartica* Lipase Oxidation Cycle



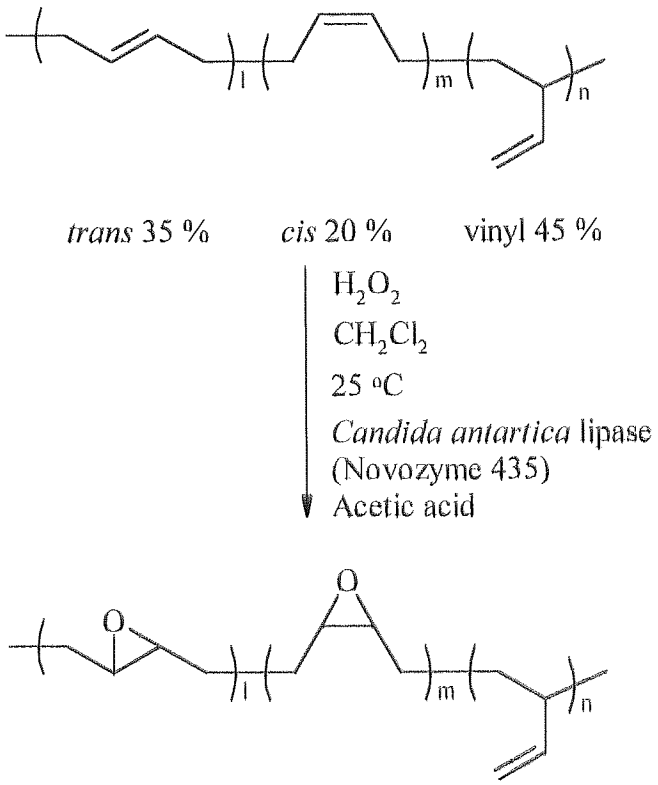
In this study by Björkling it was found that the most efficient enzyme was an immobilised lipase from *Candida antartica* (Novozyme 435).

The advantage of this enzymatic epoxidation process is that the reaction can be carried out in organic solvents and occurs under very mild condition with the potentially hazardous peroxyacid being generated *in situ* in low concentration. The simplicity of this system suggested that polymeric systems might be modified by this process as it was not necessary for the enzyme to interact with the polymer.

2.1.5. Initial Experiments

Initial experiments (section 7.5.1) were carried out to investigate the possibility of epoxidising phenyl terminated poly(butadiene) (Mn 1300) by using the method of Björkling,[96] see Figure 20. The reaction was carried out in dichloromethane at 25 °C with 10 mol % of acetic acid, 10 wt % of *Candida antartica* lipase (Novozyme 435) and a 27.5 wt % solution of hydrogen peroxide. The reaction was carried out with the exclusion of light, thus preventing ultra-violet light induced breakdown of hydrogen peroxide.

Figure 20. Epoxidation of Poly(butadiene)



The ^{1}H NMR and ^{13}C NMR spectra of poly(butadiene) are shown in Figures 21 and 22. The corresponding NMR spectra of the epoxidised polymer obtained after 24 hours are shown in Figures 23 and 24.

Figure 21. ^{1}H NMR of Poly(butadiene)

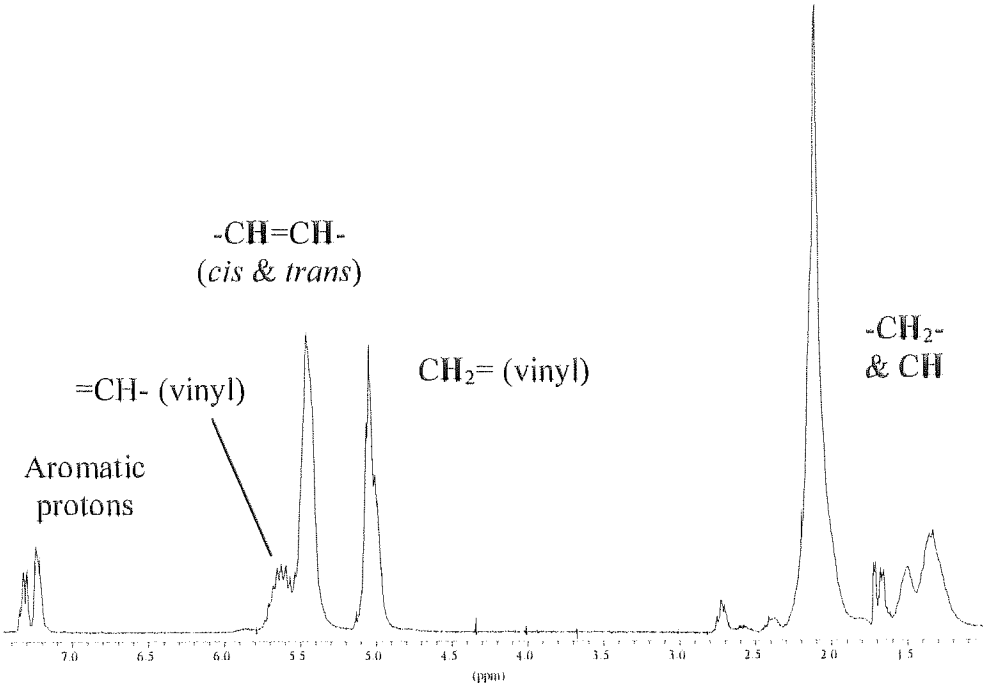


Figure 22. ^{13}C NMR of Poly(butadiene)

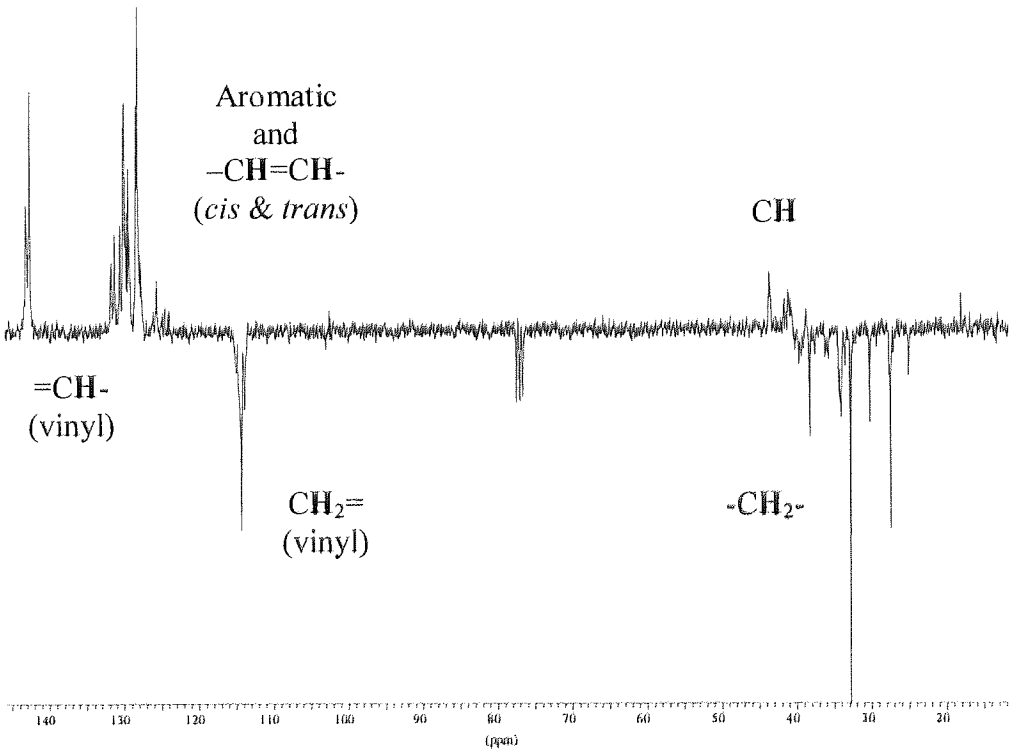


Figure 23. ^{1}H NMR of Epoxidised Poly(butadiene)

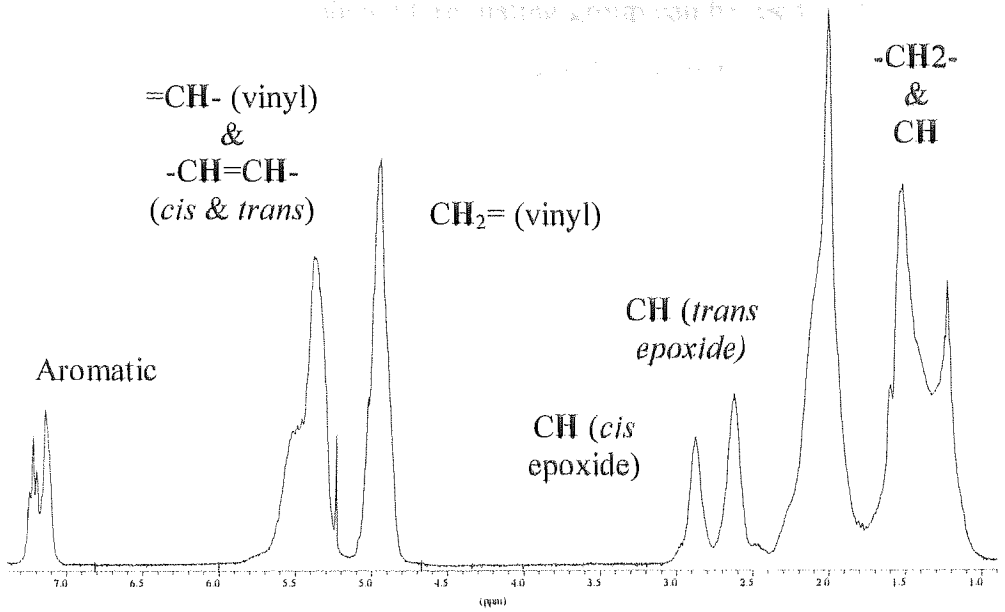
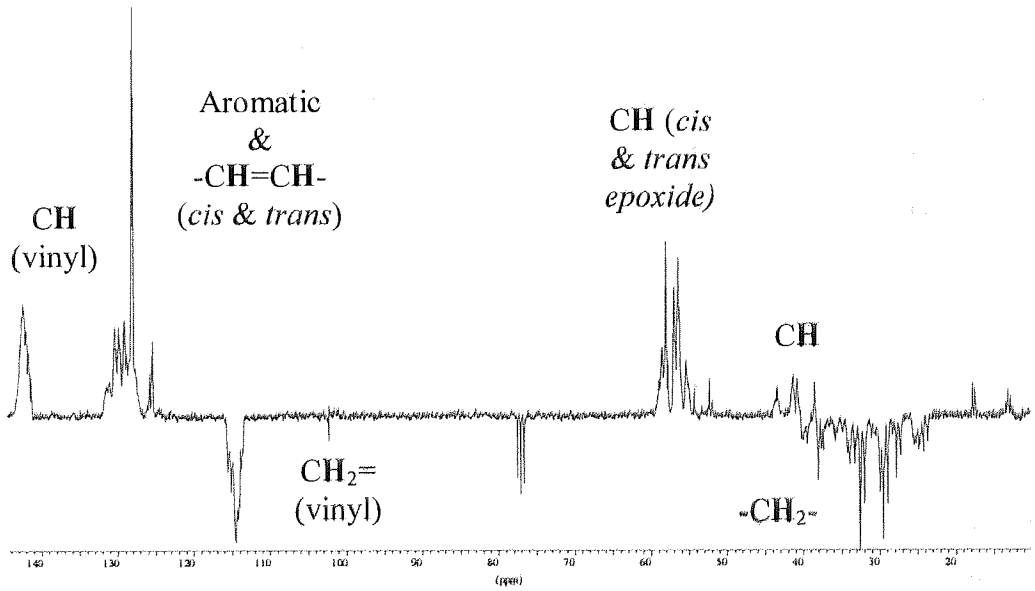


Figure 24. ^{13}C NMR of Epoxidised Poly(butadiene)



New peaks which are present in the product NMR spectra (Figures 23 and 24) can be assigned to the epoxidised polymer. The phenyl terminating group can be used to determine the extent of epoxidation and the selectivity as it is unchanged by the reaction. Each polymer chain is terminated with one phenyl group containing 5 protons, therefore, the area for one proton can be calculated from the ^{1}H-NMR spectrum. The number of vinyl =C**H**$_2$ protons can then be calculated which subsequently allows the calculation of number of vinyl =C**H**- protons. The *cis* and *trans* –C**H**=C**H**- proton peaks overlap with the vinyl =C**H**- protons but can now be calculated by simple subtraction of the known number of vinyl =C**H**- protons. Elemental analysis of the epoxidised polymer suggested that 30 % of the double bonds had been epoxidised. This is in agreement with the 30 % calculated from the ^{1}H-NMR spectrum. It is apparent from the ^{1}H-NMR spectra that only the *cis* and *trans* double bonds had been epoxidised leaving the vinyl double bonds completely untouched. As the composition of the polymer was known, it was established that 60 % of the double bonds in the polymer backbone had been epoxidised. Obviously, the elemental analysis gives only a total value for the yield of epoxide and no indication of the nature of the double bonds which had been epoxidised. Although we used elemental analysis to confirm the NMR results, and in many cases the results were in good agreement, there were also cases where the agreement was poor due to the retention of solvent in the viscous products. As the NMR analysis gave more information about the selectivity of the reaction and was reproducible, this technique was routinely used to analyse the products. GPC analysis showed that the molecular weight of the polymer was not significantly altered and therefore indicated that no chain scission or crosslinking had taken place. Control experiments containing no enzyme catalyst showed that no reaction took place.

Once a basic reaction procedure had been established, the effect of varying a number of reaction conditions was investigated. The results shown in the following tables are the results of individual experiments since it is difficult to sample multiphase systems accurately.

2.1.6. The Effect of Time on the Epoxidation of Poly(butadiene)

Reactions were carried out in dichloromethane at 25°C with 10 mol % of acetic acid, 10 wt % of enzyme and a 27.5 wt % solution of hydrogen peroxide added in one portion at the start of the reaction.

Table 1. The Effect of Time on the Epoxidation of Poly(butadiene)

Reaction Time (Hours)	Percentage Vinyl Groups Epoxidised	Percent *cis* / *trans* Groups Epoxidised by ^{1}H NMR Analysis	Percent *cis* / *trans* Groups Epoxidised by Elemental Analysis	GPC Mw
2	0	0	17	3960
6	0	24	25	4080
24	0	54	67	4240
96	0	59	54	4440

It is apparent from Table 1 that the reaction is essentially complete after 24 hours. One possible reason for this is that the enzyme becomes oxidised and inactive during the course of the reaction.

2.1.7. The Effect of Various Acids on the Epoxidation of Poly(butadiene)

Table 2. shows the effect of various acids on the epoxidation of poly(butadiene). Reactions were carried out in dichloromethane at 25°C with 10 wt % of enzyme and 27.5 wt % solution of hydrogen peroxide added in one portion at the start of the reaction. All experiments were continued for 24 hours.

Table 2. The Effect of Acids on the Epoxidation of Poly(butadiene)

Acid Used, (mol %)	Percentage Vinyl Groups Epoxidised by ^{1}H NMR analysis	Percentage *cis* / *trans* Double Bonds Epoxidised by ^{1}H NMR analysis	GPC Mw
Acetic acid, (5)	0	38	-
Acetic acid, (10)	0	59	4580
Acetic acid, (20)	0	25	-
Palmitic acid, (10)	0	26	5430
Octanoic acid, (10)	0	60	-
2,2,2-trifluoroacetic acid, (10)	0	57	-

In the initial enzyme catalysed epoxidation experiments of poly(butadiene) 10 mol % of acetic acid for each mol of double bonds present in poly(butadiene) was used. It was thought that increasing the quantity of acetic acid would increase the rate of formation of peroxyacetic acid and therefore increase the rate of epoxidation, allowing a higher degree of epoxidation to occur before the enzyme became inactive. Surprisingly, it was found by ^{1}H-NMR analysis that a lower yield of epoxide was obtained both with a higher concentration of acetic acid (20 mol %) and with a lower concentration (5 mol %). One possible explanation for this observation is that higher concentrations of acetic acid may increase the rate of enzyme deactivation, whilst at the lower concentration the rate of epoxidation is reduced and the enzyme becomes deactivated at a lower epoxide conversion.

Björkling used long-chain carboxylic acids in his development of this enzyme catalysed epoxidation procedure.[96] We found that when palmitic acid was used in place of acetic acid lower yields of epoxide were obtained. Palmitic acid produced emulsions during the work up procedure and consequently lowered yields were obtained. Octanoic acid also produced emulsions, but these were easier to break and yields comparable to those with acetic acid were obtained. The easier work up procedure for acetic acid was advantageous.

2,2,2-Trifluoroperoxyacetic acid is often the acid of choice in epoxidation reactions.[89] Reactions using 2,2,2-trifluoroacetic acid gave similar results to those using acetic acid with no increase in epoxide formation.

2.1.8. The Effect of Hydrogen Peroxide Concentration and the Method of its Addition on the Epoxidation of Poly(butadiene)

Björkling found that addition of hydrogen peroxide over the course of the reaction increased product yield by allowing the enzyme to remain active longer.[96] High hydrogen peroxide concentrations rapidly damage the enzyme by an oxidative denaturing processes. Low hydrogen peroxide concentrations are achievable by either using a more dilute solution of hydrogen peroxide, or by the Björkling method of addition over the duration of the reaction. Slow addition of hydrogen peroxide enables the enzyme to consume the oxidising agent at a rate which minimises its exposure to unreacted hydrogen peroxide. The results of this investigation are presented in Table 3. Reactions were carried out in dichloromethane at 25 °C with 10 wt % of enzyme and 10 mol % of acetic acid for 24 hours.

Table 3. The effect of Hydrogen Peroxide Concentration and the Method of its Addition on the Epoxidation of Poly(butadiene)

Concentration of H_2O_2 (%) and Method of Addition [a, b]	Percentage Vinyl Groups Epoxidised	Percent *cis* / *trans* Groups Epoxidised by ^{1}H NMR Analysis	Percent *cis* / *trans* Groups Epoxidised by Elemental Analysis
27.5[a]	0	59	54
27.5[b]	0	22	26
13.75[a]	0	55	71
13.75[b]	0	22	23

[a] hydrogen peroxide added in one portion, [b] hydrogen peroxide added dropwise through the duration of the experiment.

It can be seen from Table 3. that alterations in the concentration of the hydrogen peroxide had little effect on the yield of epoxide. In contrast, lower yields of epoxide were obtained when the oxidant was added slowly during the course of the reaction rather than in one portion at the start. The lower yields obtained with the dropwise addition of hydrogen peroxide may be attributed to the low rates of epoxidation caused by the very low yields of peroxyacid in the system.

2.1.9. The Effect of Solvents on the Epoxidation of Poly(butadiene)

The solvent is an important consideration and affects product formation and yields. Björkling found that the highest yields of peroxyacid were generated in toluene and hexane. It was therefore of interest to examine the effect of solvent on our system. Reactions were carried out with 10 wt % of enzyme, 27.5 wt % aqueous solution of hydrogen peroxide and 10 mol % acetic acid for 24 hours. The results are presented in Table 4.

Table 4. The Effect of Solvent

Solvent and Temperature (°C)	Percentage Vinyl Groups Epoxidised	Percent *cis* / *trans* Groups Epoxidised by ^{1}H NMR Analysis	Percent *cis* / *trans* Groups Epoxidised by Elemental Analysis
Toluene at 25 °C[a]	0	44	-
Toluene at 25 °C[b]	0	58	48
Hexane at 25 °C[a]	0	0	-
Hexane at 25 °C[b]	0	5	12

[a] hydrogen peroxide added in one portion, [b] hydrogen peroxide added dropwise through the duration of the experiment.

One interesting result observed is that in toluene a higher yield of epoxide was obtained when the hydrogen peroxide was added dropwise over the course of the reaction rather than in one portion at the beginning, whereas, with dichloromethane the opposite effect was observed. Presumably, the relative rates of denaturation and epoxidation are altered in toluene. The maximum yield of epoxide was almost identical with both toluene and dichloromethane although the method of hydrogen peroxide addition differed. Surprisingly, no appreciable reaction in hexane was observed. One difference between our system and that of Björkling was that we used acetic acid whereas he used long chain acids. It is possible that the lower solubility of peroxyacetic acid in hexane compared with peroxypalmitic acid could be an important factor.

2.1.10. The Effect of Temperature on the Epoxidation of Poly(butadiene)

The effect of temperature on this reaction was investigated. Reactions were carried out with 10 mol % acetic acid, 27.5 wt % aqueous solution of hydrogen peroxide and 10 wt % of enzyme for 24 hours. The results of these studies are presented in Table 5.

Table 5. The Effect of Temperature on the Epoxidation of Poly(butadiene)

Solvent and Temperature (°C)	Percentage Vinyl Groups Epoxidised	Percent *cis* / *trans* Groups Epoxidised by ^{1}H NMR Analysis	Percent *cis* / *trans* Groups Epoxidised by Elemental Analysis
[a]CH_2Cl_2 at 25 °C	0	59	54
[a]CH_2Cl_2 at 37 °C	0	19	21
[b]CH_2Cl_2 at 25 °C	0	22	26
[b]CH_2Cl_2 at 37 °C	0	14	17
[b]Toluene at 25 °C	0	58	48
[b]Toluene at 37 °C	0	33	34
[b]Hexane at 25 °C	0	5	12
[b]Hexane at 37 °C	0	9	13

[a] addition of hydrogen peroxide in one portion at the start of the reaction, [b] addition of hydrogen peroxide dropwise throughout the reaction.

It can be seen from Table 5, that an increase in temperature reduced the yield of epoxide in both dichloromethane and toluene. There could be two possible explanations for this observation. The higher temperature could be either increasing the rate at which the enzyme is being denatured, or alternatively it could be accelerating the decomposition of the hydrogen peroxide. Both of these eventualities would decrease the levels of the epoxide formed.

2.1.11. The Effect of Amount of Enzyme on the Epoxidation of Poly(butadiene)

Given that the enzyme is crucial to the *in situ* synthesis of peroxyacetic acid, it was a logical progression of the research to investigate the optimal quantities of enzyme catalyst required in the system whilst keeping other reaction variables constant. Experiments were carried out in dichloromethane at 25 °C with 10 mol % of acetic acid and a 27.5 wt % aqueous solution of hydrogen peroxide for 24 hours, see Table 6.

Table 6. The Effect of Enzyme

Quantity of Enzyme (wt %)	Percentage Vinyl Groups Epoxidised	Percent *cis* / *trans* Groups Epoxidised by ^{1}H NMR Analysis	Percent *cis* / *trans* Groups Epoxidised by Elemental Analysis
5[a]	0	38	-
10[a]	0	59	54
20[a]	0	54	67
5[b]	0	20	24
10[b]	0	22	26
20[b]	0	26	-
20[b, c]	0	51	53
10[b, d]	0	0	3

[a] addition of hydrogen peroxide in one portion at the start of the reaction, [b] addition of hydrogen peroxide drop wise throughout the reaction, [c] 10 wt % of enzyme was added. After 24 hours a further 10 wt % of enzyme was added and the reaction continued for a further 24 hours, [d] reused enzyme.

Doubling the quantity of enzyme would be expected to increase the rate of reaction. It seemed likely that this higher reaction rate would lead to a higher yield of epoxide before the enzyme became inactive. The percentage of backbone epoxidation dropped below the previously achieved levels when the quantity of enzyme was halved. This lower yield of epoxide can be explained by a lower reaction rate. The amount of epoxide formed before the smaller quantity of enzyme became inactive would be expected to be reduced. It is unclear why the yield of epoxide is essentially unchanged when the quantity of enzyme is doubled in the drop wise experiments. A possible explanation is that there is no oxidant remaining after 24 hours. This explanation can be discounted as hydrogen peroxide could be detected (sodium metabisulfate and starch and potassium iodide test strips) after each reaction. Also, addition of a second 10 wt % of enzyme and allowing the reaction to proceed for a further 24 hours resulted in a substantial increase in the yield of epoxide. For the first portion of the enzyme the hydrogen peroxide was added dropwise over the course of the reaction and it is apparent from Table 6 that this reaction gives a polymer with 22 % of the *cis* and *trans* double bonds epoxidised. The second portion of enzyme would be in a similar system to that where the oxidant is added in one portion at the beginning of the reaction. Table 6 shows that under these conditions 59 % of the *cis* and *trans* double bonds should be epoxidised. It would therefore be expected that the total yield of *cis* and *trans* epoxide would be approximately 83 %. Our results showed that only 51 % of these double bonds were in fact epoxidised. This value is very similar to the maximum yield that has been obtained with this enzyme catalysed process. This suggests that the double bonds of the polymer are no longer available for epoxidation since the conformation of the polymer changes during the epoxidation reaction. Given that the system is a well mixed combination of an aqueous phase and an organic phase (in which the polymer is dissolved), it seems probable that the epoxidised polymer would adopt conformations in which the more hydrophilic epoxide groups are able to interact with the aqueous phase whilst the hydrophobic alkenes are buried within the centre of the polymer matrix. This would have the effect of rendering unepoxidised alkene groups inaccessible for further reactions. In support of this, Zuchowska reported that the epoxidation of poly(butadiene) with concentrated acetic acid and 60 wt % aqueous hydrogen peroxide resulted in a decrease in rate of epoxidation as the reaction proceeded.[95] Zuchowska suggested that side reactions involving ring opening were responsible for the rate decrease with time. It is unlikely that this explanation is applicable to our system as very mild reaction

conditions were used and FT-IR, ^{1}H-NMR and ^{13}C-NMR analysis showed no evidence of ring opening. Conformational changes appear to be a more likely explanation for the cessation of the reaction in our case.

Reactions carried out with recovered enzyme gave no epoxidation demonstrating that the enzyme becomes denatured within 24 hours.

2.1.12. Epoxidation of Poly(butadiene) with Peroxyacetic Acid

To demonstrate the importance and benefits of the mild enzyme catalysed system, two reactions were carried out in dichloromethane at 25 °C, one reaction with a 32 wt % solution of peroxyacetic acid in acetic acid without enzyme and a second in the presence of 10 wt % of enzyme. The results of these studies are shown in Table 7.

Table 7. Peroxyacetic Acid Oxidation of Poly(butadiene)

Reaction Conditions	Percentage Vinyl Groups Reacted by ^{1}H NMR analysis	Percentage *cis* / *trans* Double Bonds Reacted by ^{1}H NMR analysis	GPC Mw
10 wt % Enzyme	100	100	1750
No Enzyme	100	100	-

These reactions both gave identical products in which all of the double bonds were completely epoxidised non-selectively. FT-IR spectra of the products showed evidence of ring opened products (3445 cm^{-1} (OH) and 1730 cm^{-1} (ester)).

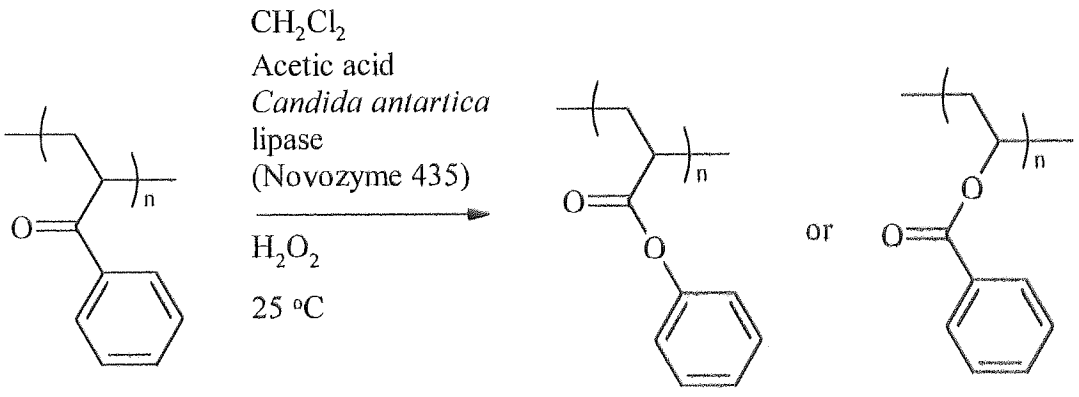
2.2. Baeyer-Villiger Oxidation of Ketone Polymers

Roberts and co-workers exploited the lipase-catalysed peroxyacid formation, developed by Björkling, in Baeyer-Villiger reactions.[98] Roberts was able to show that Baeyer-Villiger oxidation of cyclic ketones using this approach took place with only slightly reduced yields (typically only 5-10 % smaller) compared with those obtained with *m*-chloroperoxybenzoic acid. Since this system had been successful for the epoxidation of poly(butadiene), it was thought that it might also be applicable to the Baeyer-Villiger oxidation of poly(vinyl phenyl ketone) (Mn 750) and poly(methyl vinyl ketone) (Mn 6270).

2.2.1. Baeyer-Villiger Oxidation of Poly(vinyl phenyl ketone)

The migratory aptitude of the phenyl group and the secondary alkyl group in poly(vinyl phenyl ketone) are similar.[99] The Baeyer-Villiger oxidation of phenyl isopropyl ketone with trifluoroperacetic acid gives a mixture of the two esters. The ester in which the isopropyl migrates predominates, this ester being formed in twice the yield of the other ester.[99] Figure 25 shows the two possible products obtained from the Baeyer-Villiger oxidation of poly(vinyl phenyl ketone).

Figure 25. Baeyer-Villiger Oxidation of Poly(vinyl phenyl ketone)



The Baeyer-Villiger reactions were carried out in dichloromethane at 25 °C with 10 mol % acetic acid, 10 wt % *Candida antartica* lipase (Novozyme 435) and hydrogen peroxide added in one portion at the start of the reaction, see Table 8.

Table 8. Baeyer-Villiger Oxidation of Poly(vinyl phenyl ketone) Products

Time (Hours)	Reaction Conditions (Oxidant, Acid, Enzyme)	Percentage Oxidation by Elemental Analysis
2	H_2O_2 (27.5 wt %), acetic acid, Novozyme 435 (10 wt %)	7.4
6	H_2O_2 (27.5 wt %), acetic acid, Novozyme 435 (10 wt %)	12.7
24	H_2O_2 (27.5 wt %), acetic acid, Novozyme 435 (10 wt %)	7.3
96	H_2O_2 (27.5 wt %), acetic acid, Novozyme 435 (10 wt %)	9.8
168	H_2O_2 (27.5 wt %), acetic acid, Novozyme 435 (10 wt %)	-
24	H_2O_2 (13.75 wt %), acetic acid, Novozyme 435 (10 wt %)	-
24	H_2O_2 (13.75 wt %), F_3CCO_2H, Novozyme 435 (10 wt %)	-
24	H_2O_2 (27.5 wt %), acetic acid, Novozyme 435 (20 wt %)	-
24	Peroxyacetic acid (32 wt %), Novozyme 435 (10 wt %)	-

The ^1H NMR and ^{13}C NMR spectra of poly(vinyl phenyl ketone) are shown in Figures 26 and 27. The corresponding NMR spectra for the product obtained form the reaction of H_2O_2 (27.5 wt %) and Novozyme (10 wt %) after 24 hours of reaction are shown in Figures 28 and 29. The NMR spectra for all of the other reactions in Table 8 were identical.

Figure 26. ^{13}C NMR of Poly(vinyl phenyl ketone)

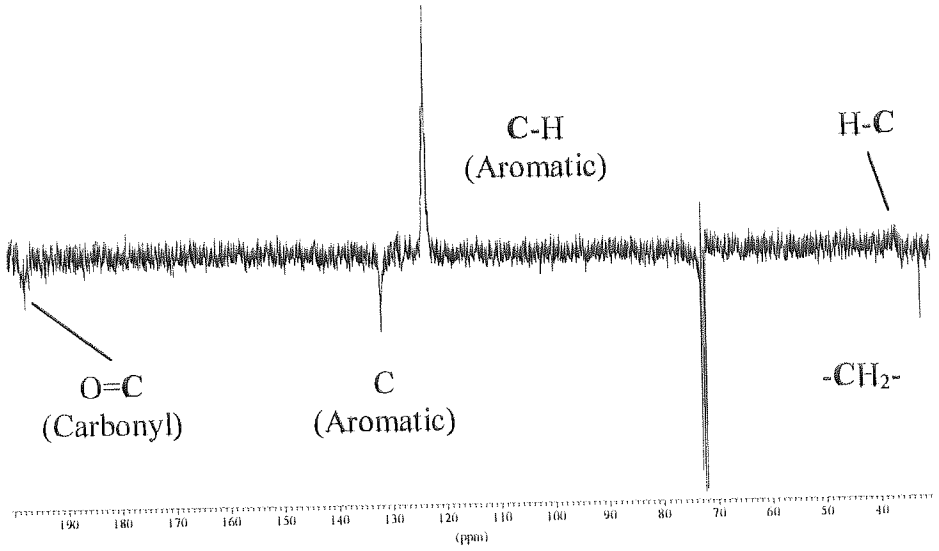


Figure 27. ^{1}H NMR of Poly(vinyl phenyl ketone)

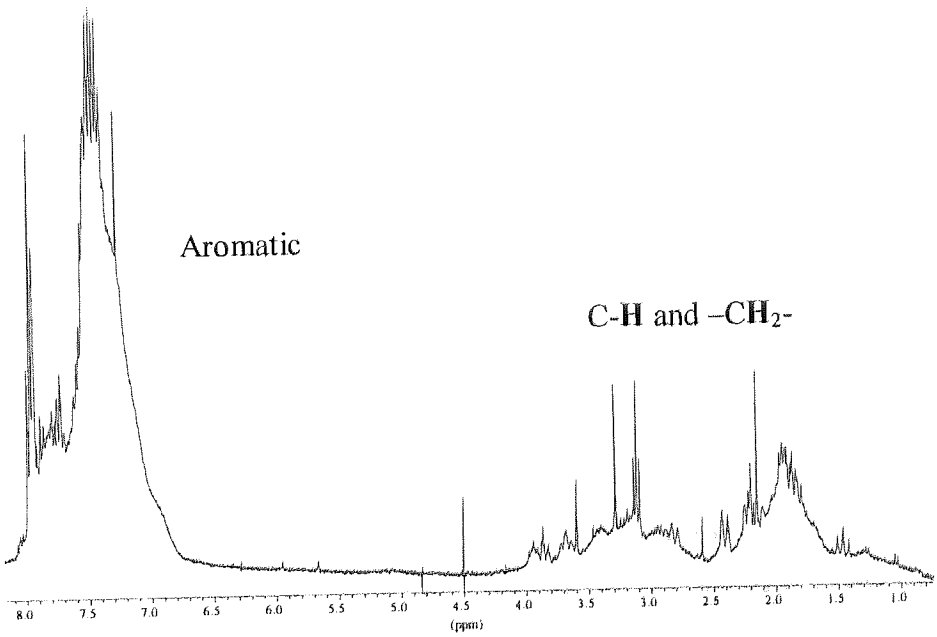


Figure 28. ^{13}C NMR of Oxidised Poly(vinyl phenyl ketone)

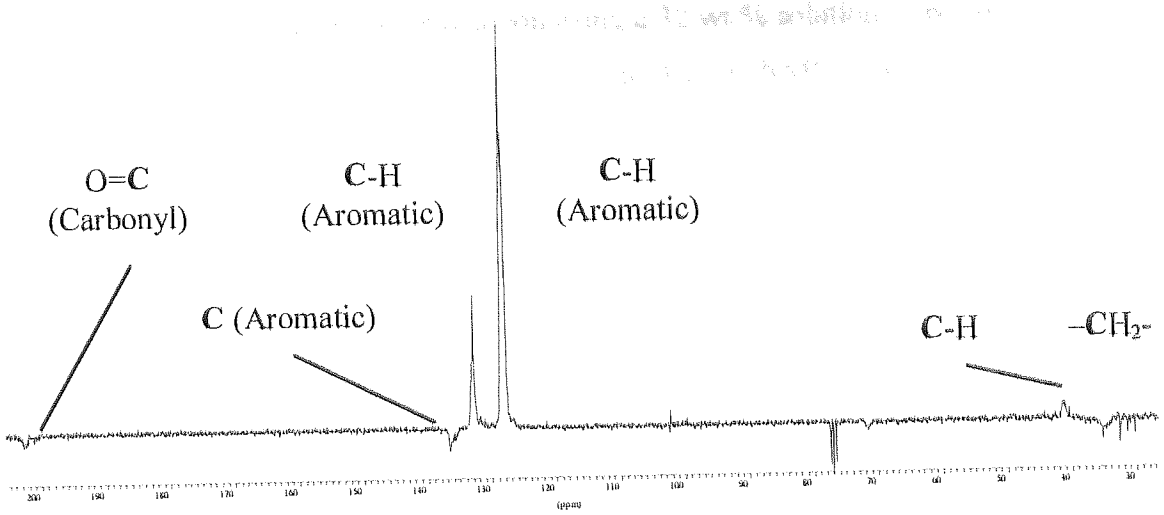
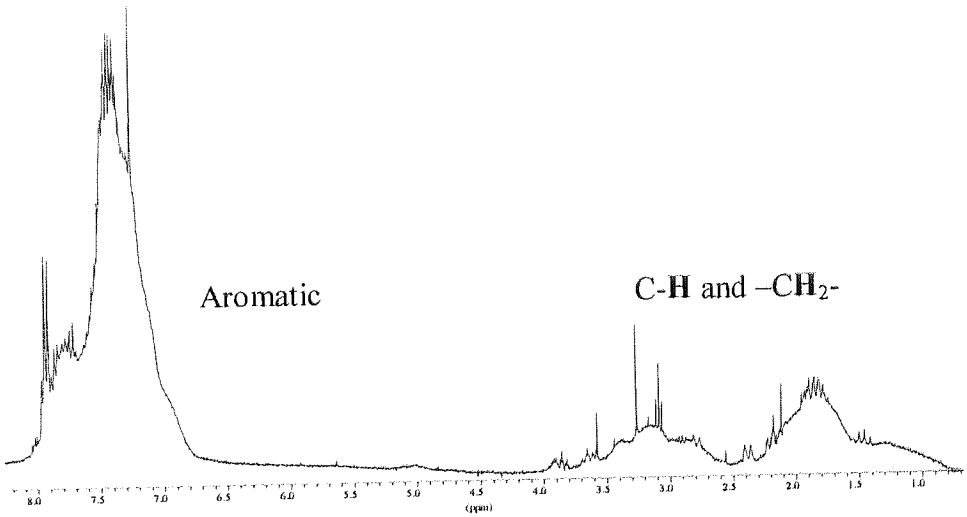


Figure 29. ^{1}H NMR of Oxidised Poly(vinyl phenyl ketone)

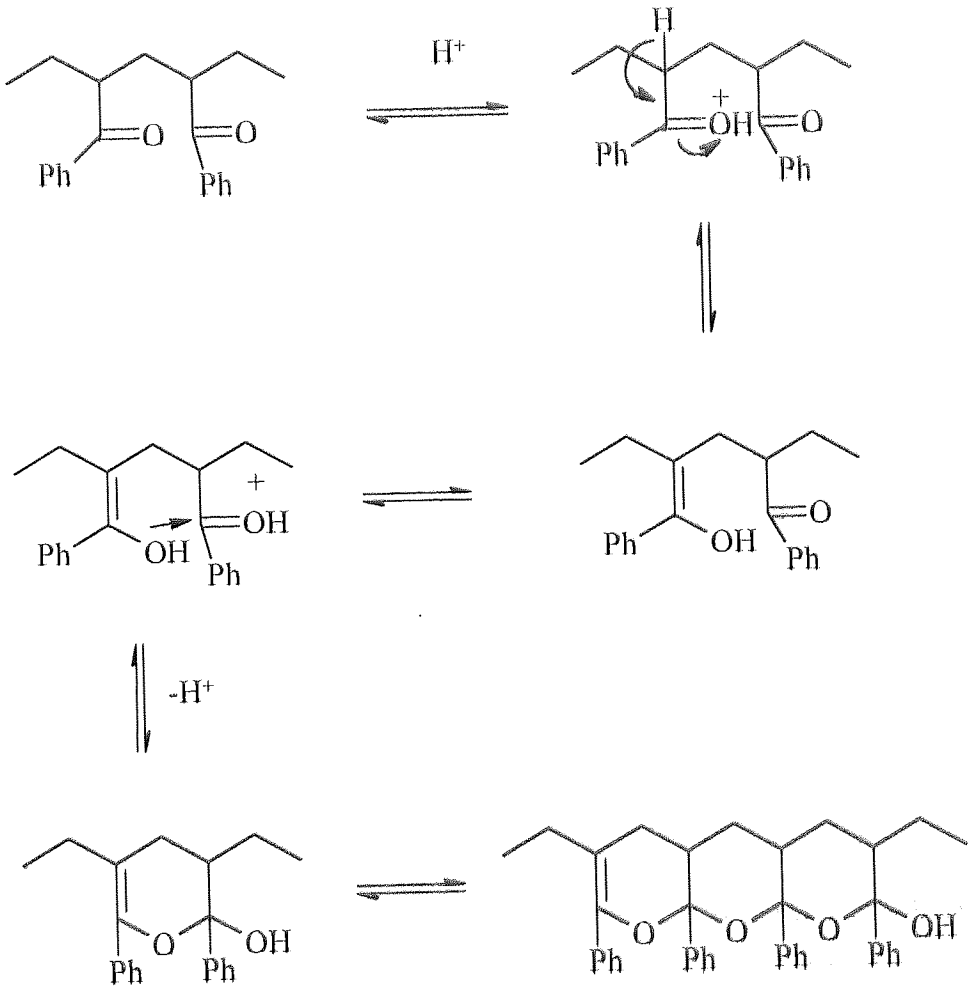


The ^{1}H NMR spectra of poly(vinyl phenyl ketone) and its oxidation products were extremely broad and no useful information about the outcome of the reaction could be obtained. However, examination of the ^{13}C NMR spectra showed the appearance of a second aromatic peak, see Figure 28.

Surprisingly, control reactions carried out without the enzyme also gave identical results. In addition the product obtained from a reaction using a 32 wt % solution of peroxyacetic acid in acetic acid as oxidant also gave a product with a similar ^{13}C NMR spectrum. Whilst the new peak in the ^{13}C NMR spectra of the products might be attributed to the formation of ester from a Baeyer-Villiger reaction, infrared spectra of the products shows no evidence of ester carbonyl groups.

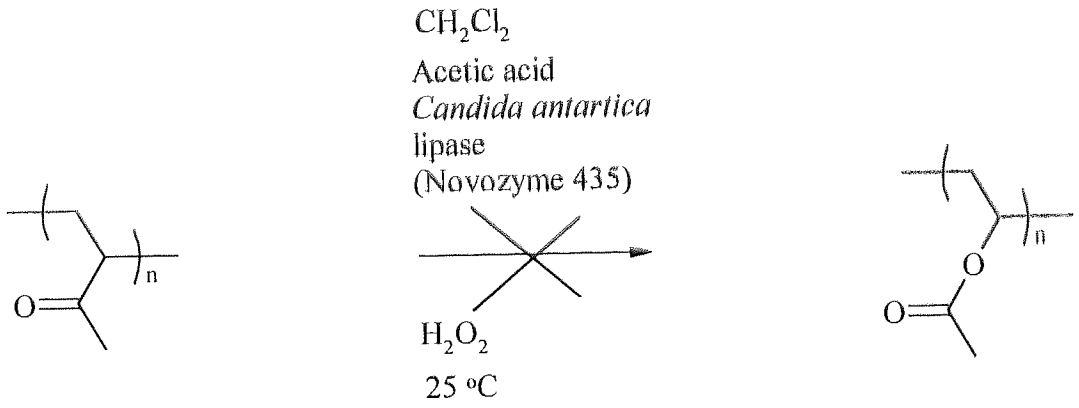
The common feature of all of these reactions is the presence of acid. It is possible that under these conditions the ketone groups are modified into ketals, see Figure 30.

Figure 30. Formation of Ketals



This reaction needs only catalytic quantites of acid. It would be possible for a number of adjacent ketones to react in this manner forming a small sequence of stable tetrahydropyran rings. In support of this, the enzyme catalysed transcyanation reactions which were carried out on this same polymer and are described in Chapter 5 gave products with identical spectra to those of the Baeyer-Villiger reactions. Catalytic quantities of acid were also used in these transcyanation reactions. In contrast, attempts to carry out Baeyer-Villiger reactions with poly(vinyl methyl ketone) (Mn 6270) using the same reaction conditions resulted in isolation of the unchanged polymer, see Figure 31. It is possible that in poly(vinyl phenyl ketone) the aromatic ring stabilises the enol formed, allowing subsequent ketal formation.

Figure 31. Baeyer-Villiger Oxidation of Poly(methyl vinyl ketone)



The NMR spectra showed that no reaction had occurred. It is apparent from these studies that it is the nature of the polymers which prevent reaction occurring rather than the use of an enzyme catalysed system.

2.3. Conclusion

It was found that an immobilised enzyme from *Candida antartica* can be used to selectively epoxidise poly(butadiene) in organic solvents under very mild conditions. The reaction shows a high selectivity for the *cis* and *trans* double bonds of the polymer backbone leaving the pendent vinyl groups untouched. It was found that up to 59 % of the *cis* and *trans* double bonds could be epoxidised.

The effect of varying a number of parameters was investigated but higher yields of epoxide could not be obtained. These results showed that the effect of varying even a single paramer did not lead to a readily predictable outcome. This is probably due to the complex nature of this 3 phase system in which several interdependent processes are occurring simultaneously. However, these results do suggest that the inability to obtain higher yields of epoxide is probably due to changes in the conformational properties of the partially epoxidised polymer.

This work is the first example of an enzyme catalysed modification of the backbone of a synthetic polymer. Part of this work has been published as a preliminary communication.[100]

Attempts to use the same enzyme catalysed system to carry out Baeyer-Villiger oxidations of poly(vinyl phenyl ketone) and poly(vinyl methyl ketone) were unsuccessful. Poly(vinyl phenyl ketone) was modified but in a non-enzymatic process. Given that similar results were obtained in a conventional chemical reaction with peracetic acid it can be concluded that the lack of reactivity is a property of the polymers and not the result of the enzymatic reaction.

Chapter 3.

Esterification of Hydroxyl Functionalised Polymers

3.0. Esterification of Hydroxyl Functionalised Polymers

Ritter's work on the acylation of "comb-like" methacrylate polymers showed that the esterification of long pendent side chains ending with hydroxyl or carboxyl functional groups was possible.[9] Morrow was also able to demonstrate the use of polymers as substrates in the resolution of chiral esters by lipase-catalysed transesterification of the terminal hydroxyl groups of poly(ethylene glycol).[86, 87] The work of Ritter and of Morrow was limited to polymers containing long pendent side chains with reacting groups distant from the polymer backbone, or of polymers terminated with a reactive group.

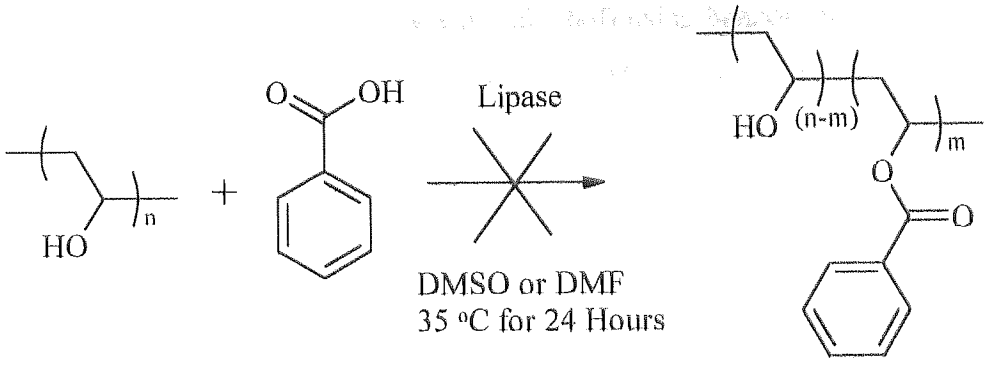
It was of interest to investigate the application of cheap and readily available lipases to the esterification and transesterification reactions of polymers with functional groups much closer to the polymer backbone. Two approaches were investigated, the first was the transesterification and esterification of polymers containing hydroxyl groups and the second the transesterification of activated esters of poly(methacrylic) acid. The first of these approaches is discussed in this chapter whilst the second approach is described in chapter 4.

3.1. Esterification of Poly(vinyl alcohol)

Poly(vinyl alcohol) and benzoic acid were selected for initial investigations as they are cheap and readily available. Benzoic acid and its esters are readily identifiable in NMR spectra which assists product analysis.

The highly polar nature of both starting materials dictated that polar solvents were required. The use of aqueous systems would promote the reverse hydrolysis reaction and were avoided. It was found that dimethyl sulphoxide (DMSO) and dimethyl formamide (DMF) were suitable solvents, but it was necessary to heat the reaction mixture to 80 °C for 1 hour to dissolve the poly(vinyl alcohol). The reaction mixture was then cooled to 35 °C before addition of porcine pancreatic lipase. The proposed reaction is shown in Figure 32.

Figure 32. Esterification of Poly(vinyl alcohol)



Under these conditions no reaction was observed with only starting materials being isolated from the reaction. The reason for the lack of reaction, is possibly due to the highly polar nature of the DMSO and DMF solvents denaturing the enzyme by displacement of enzyme bound water. Additionally, the highly polar nature of the polymer is likely to increase its interaction with polar groups on the enzyme and inhibit its free diffusion to and from the enzyme's active site. Also, the relatively bulky nature of the polymer might inhibit the reaction.

3.2. Modification of Poly(vinyl alcohol-co-ethylene)

It was thought that the use of poly(vinyl alcohol-co-ethylene) in place of poly(vinyl alcohol) might solve the solubility problems by reducing the number of polar groups present, thereby allowing a wider variety of organic solvents to be investigated. In addition, Ritter had found that spacer groups in the polymer backbone increased the yield of modified side chain.[9, 86]

Unfortunately, poly(vinyl alcohol-co-ethylene) was found to be highly crystalline, and posed an equal solubility problem to that of poly(vinyl alcohol). It was still necessary to dissolve the polymer in hot DMSO or DMF and then allow the mixture to cool to 35 °C before addition of enzyme.

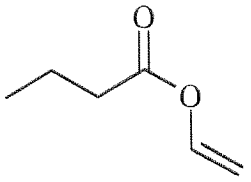
3.2.1. Esterification of Poly(vinyl alcohol-co-ethylene)

Esterification reactions described for poly(vinyl alcohol) using benzoic acid were repeated for poly(vinyl alcohol-co-ethylene) in both DMSO and DMF. Starting materials were isolated with no evidence of any reaction having taken place. Due to the lack of reactivity, the use of activated esters as substrates was considered.

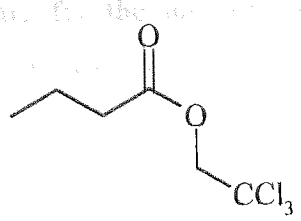
3.2.2. Activated Esters

A number of activated esters have been developed in order to promote transesterification reactions and limit the reverse reactions.[101, 102] Amongst the activated esters which have been used in enzymatic reactions are enol esters (vinyl esters),[103] 2,2,2-trifluoroethyl esters, 2,2,2-trichloroethyl esters.[104] Anhydrides,[105] thiocyanates, phenolic esters[106] and thiol acids[107] have also been used to promote formation of esters. Some of these activated species are shown in Figure 33.

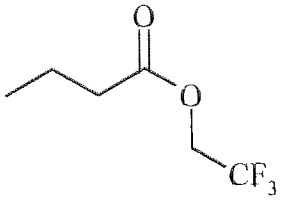
Figure 33. Activated Esters



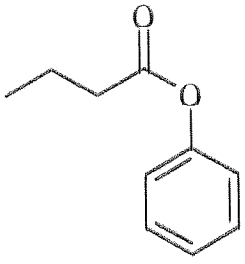
Vinyl ester



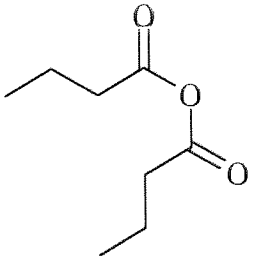
2,2,2-trichloroethyl ester



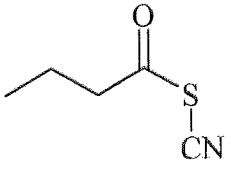
2,2,2-trifluoroethyl ester



Phenyl ester



Anhydride



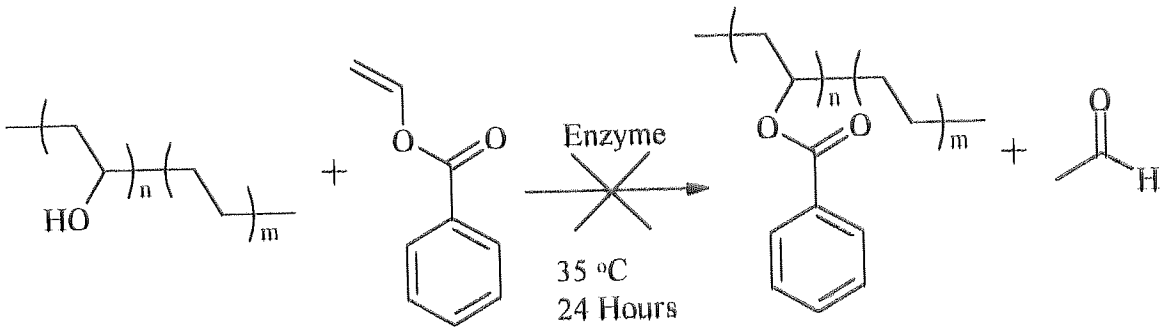
Thiocyanate

On transesterification vinyl esters release highly volatile non-nucleophilic acetaldehyde and thiocyanates release gaseous hydrogen thiocyanate on reaction with alcohols making these reactions irreversible. The other activated esters shown in Figure 33. release weakly nucleophilic alcohols on transesterification, limiting the reverse reaction.

Problems have been encountered with some of these systems, for instance acetaldehyde released from the reaction of vinyl esters form Schiff's bases with the lysine residues on the enzyme.[108] Removal of acetaldehyde from the system by molecular sieve or by evaporation allows these systems to be used effectively.

Vinyl esters are very reactive and their transesterification reactions are irreversible. Vinyl benzoate was selected as a suitable activated ester for the investigation of the enzyme catalysed modification of hydroxyl containing polymers, see Figure 34.

Figure 34. Transesterification with Activated Esters



Reaction of vinyl benzoate with poly(vinyl alcohol-co-ethylene) for 24 hours gave only starting materials. Extension of the reaction time to 96 hours again gave no reaction. Changing the enzyme from porcine pancreatic lipase to *Candida rugosa* lipase (CRL) also gave unchanged starting material.

Similar explanations to those previously discussed for the esterification reactions can be advanced to explain the lack of reactivity in these systems.

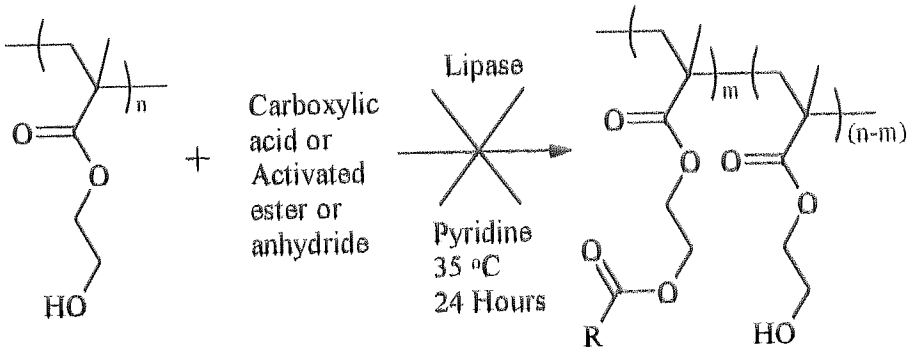
3.3. Transesterification of Poly(2-hydroxyethyl methacrylate)

Poly(2-hydroxyethyl methacrylate) was selected as an alternative hydroxyl containing polymer. It was thought that extension of the hydroxyl groups out from the polymer backbone on a flexible pendent side chain would make this polymer more soluble in less polar solvents. In fact pyridine was the least polar solvent in which the polymer dissolved. The advantages of this polymer were that it had primary hydroxyl groups which were separated from the backbone by a short flexible spacer. 2-Hydroxyethyl methacrylate is a cheap and readily available monomer and was polymerised by the addition of the free radical initiator α,α'-azobisisobutyronitrile (AIBN) with subsequent heating at 60 °C in degassed toluene for 24 hours. The polymer was obtained with a Mn of 64600.

Poly(2-hydroxyethyl methacrylate) was reacted with benzoic acid and vinyl benzoate with both PPL and CRL enzymes but no reaction occurred.

A number of other activated esters were reacted with poly(2-hydroxyethyl methacrylate) in pyridine as shown in Figure 35. The reaction of vinyl acetate, vinyl pivalate and benzoic anhydride were investigated but in each case only starting materials was isolated from the reaction. Benzoic anhydride was hydrolysed to benzoic acid during the work up procedure and only unreacted polymer was isolated as product.

Figure 35. Esterification of Poly(2-hydroxyethyl methacrylate)



3.4. Conclusion

Ritter was able esterify "comb-like" polymers in which the reactive group was at the end of a 16 atom pendent chain.[9, 86] His investigations showed that spacing groups along the polymer backbone and long pendent side chains aided esterification. Our results using the short pendent chains together with Ritter's investigations suggest that the reactions are dominated by steric factors preventing reaction taking place. It would be of interest to establish the minimum length of spacing groups and pendent side chain required for the reaction to occur.

Chapter 4.

Modification of Active Ester Functionalised Polymers

4.0. Modification of Active Ester Functionalised Polymers

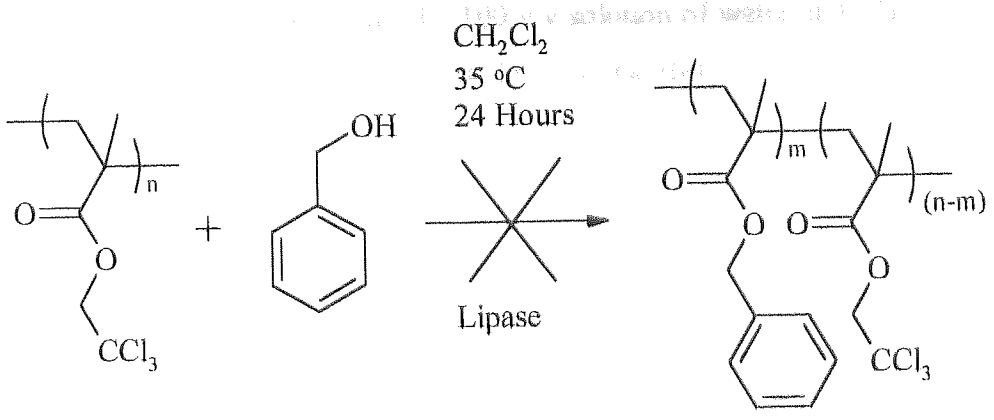
Following the research using polymers containing hydroxyl groups close to the polymer backbone, exploratory studies were carried out to discover if activated esters close to the backbone could be modified. The activated ester polymers chosen were poly(2,2,2-trichloroethyl methacrylate), poly(2,2,2-trifluoroethyl methacrylate), poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) and poly(*tert*-butyl acrylate). The 2,2,2-trichloroethyl methacrylate and 2,2,2-trifluoroethyl methacrylate monomers were synthesised from methacrylic acid and the corresponding alcohol using acid catalysed esterification under reflux with a Dean and Stark trap to remove water as it was formed. *tert*-Butyl acrylate was commercially available from Aldrich. The monomers were polymerised with a free radical initiator (AIBN) to give poly(2,2,2-trichloroethyl methacrylate) (Mn 23000), poly(2,2,2-trifluoroethyl methacrylate) (Mn 2570) and poly(*tert*-butyl acrylate) (Mn 19900). Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) (Mn 12500) was commercially available from Aldrich. Previous work in our laboratory had shown that the activated ester monomers chosen react readily in enzyme catalysed transesterification systems. It was thought that the polymers might be suitable substrates for transesterification reactions.

A variety of nucleophiles were selected for the investigations of the modification of the activated ester polymers. The reactions were all conducted at a temperature of 35 °C. With the exception of reactions using sugar and water as nucleophiles, all reactions were carried out in dichloromethane. Reactions involving sugars were conducted in pyridine and the reaction using water was conducted in tetrahydrofuran.

4.1. Modification of Poly(2,2,2-trichloroethyl methacrylate)

Initial investigations of the enzyme catalysed transesterification of poly(2,2,2-trichloroethyl methacrylate) with benzyl alcohol used porcine pancreatic lipase as a catalyst at 35 °C in dichloromethane gave no observable reaction, see Figure 36.

Figure 36. Transesterification of Poly(2,2,2-trichloroethyl methacrylate)



Repeating the reaction with *Candida rugosa* lipase also resulted in no observable reaction.

Given the lack of reactivity of benzyl alcohol in the system, it was thought a more powerful nucleophile might enable a transesterification reaction to take place. 2-Phenylethylalcohol is a more powerful nucleophile than benzyl alcohol by virtue of the fact that the hydroxyl group is further away from the aromatic ring and therefore less affected by the electron withdrawing properties of the aromatic ring.

Transesterification reactions with poly(2,2,2-trichloroethyl methacrylate) and 2-phenylethylalcohol catalysed with either porcine pancreatic lipase or *Candida rugosa* lipase were unsuccessful.

Given the lack of success with alcohols as nucleophiles, attention was turned to the more reactive amines. Aniline was used in investigations as a possible nucleophile for modifying poly(2,2,2-trichloroethyl methacrylate). No reaction was observed to take place, with either porcine pancreatic lipase or *Candida rugosa* lipase as catalysts. It was thought that 2-phenylethylamine being a more powerful nucleophile than aniline might be more successful. The aniline experiments were repeated with 2-phenylethylamine as the nucleophilic component of the system. Unfortunately, no reaction appeared to take place with only starting materials being isolated.

Other investigations using nucleophiles such as glucose (in an attempt to synthesise a potentially biocompatable polymer), 1-butanol and 1-butylamine as nucleophiles were also unsuccessful.

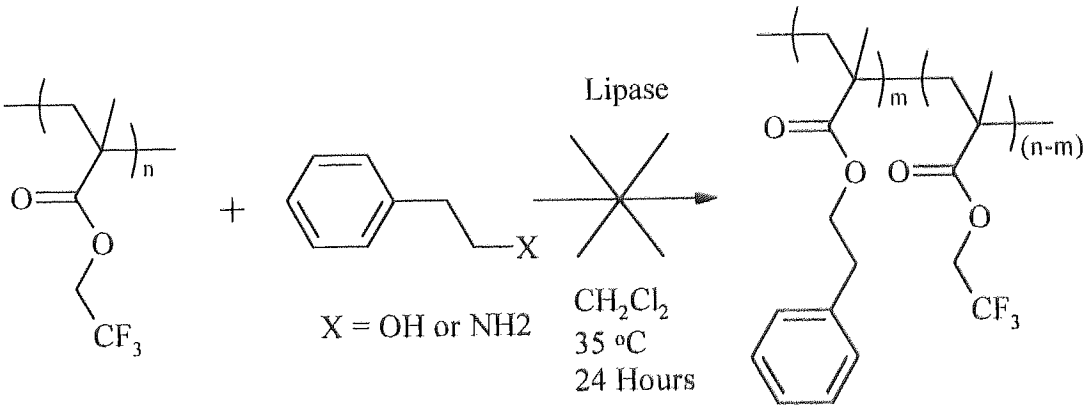
An enzyme catalysed hydrolysis of poly(2,2,2-trichloroethyl methacrylate) was investigated. The polymer was found to dissolve in a 1 : 100 v/v solution of water and THF. However, no reaction was observed with porcine pancreatic lipase as catalyst.

4.2. Modification of Poly(2,2,2-trifluoroethyl methacrylate)

2,2,2-Trifluoroethyl esters are more reactive than the 2,2,2-trichloroethyl esters. In addition to the higher activity, the 2,2,2-trifluoroethyl group is more electron withdrawing than the chlorine analogue and therefore the alcohol released during reactions with this ester are less nucleophilic and therefore inhibit the reverse reaction more effectively than the 2,2,2-trichloroethyl alcohol. It was hoped that these combined factors would facilitate a reaction with this polymer.

The reactions of poly(2,2,2-trichloroethyl methacrylate) with 2-phenylethylalcohol and 2-phenylethylamine were studied but no reaction occurred with porcine pancreatic lipase or *Candida rugosa* lipase as catalysts, see Figure 37.

Figure 37. Transesterification of Poly(2,2,2-trifluoroethyl methacrylate)



4.3. Modification of Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid)

Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) was considered as a potentially suitable polymeric substrate that might be transesterified. The *tert*-butyl ester of the polymer was of particular interest as the bulky *tert*-butyl alcohol is an excellent leaving group and also a poor nucleophile.

A variety of nucleophiles were examined in the reaction of poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) with either porcine pancreatic lipase or *Candida rugosa* lipase as the catalysts.

Benzyl alcohol, 1-butanol and benzyl mercaptan were unreactive. The reaction of 2-phenylethylamine with poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) was investigated with both porcine pancreatic lipase and *Candida rugosa* lipase as catalysts, see Figure 38. The ^{13}C NMR and ^{1}H NMR spectra of the products indicated that a reaction had taken place as the signal for the *tert*-butyl group was greatly reduced. The ^{13}C NMR and ^{1}H NMR spectra of the starting polymer and product obtained using PPL are shown in Figures 39-42.

Figure 38. Reaction of Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) with 2-Phenylethylamine

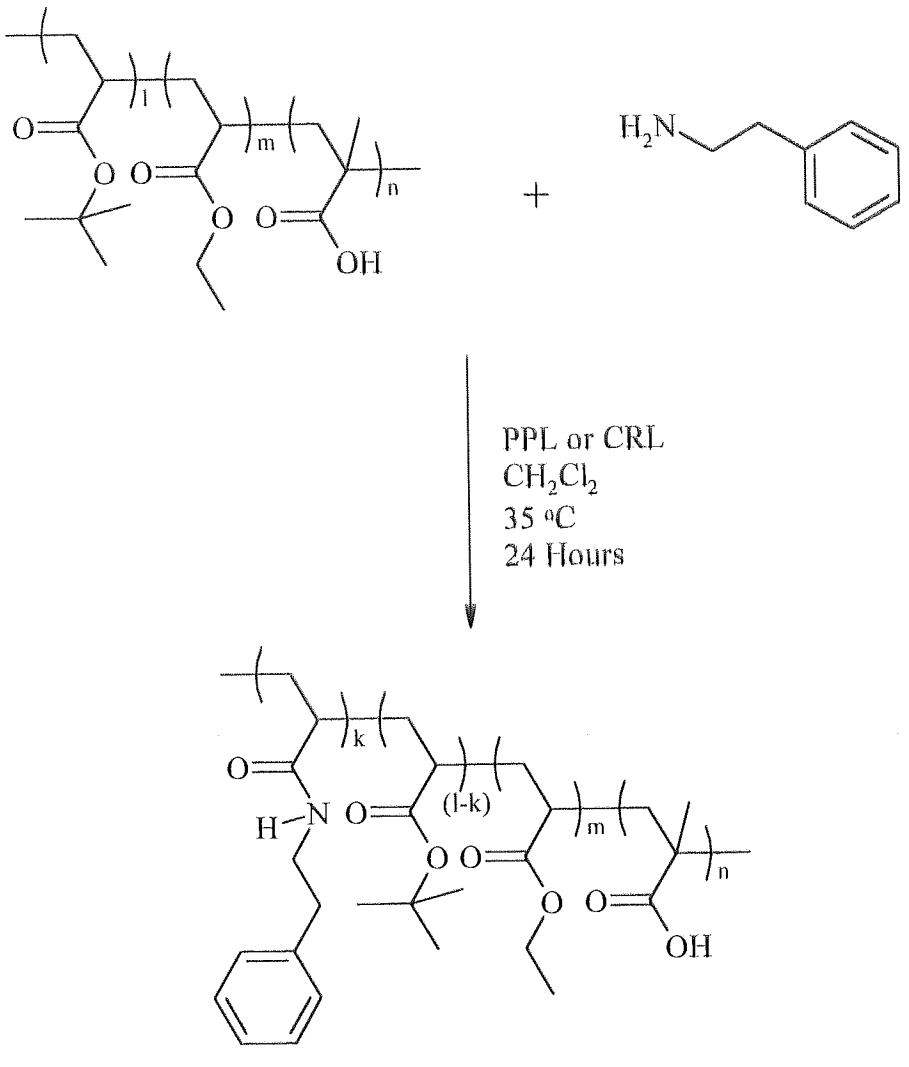


Figure 39. ^{13}C NMR of Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid)

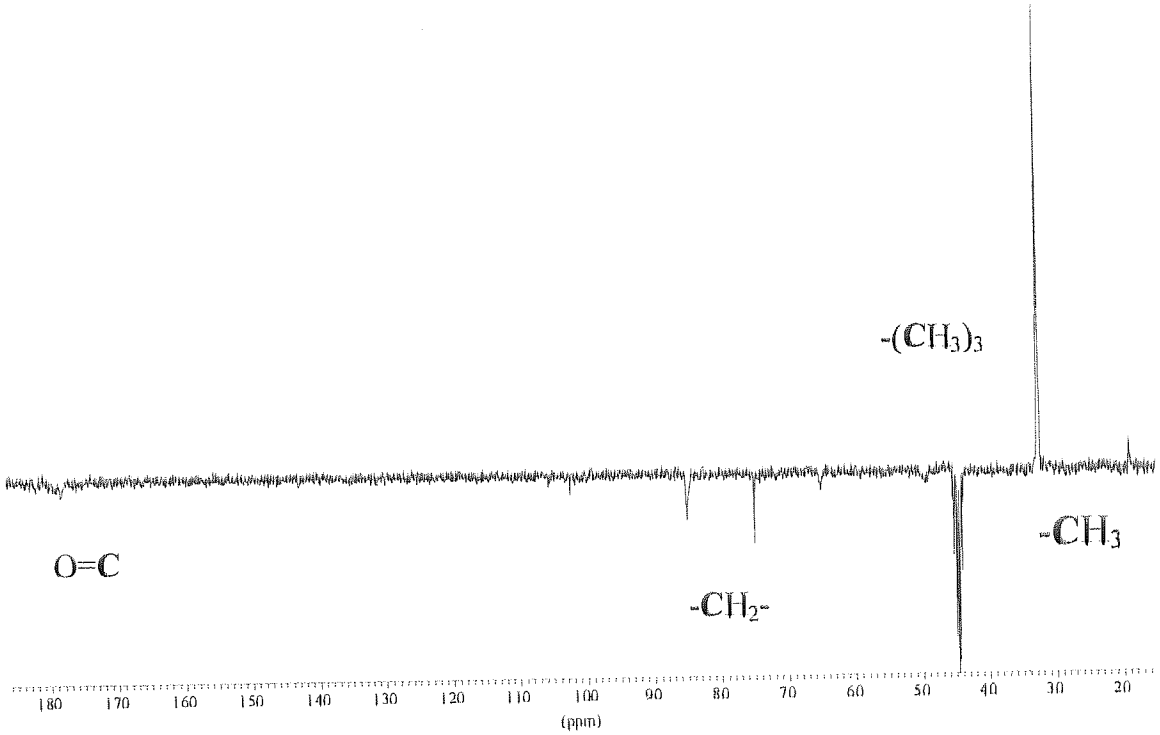


Figure 40. ^{1}H NMR of Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid)

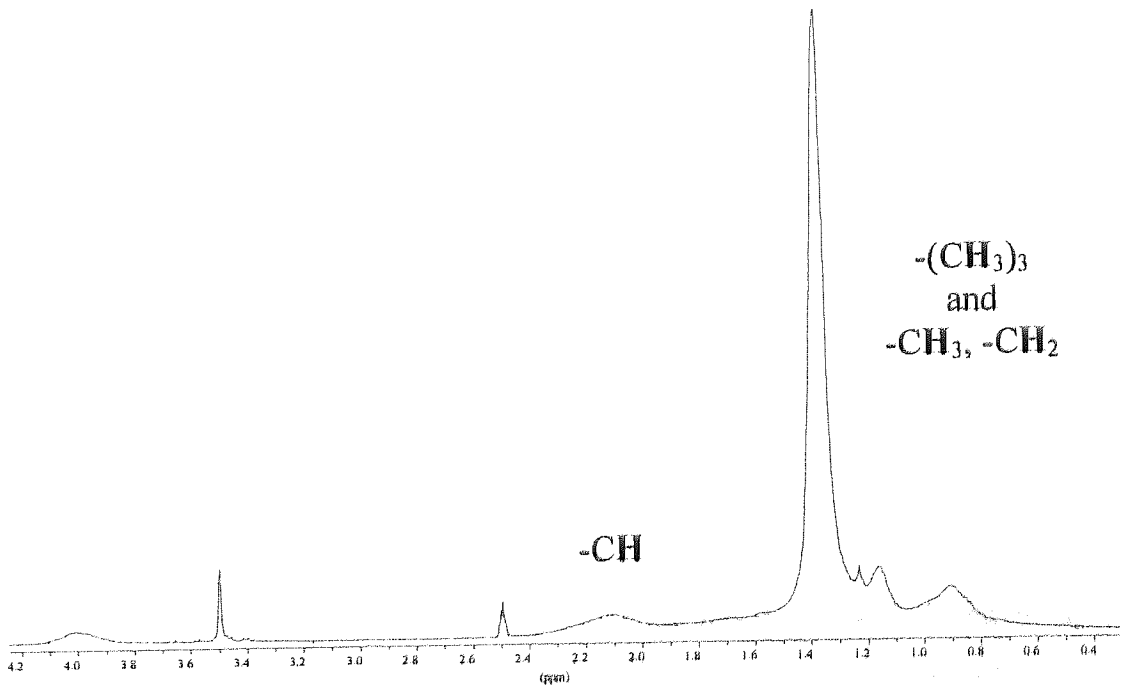


Figure 41. ^{13}C NMR of the Reaction of 2-Phenylethylamine with Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid)

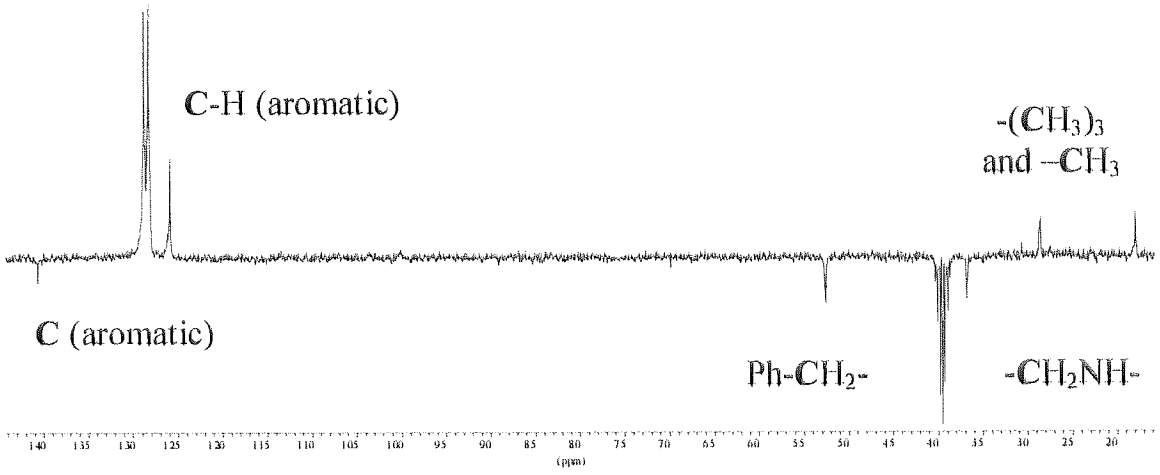
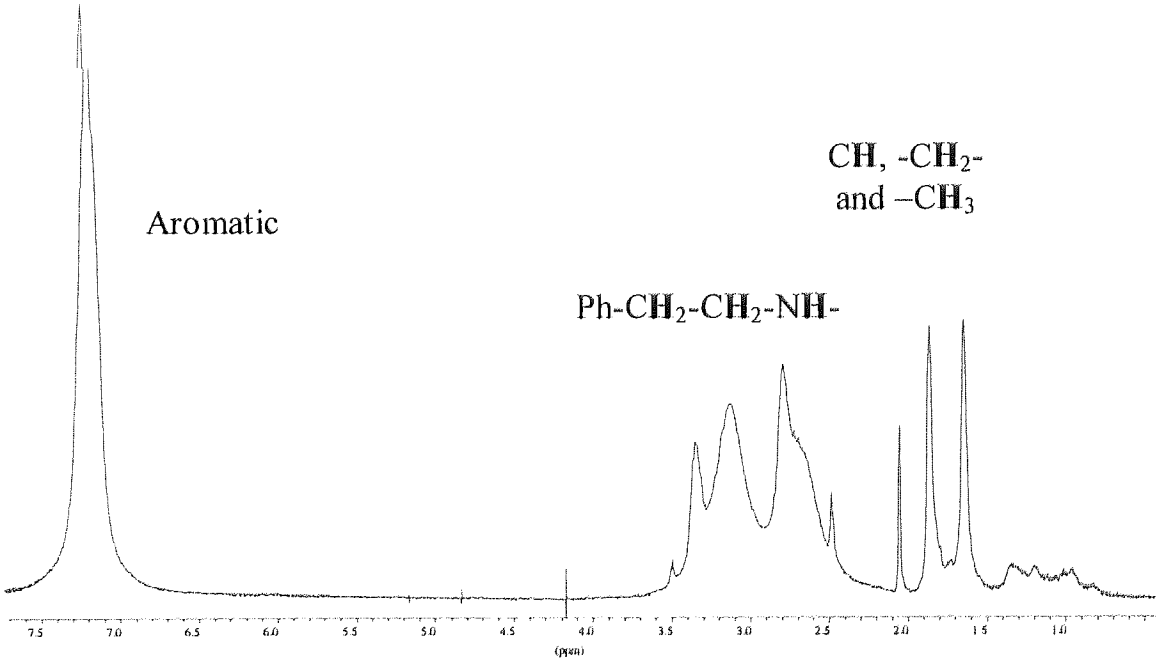


Figure 42. ^{1}H NMR of the Reaction of 2-Phenylethylamine with Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid)



The peak corresponding to the methyl groups of the *tert*-butyl alcohol unit is no longer present in the product and a new aromatic peak indicating the presence of a product derived from 2-phenylethylamine can be seen, see Figures 42 and 43.

A control experiment was carried out under identical conditions except that no enzyme was present. The same results were observed for these reactions indicating that the enzyme catalyst plays no part in the reaction. The ^{1}H NMR and ^{13}C NMR spectra of this control experiment are shown in Figures 43 and 44.

Figure 43. ^{13}C NMR of the Reaction if 2-Phenylethylamine with Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) with no Enzyme Present

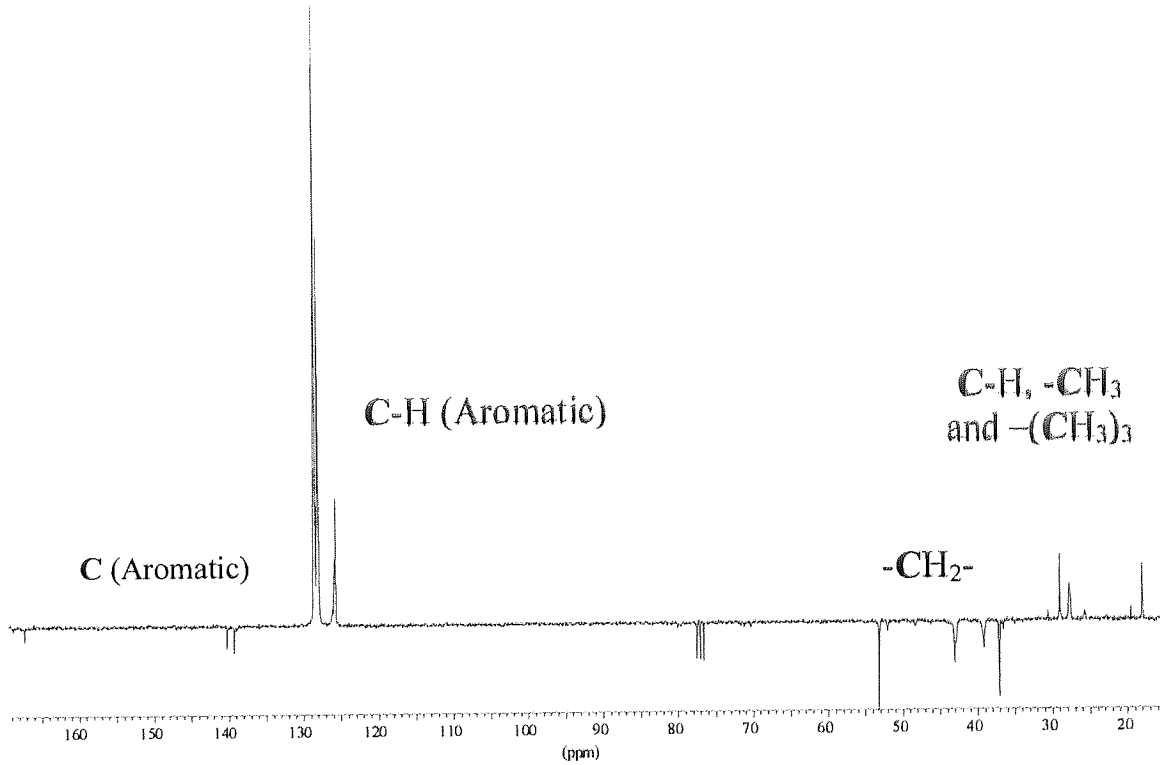
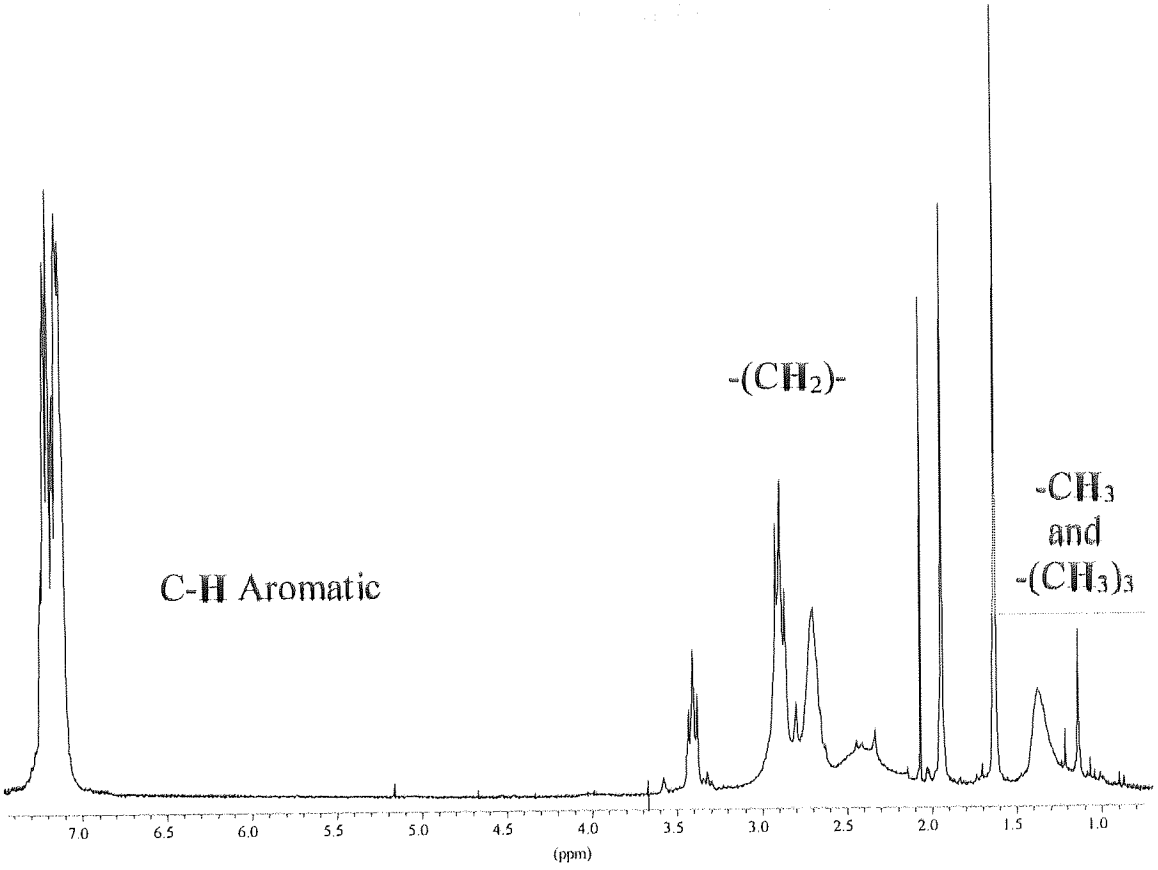


Figure 44. ^{1}H NMR of the Reaction of 2-Phenylethylamine with Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) with no Enzyme Present



4.4. Modification of Poly(*tert*-butyl acrylate)

It was observed in the previous system that substitution of the *tert*-butyl groups gave an amide both with and without enzyme catalysis. We therefore decided to investigate the corresponding reactions of poly(*tert*-butyl acrylate), which in contrast to poly(*tert*-butyl acrylate-co-ethyl acrylate-co-acrylic acid) is of defined structure.

This polymer was employed as the polymer substrate with the nucleophiles 2-phenylethyl alcohol, 1-butanol, 2-phenethylamine, 1-butylamine, diethylamine, benzylmercaptan, 1-butanethiol, glucose and L-leucine methyl ester hydrochloride in the solvents toluene, diethylether and pyridine. Porcine pancreatic lipase was used as a catalyst at 35 °C for 24 hours.

Comparison of the NMR spectra for the unreacted polymer, see Figures 46 and 47, with the NMR spectra of the product obtained from the reaction with 2-phenylethylamine in the presence of catalyst for 24 hours, see Figures 48 and 49, suggest that some reaction between 2-phenethylamine may have taken place, see Figure 45. The other nucleophiles investigated did not react and only starting materials were recovered. The same reaction carried out in diethyl ether as solvent gave similar results.

Figure 45. The Reaction of 2-Phenylethylamine with Poly(*tert*-butyl acrylate)

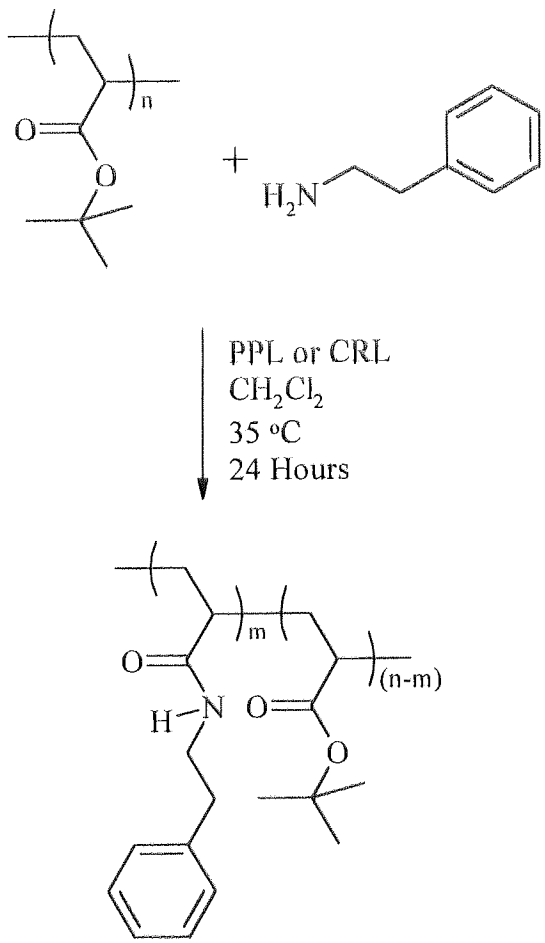


Figure 46. ^{13}C NMR of Poly(*tert*-butyl acrylate)

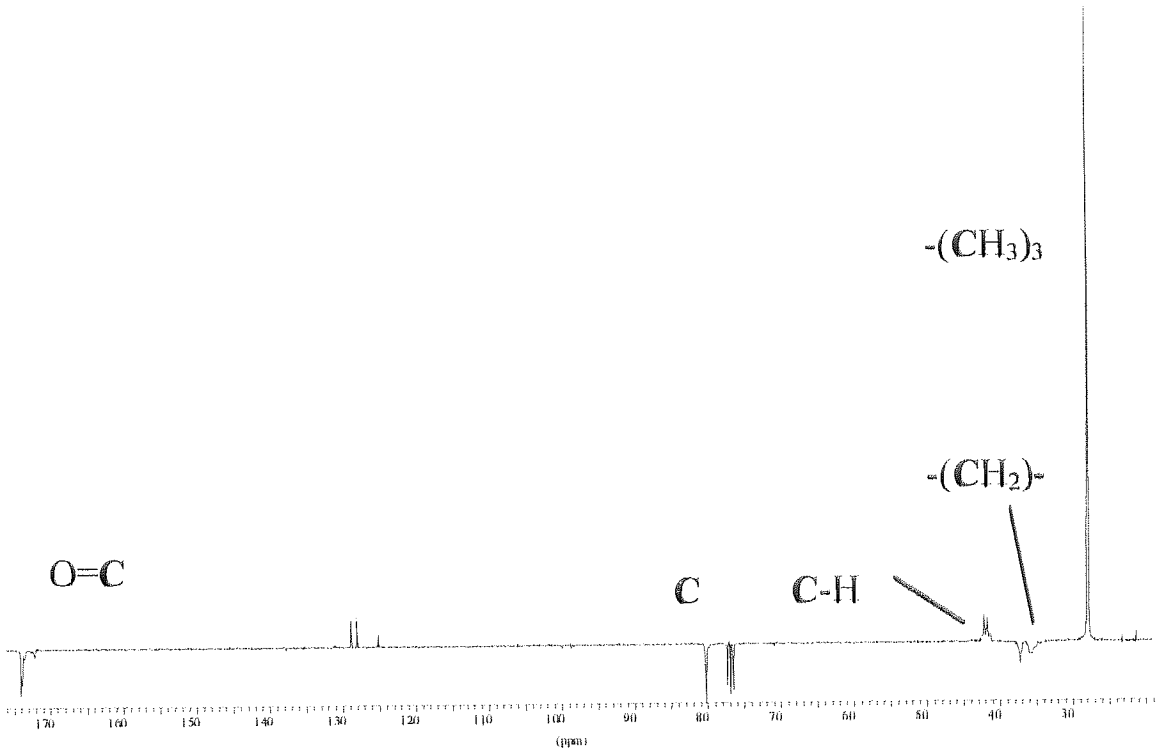


Figure 47. ^{1}H NMR of Poly(*tert*-butyl acrylate)

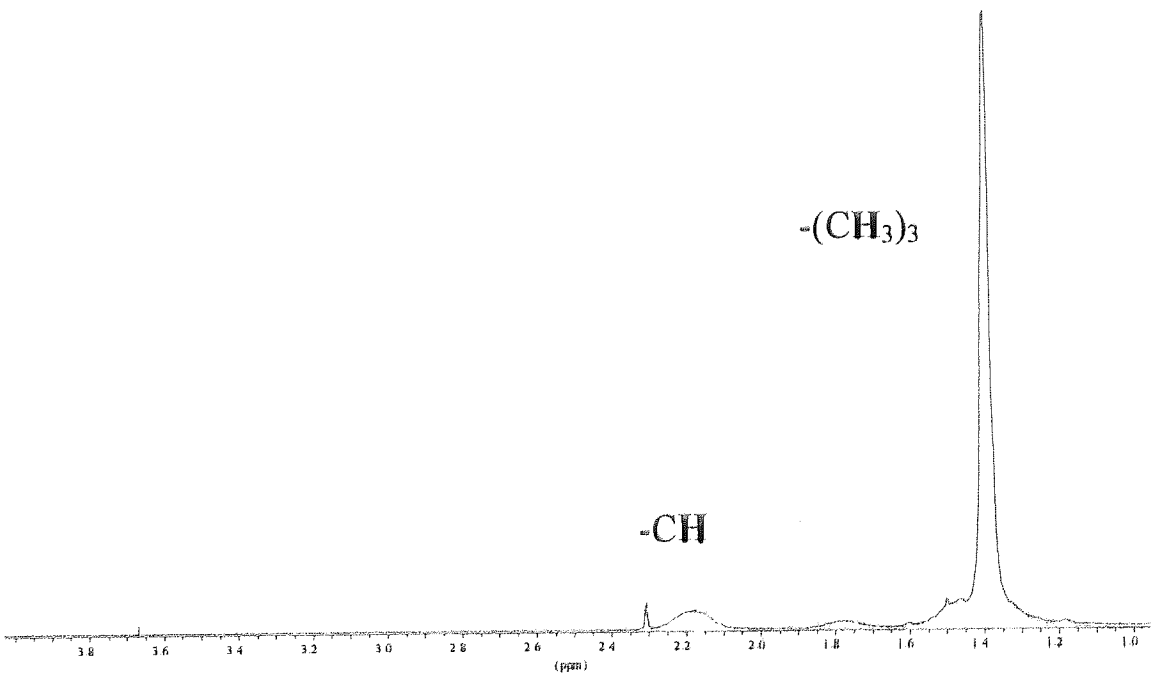


Figure 48. ^{13}C NMR of the Reaction of 2-Phenylethylamine with Poly(*tert*-butyl acrylate) Carried out in CH_2Cl_2

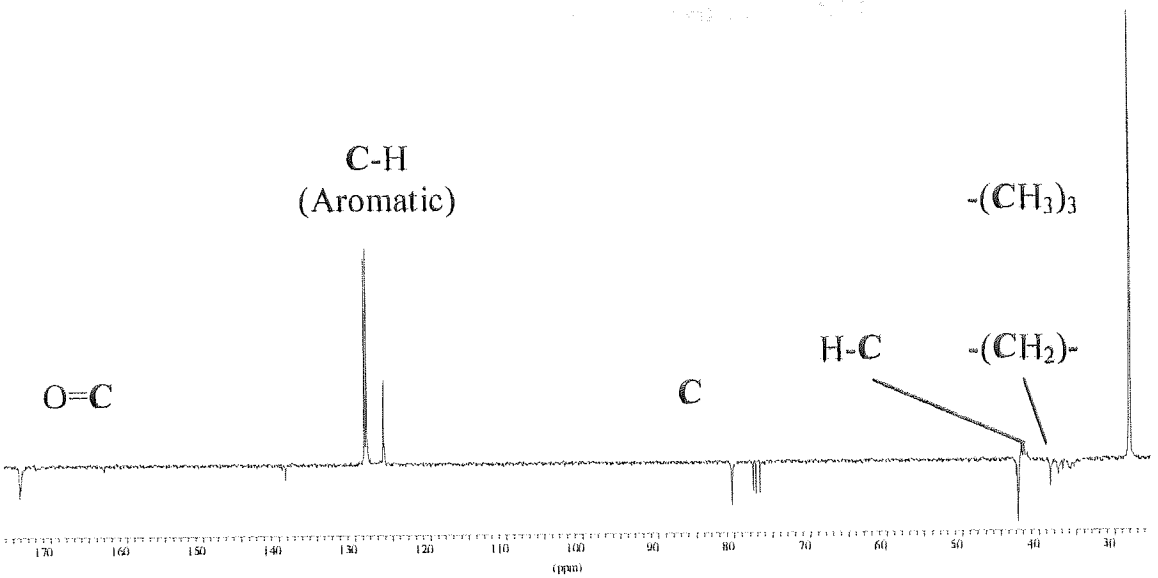
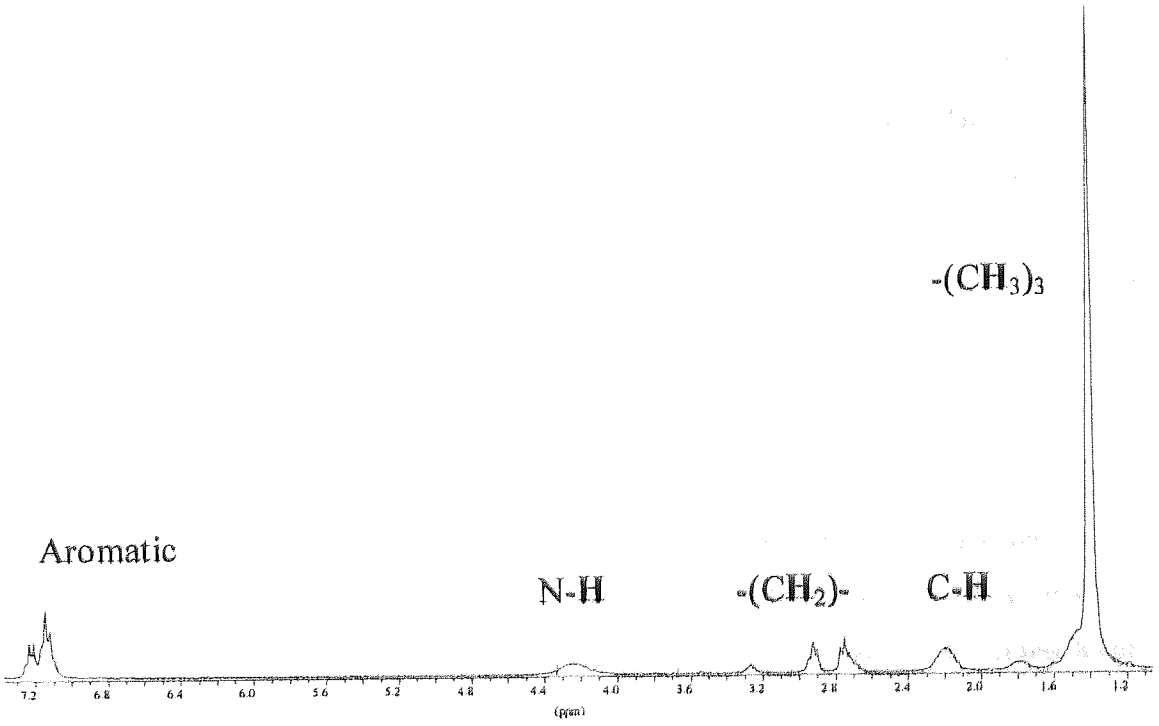


Figure 49. ^{1}H NMR of the Reaction of 2-Phenylethylamine with Poly(*tert*-butyl acrylate) Carried out in CH_2Cl_2



Signals arising from the ethyl group (-CH_2-CH_2-) of the nucleophile were found in the ^1H NMR and ^{13}C NMR spectra of the product. In addition to these peaks aromatic peaks were also observed. None of the above peaks were observed in the NMR spectra of the control experiments which were carried out in the absence of enzyme. A slight decrease in the signal arising from the *tert*-butyl group was observed. Taken together, these facts suggest that an enzyme catalysed reaction had taken place. However, the amount of material originating from the nucleophile is much greater than can be accounted for by the corresponding decrease in the *tert*-butyl signal. It therefore seems reasonable to conclude that no reaction has actually occurred and that the signals for the nucleophile arise from the residual starting material trapped within the polymer matrix.

The lack of reactivity of the *tert*-butyl groups in poly(*tert*-butyl acrylate) is surprising considering the high reactivity of these groups in poly(*tert*-butyl acrylate-co ethyl acrylate-co-methacrylic acid). It seems likely that the ethyl acrylate and methacrylic acid groups act as spacers allowing the nucleophile greater access to the *tert*-butyl ester.

4.5. Conclusion

The use of activated ester to promote the enzyme catalysed modification of polymers was investigated. The reactions of poly(2,2,2-trichloroethyl methacrylate), poly(2,2,2-trifluoroethyl methacrylate), poly(*tert*-butyl acrylate-co ethyl acrylate-co-methacrylic acid) and poly(*tert*-butyl acrylate) with a variety of alcohol, amine and thiol nucleophiles in the presence of porcine pancreatic lipase and *Candida rugosa* lipase were all unsuccessful. Whilst poly(*tert*-butyl acrylate-co ethyl acrylate-co-methacrylic acid) was similarly unreactive, reaction did occur with 2-phenylethyl amine but in a non-enzymatic process.

Ritter successfully modified carboxyl groups near the end of long pendent chains. In addition, polymer molecules are suitable substrates in enzyme catalysed polyester synthesis. It seems that steric factors are responsible for the lack of reaction in our systems. It would be of interest to investigate the effect of spacers in the main chain and the minimum length of spacer required in the side chain.

Chapter 5.

Miscellaneous Modifications of Polymers

5.0. Miscellaneous Modification of Polymers

5.1. Oxynitrilases

S and R oxynitrilases catalyse the addition of HCN to aldehydes and ketones,[109,110] see Figure 50.

Figure 50. Transcyanation

R" = H, Alkyl or Aromatic

Either isomer can form depending on the enzyme chosen.

Little is known of the mechanism of oxynitrilase enzymes. It has been speculated that a flavin group located near the active site binds to free HCN, forming a reactive 4a-cyano-flavin intermediate.[111] Both S and R directing enzymes are known and enable the synthesis of either enantiomer. Whilst research on enzyme catalysed transcyanation reactions of aldehydes has been carried out, comparatively little work has been carried out with ketones as substrates. More recent work by Effenberger confirmed that ketones are equally reactive and pose little by way of additional problems.[110]

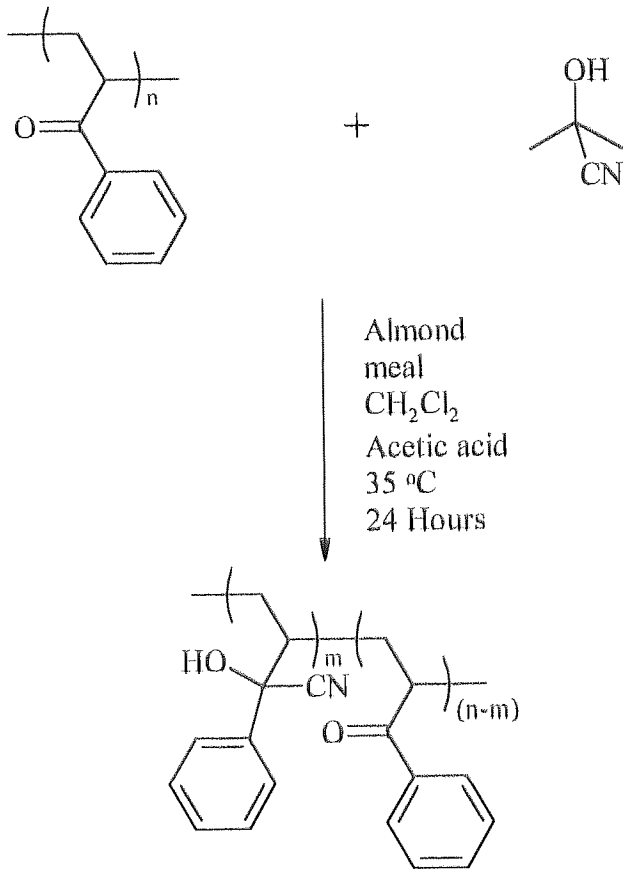
These enzymes are found to give the corresponding S or R cyanohydrin in high optical purity. In organic solvents the competing non-enzymatic addition of HCN is suppressed leading to high enantiomeric excesses.[112] Organic solvents reduce the quantity of free HCN dissolved in them, therefore limiting non-enzymatic addition of HCN. A further improvement on this reaction system uses acetone cyanohydrin as a safer source of HCN to give products of high enantiomeric purity, with better consistency than similar conditions using hydrogen cyanide solution as the cyanide source.[113] This is achieved because acetone cyanohydrin releases only low levels of HCN into solution, and reduces further the possibility of non-enzymatic addition of free HCN to the carbonyl group. The use of a biphasic system of buffer solution and organic solvent also enhances the enantiomeric excess obtained.[114] This is because products and starting materials partition into the organic phase in preference to the aqueous phase, and are then not being exposed to free HCN. Water-soluble reactants gave racemic products with hardly any observable selectivity, conversely, substrates whose water solubility's are low give almost enantiomerically pure products.

5.1.1. Transcyanation of Poly(vinyl phenyl ketone)

Poly(vinyl phenyl ketone) is a readily available and cheap polymer purchased from Aldrich and an ideal candidate for transcyanation. Almond meal can be easily prepared or purchased from Sigma and requires no further treatment before use as catalyst in the reaction. For purposes of safety and convenience acetone cyanohydrin was used as a source of HCN.

Poly(vinyl phenyl ketone) was dissolved in dichloromethane together with acetone cyanohydrin, catalytic amounts acetic acid and almond meal were then added and the reaction stirred at 35 °C for 24 hours, see Figure 51.

Figure 51. Transcyanation of Poly(vinyl phenyl ketone)



The NMR spectra of the starting materials are shown in Figures 52 and 53 and NMR spectra of the product are shown in Figures 54 and 55.

Figure 52. ^{13}C NMR of Poly(vinyl phenyl ketone)

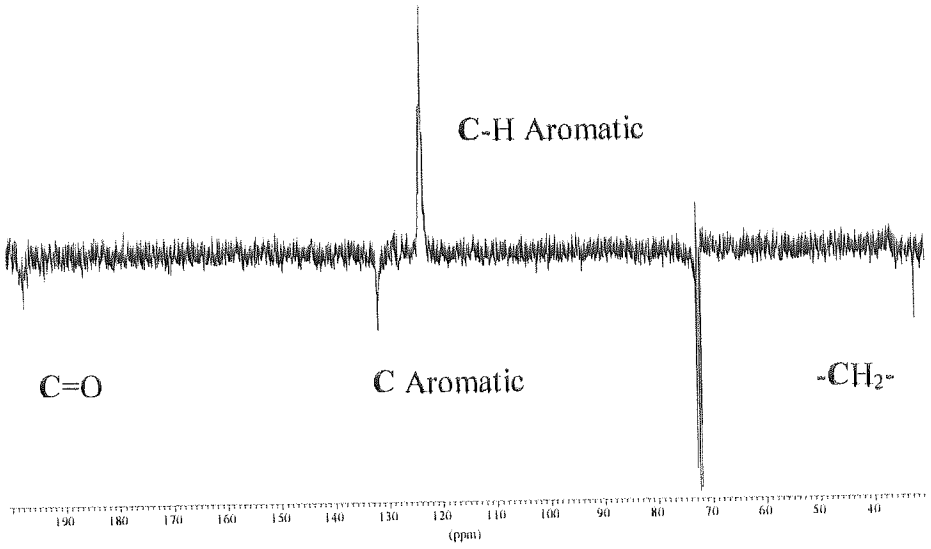


Figure 53. ^{1}H NMR of Poly(vinyl phenyl ketone)

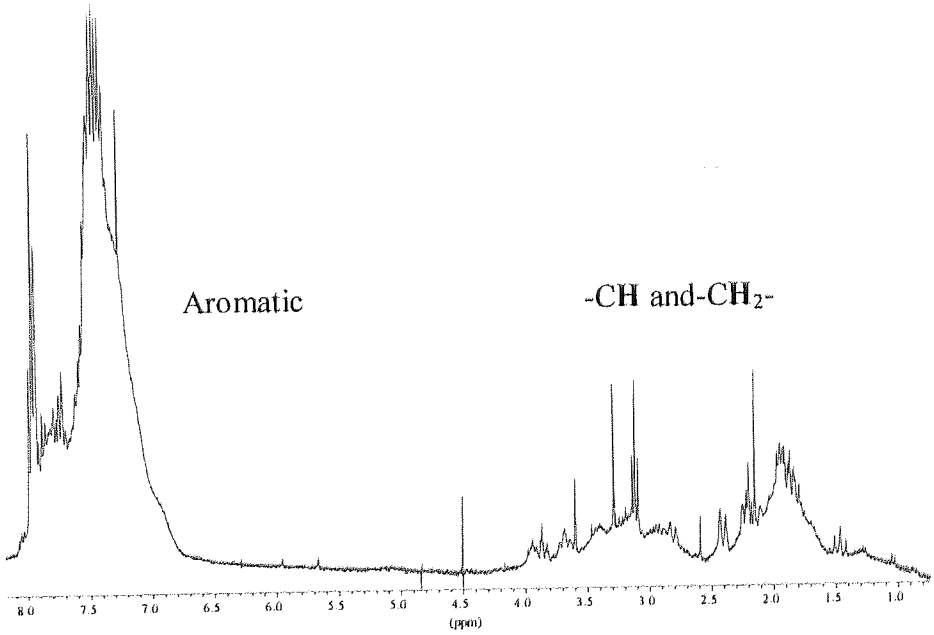


Figure 54. ^{13}C NMR of Transcyanation of Poly(vinyl phenyl ketone)

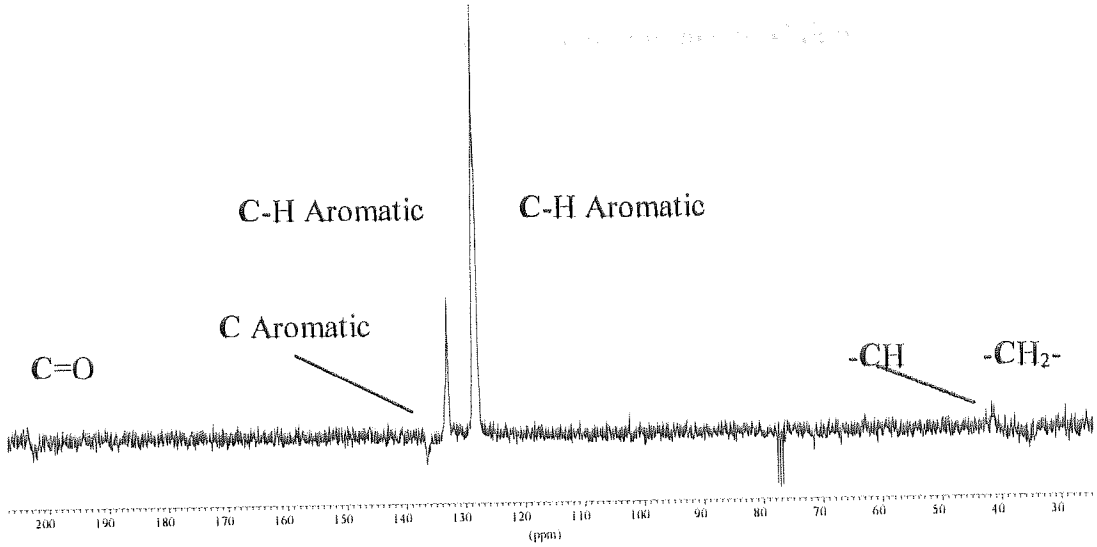
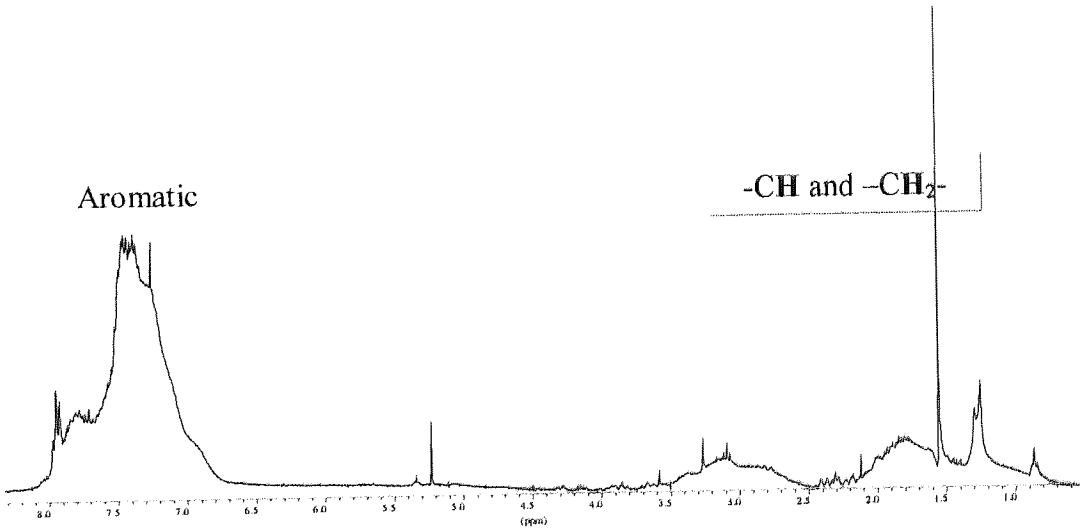


Figure 55. ^{1}H NMR of Transcyanation of Poly(vinyl phenyl ketone)



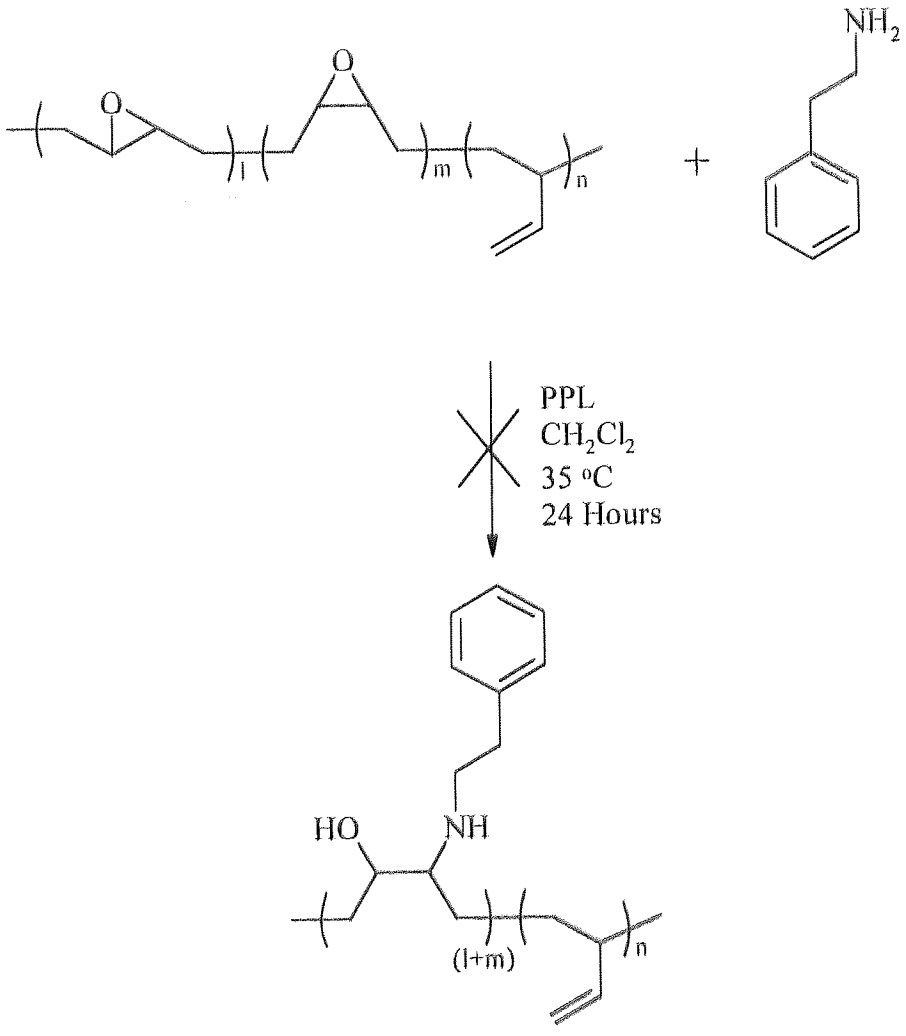
^{1}H NMR spectra of the transcyanation products were too broad to be of use whilst the ^{13}C NMR spectra of the products of transcyanation of poly(vinyl phenyl ketone) showed the appearance of a second aromatic peak indicating that a reaction had occurred. Control experiments with no enzyme present gave the same NMR results indicating that a non-enzymatic reaction was taking place. The ^{13}C NMR spectra is identical to those of the Baeyer-Villiger reactions of poly(vinyl phenyl ketone). This suggests that acid catalysed ketal formation also occurs in this transcyanation reaction as discussed for the Baeyer-Villiger reaction in section 2.2.

5.2. Ring Opening of Epoxidised Poly(butadiene)

Cole-Hamilton found that strong bases and extreme forcing conditions were required to open the epoxide ring of partially epoxidised poly(butadiene).[115] It is known that porcine pancreatic lipase is able to catalyse the opening of epoxide rings with amines.[7] It was therefore of interest to see if porcine pancreatic lipase would catalyse the opening of the epoxide rings of partially epoxidised poly(butadiene).

Epoxidised poly(butadiene) and one of a variety of nucleophiles were dissolved in dichloromethane, porcine pancreatic lipase added and the reaction stirred for 24 hours at 35 °C, see Figure 56.

Figure 56. Ring Opening of Epoxidised Poly(butadiene)



Reactions carried out with 2-phenylethylamine, 2-phenylethyl alcohol and benzyl mercaptan led to the isolation of unchanged polymer. The reactions were repeated with diethyl ether solvent but similar results were obtained.

Although this result is consistant with Cole-Hamilton's observations that the epoxide rings of epoxidised poly(butadiene) are difficult to open with many nucleophiles and require extremely strong forcing conditions, it is likely that the polymer is not a suitable substrate for this enzyme.[115]

5.3. Glycosidation and Transglycosidation of Hydroxyl Containing Polymers

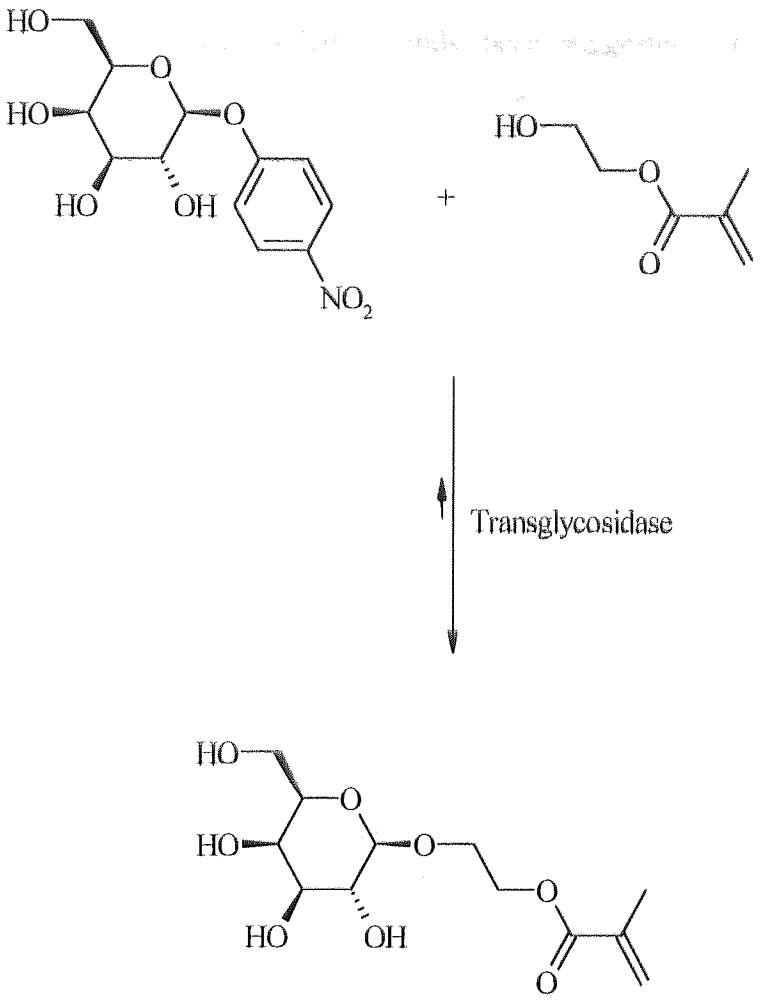
5.3.1. Glycosidases

The glycosidases cleave glycosidic bonds in the catabolism of carbohydrates. Two types of glycosidases exist; the *exo*-glycosidases that cleave terminal sugar residues and *endo*-glycosidases which cleave any glycosidic bonds other than terminal glycosidic bonds. The natural nucleophilic substrate for these enzymes is water, but this can be replaced by many other nucleophiles such as alcohols and amines.[116]

Use of these enzymes allows the stereochemistry at the anomeric centre to be completely controlled through the choice of the appropriate enzyme as both α and β-glycosidase are available.

Glycosidases have been used to couple a variety of galactosyl donors, to selected glycosyl acceptors *via* a glycosidic ether link[117] as is shown in Figure 57.

Figure 57. Transglycosidation

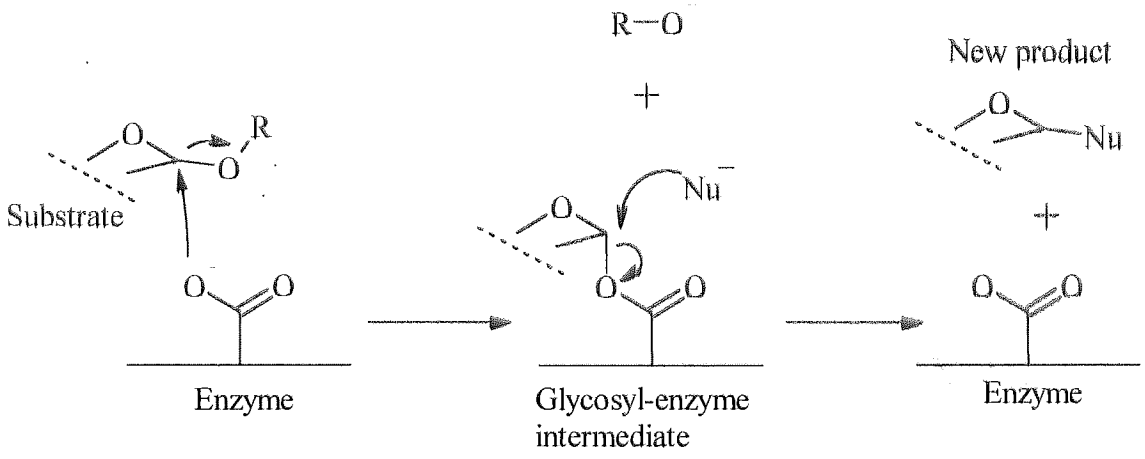


The chemical transformations of carbohydrates require complicated regioselective protection and deprotection of the hydroxyl groups on the sugar moieties. In addition to this, complete stereocontrol of the glycoside bond forming reaction has not yet been achieved with traditional chemical methods. Use of enzymes provides a solution to both of these problems. Glycosidases are able to differentiate between α and β glycosidic links.[118] Just as activated esters are used in transesterification reactions a similar approach can be used to activate sugars. For example, the 4-nitrophenyl group in 4-nitrophenyl-β-D-galactopyranoside is a good leaving group and activates the sugar.[118] This use of activated sugars forces the reaction equilibrium in the desired forward direction and minimises the possibility of competitive reverse reactions.

5.3.2. Mechanism of Glycosidase Enzyme-Substrate Interaction

The mechanism shown in Figure 58 has recently been suggested for the hydrolysis of glycosidic links by β-glucosidase from *Agrobacterium*.[119] The mechanism entails the carboxyl moiety of a glutamic acid residue, attacking the glycoside donor, forming a covalent glycosyl enzyme-substrate intermediate. The enzyme-substrate intermediate is then attacked by a nucleophile, thus forming a new glycosidic-link.

Figure 58. Mechanism of β-Glucosidase Enzyme

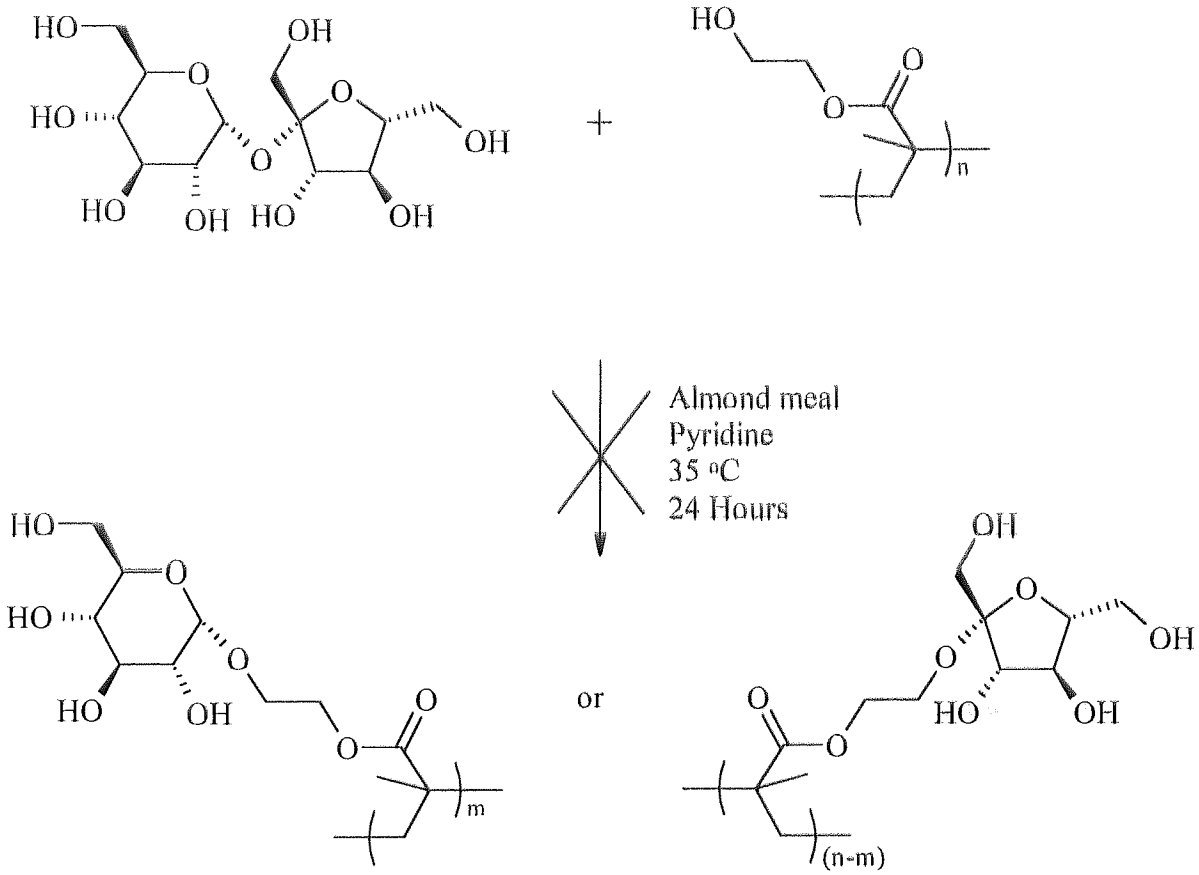


5.3.3. Transglycosidation of Poly(vinyl alcohol) and Poly(2-hydroxyethyl methacrylate)

Polymers containing natural product components such as sugars are of interest as they may be biocompatable and could have potential uses in biological systems. Poly(vinyl alcohol) and poly(2-hydroxyethyl methacrylate) were used to investigate the transglycosidation reactions with sucrose, lactose, sucrose octaacetate and 2-nitrophenyl-β-D-galactopyranoside. These reaction systems utilised transglycosidase from bitter almond meal and commercially available purified lactase enzyme as catalysts for the formation of the glycosidic links between the polymers and various sugar moieties. Poly(vinyl alcohol) was dissolved in DMSO by heating to 80 °C and then allowed to cool to 35 °C before the sugar and enzyme were added. Poly(2-hydroxyethyl methacrylate) and the sugar being reacted were dissolved in pyridine at 35 °C and the enzyme then added. The reaction mixtures were then stirred for 24 hours before isolation of product.

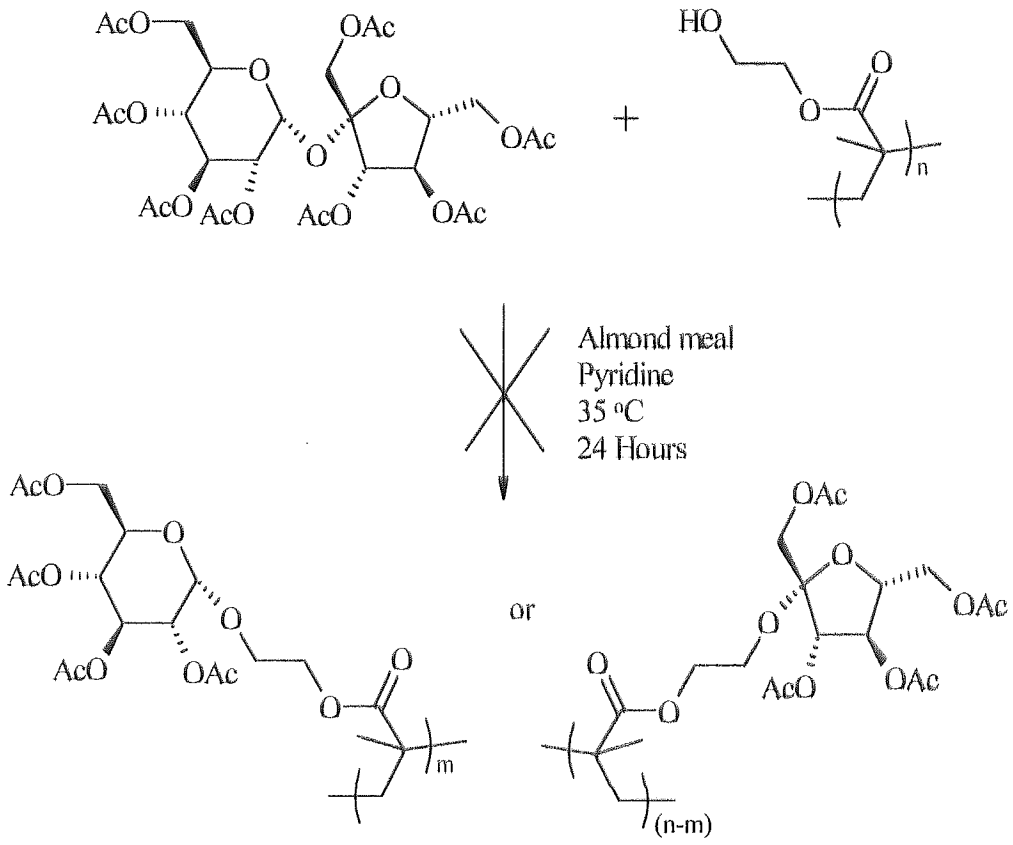
The transglycosidation reaction between poly(2-hydroxyethyl methacrylate) and sucrose, see Figure 59, was investigated, but NMR analysis revealed that no reaction appeared to have taken place.

Figure 59. Transglycosidation of Poly(2-hydroxyethyl methacrylate)



It was thought that this lack of success maybe a result of the limited solubility of sucrose. To improve solubility sucrose octaacetate was synthesised. One drawback to this approach was that there was the possibility that this would result in the sugar derivative being unable to bind to the active site of the enzyme. Sucrose octaacetate was synthesised by dissolving sucrose in acetic anhydride and heating on a water bath. It was reacted under the same conditions as had been used for sucrose, see Figure 60.

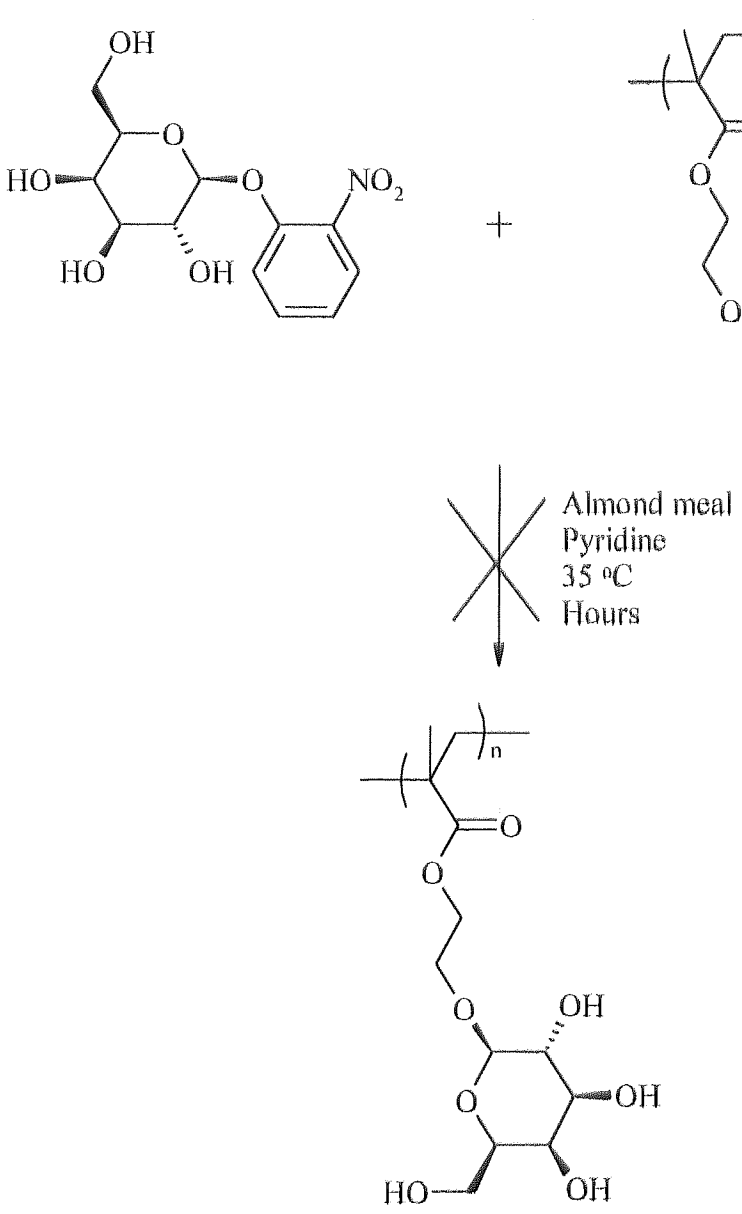
Figure 60. Transglycosidation of Poly(2-hydroxyethyl methacrylate) with Sucrose Octaacetate



Unfortunately, this experiment did not give any transglycosidation reactions with only starting materials being isolated.

Activated sugars such as 2-nitrophenyl-β-D-galactopyranoside have been successfully used in transglycosidation reactions. The 2-nitrophenyl group is electron withdrawing and a poor nucleophile, the reaction is driven in the desired direction making it irreversible, in a similar manner to transesterification reactions using activated esters. Figure 61 shows the transglycosidation of poly(2-hydroxyethyl methacrylate) with the activated sugar 2-nitrophenyl-β-D-galactopyranoside.

Figure 61. Transglycosidation of Poly(2-hydroxyethyl methacrylate) with 2-Nitrophenyl-β-D-galactopyranoside

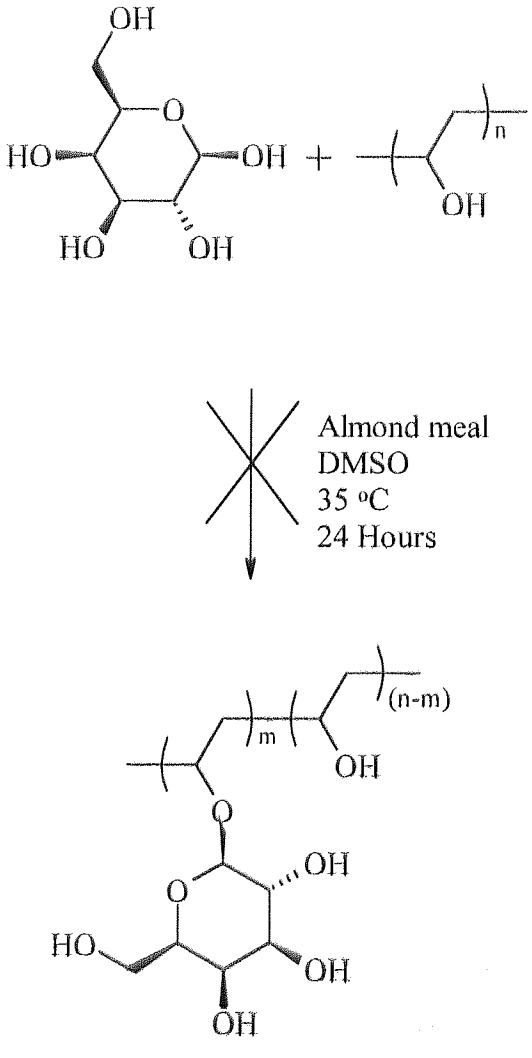


No evidence of any reaction was observed and only starting materials were recovered.

5.3.3.1. Glycosidation of Poly(vinyl alcohol)

Glycosidation and transglycosidation of poly(vinyl alcohol) with β-D-glucose and β-D-lactose were carried out by a similar reaction procedure to that used previously for the transglycosidation reactions of poly(2-hydroxyethyl methacrylate). Dimethyl sulfoxide was used as a solvent because of the solubility problems encountered with poly(vinyl alcohol), see Figure 62.

Figure 62. Glycosidation of Poly(vinyl alcohol) with β-D-Glucose



In the case of D-glucose only starting materials were recovered, no product formation was observed. NMR analysis of the product isolated from the reaction of β-D-lactose with poly(vinyl alcohol) showed no evidence of polymer modification but did reveal that the sugar component of the system had changed. The new complex NMR spectrum suggested epimerisation of β-D-lactose and possibly glycosidation reactions taking place between the individual sugar units but showed the polymer to be unchanged.

All glycosidation and transglycosidation reactions attempted proved to be unsuccessful even when activated glucose was used. As the monomers used here are known to undergo glycosidation and transglycosidation reactions it is suspected that the bulk of the polymer and the associated steric hindrance is responsible for the lack of reactivity of these systems. NMR evidence indicating modification of the sugar moiety was not accompanied by any evidence of polymer modification having taken place and can therefore be explained by epimerisation and transglycosidation reactions occurring between sugar units.

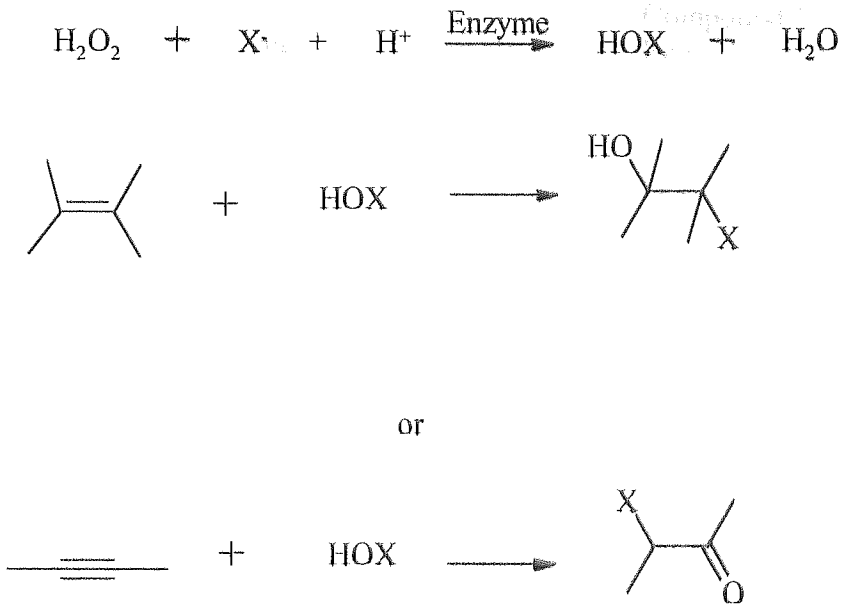
5.4. Halohydroxylation

5.4.1. Haloperoxidases

The haloperoxidase enzymes are named chloro-, bromo-, or iodoperoxidases depending on the most electronegative halide they can oxidise.[120] Haloperoxidases have been detected in many different organisms including mammals, birds, plants, algae, moulds and bacteria. Although it is known that halogenated metabolites appear to play a fundamental role in many organism's defence mechanisms, not much is known about the contribution of the enzyme to the formation of C-X (X= I, Br, Cl) bonds.[121, 122] To date, no enzyme system has been found that is able to catalyse the addition of F to alkenes and alkynes, although fluorinated compounds are known in nature.

The chloroperoxidase from *Caldariomyces fumago* is commercially available. The enzyme is thought to catalyse the *in situ* synthesis of HOX (from H_2O_2 and KX) which is the active halogenating species in solution. The HOX can react with alkene and alkynes *via* a Markownikoff addition to give halohydrins and α-halo ketones respectively, see Figure 63.

Figure 63. Halohydrin and α-halo ketone Formation



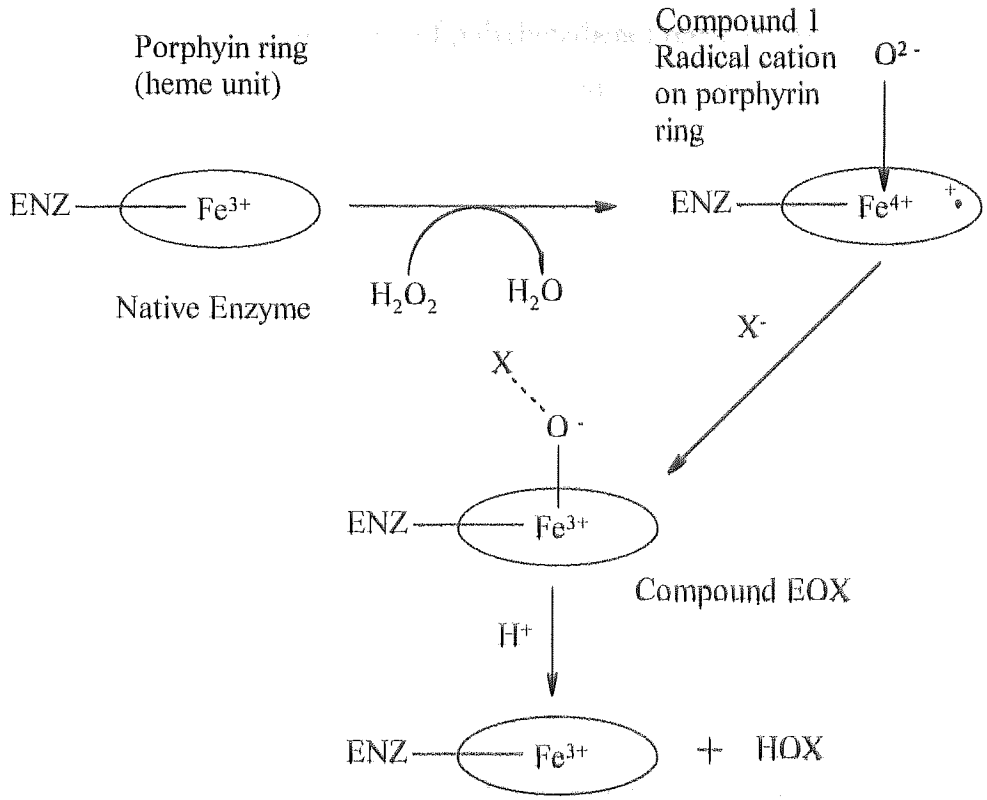
The system can also be manipulated to give a dihalogenated product rather than halohydrin products.[123] It was found that whilst systems using low concentrations of halide ion (20 mM) produced halohydrins, high concentration of halide ions (2000 mM) produced dihalides.

One disadvantage of chloroperoxidase is that it requires an aqueous buffer of pH ~ 3 in order to operate. In an effort to increase its stability under a wider variety of reaction conditions, chloroperoxidase has been immobilised on talc.[124] The enzyme-talc combination was used to synthesise a halohydrin with no oxidative by-products being formed. The immobilised enzyme allowed larger quantities of hydrogen peroxide and substrate to be used. The enzyme was recoverable and reusable.

5.4.2. Mechanism of Haloperoxidase Enzyme-substrate Interaction

Haloperoxidase possesses a ferriprotoporphyrin IX group. Both the iron (III) ion and the porphyrin group play an important role[49] see Figure 64.

Figure 64. Mechanism of Haloperoxidase Catalysis



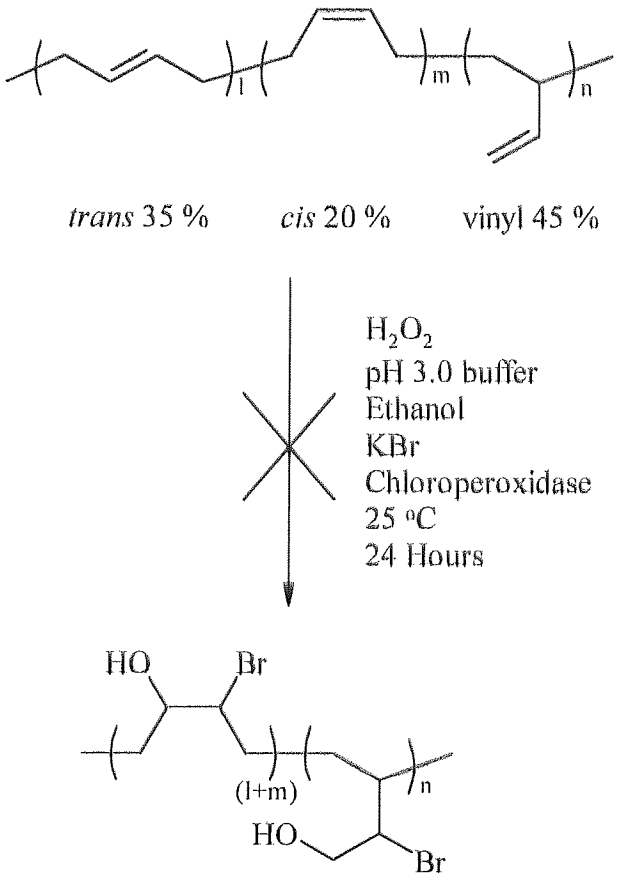
Hydrogen peroxide reacts with the native enzyme, oxidising the heme unit (compound 1) as shown in Figure 64. The iron ion has a formal charge of 4+ and the porphyrin ring is transformed into a radical cation. Compound 1 reacts with oxidisable substrates, such as halide ions, to form compound EOX. Two electrons are transferred from the halide ion to the iron (IV) and the porphyrin radical cation, thereby oxidising the halide to the X^+ ion. The existence of compound EOX has not been conclusively proven, but evidence for its existence is increasing.[121] It is thought that the transient postulated compound of EOX decomposes, liberating an oxidised halide species as a reactive halogenating agent. The exact nature of the halide species is difficult to determine as it exists in an equilibrium of HOX, X_2 and X_3^- in aqueous solution.

5.4.3. Halohydroxylation of Poly(butadiene)

The successful enzyme catalysed epoxidation of poly(butadiene) relied on using an enzyme to catalyse the *in situ* formation of the peroxyacetic acid oxidant. In this reaction the enzyme played no part in the actual epoxidation step. Our studies of the modification of synthetic polymers in which the polymer was required to interact directly with the enzyme were all unsuccessful. Chloroperoxidase from *Caldariomyces fumago* can be used to catalyse the *in situ* synthesis of HOX and it was hoped that HOX would then react with the alkene groups of poly(butadiene) in a conventional chemical process, with no interaction between the alkene and the enzyme being required. This seemed to be a promising system which might allow the halogenation of poly(butadiene) under very mild conditions. One drawback of this enzyme is that it needs to operate in an aqueous buffer at pH ~ 3.0. This posed solubility problems due to the hydrophobic nature of poly(butadiene). However, previous studies of the halohydroxylation of simple unsaturated hydrocarbons illustrated that hydrophobic compounds were reactive in this system.[49, 122, 123]

Chloroperoxidase was used in a pH 3.0 buffer solution, with aqueous hydrogen peroxide and KBr. Ethanol (30 % v/v) was added to the reaction mixture to assist mixing of the hydrophobic poly(butadiene). It was hoped that rapid stirring of this two phase system would enable the HOBr formed to react with the double bond of poly(butadiene) and form the halohydrin adduct as shown in Figure 65.

Figure 65. Bromohydroxylation of Poly(butadiene)



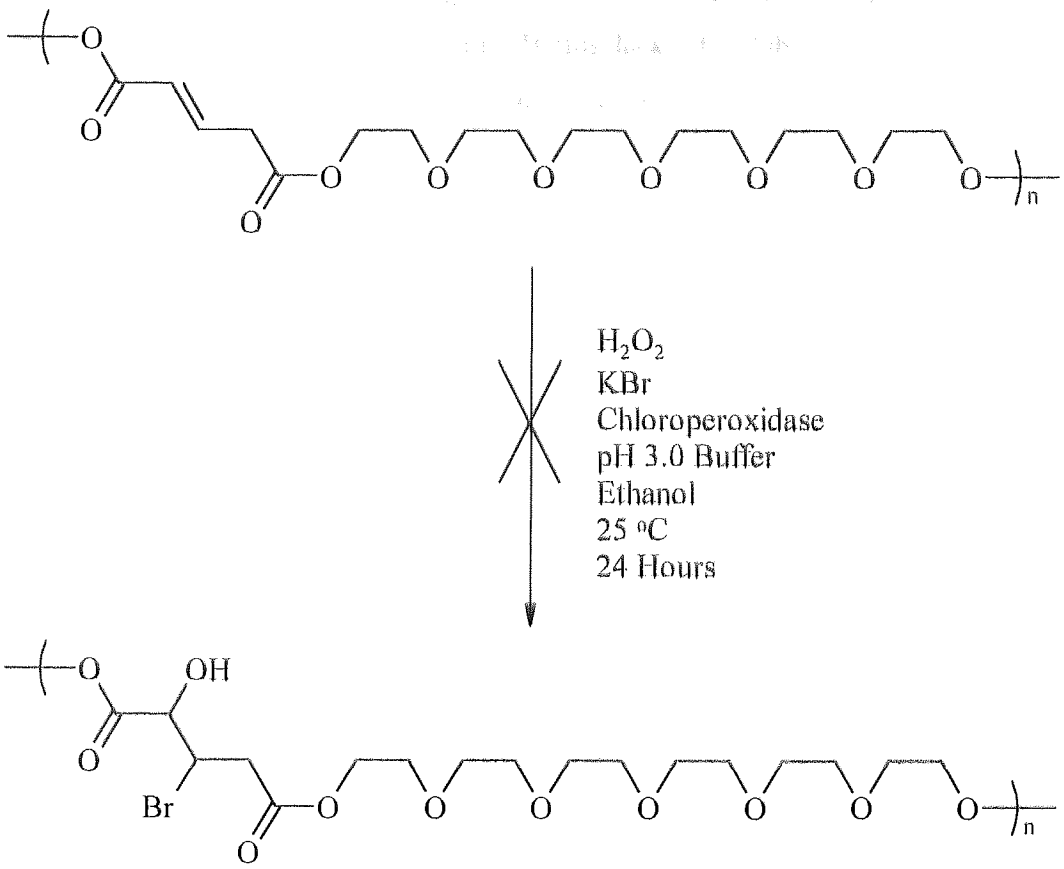
Unfortunately poly(butadiene) was not able to mix well in the system and starting material was recovered from the reaction with no apparent evidence of any halohydrin formation taking place. Any HOBr that was formed, appeared not have been able to diffuse to and react with the alkene groups.

5.4.4. Halohydroxylation of Poly(hexaethylene glycol fumarate)

The solubility problems encountered with poly(butadiene) led us to prepare a potential water soluble polymer to act as a substrate. Poly(hexaethylene glycol fumarate) was synthesised from fumaroyl dichloride and hexaethylene glycol.

The reaction employed in the previous bromohydroxylation was repeated, see Figure 66. Whilst dispersal of the polymer was improved relative to poly(butadiene), no reaction was observed.

Figure 66. Bromohydroxylation of Poly(hexaethylene glycol fumarate)



In addition to the solubility problems the electron withdrawing effect of the carbonyl groups adjacent to the alkene group may render the alkene unreactive.

5.5. Conclusion

The conversion of the ketone groups in poly(vinyl phenyl ketone) to cyanohydrins in the presence of almond meal from bitter almonds gave products with ^{13}C NMR spectra identical to those obtained in the Baeyer-Villiger reactions of this polymer discussed in Chapter 2.

Attempts to catalyse the ring opening of epoxidised poly(butadiene) with porcine pancreatic lipase as catalyst were not successful.

Transglycosidation reactions of poly(2-hydroxyethyl methacrylate) and poly(vinyl alcohol) with a variety of sugars were also unsuccessful. It seems likely that steric hindrance is the principle cause for the lack of reactivity in all of these reactions.

Chloroperoxidase was used to catalyse the *in situ* synthesis of HOBr. This failed to react with poly(butadiene) and poly(hexaethylene glycol fumarate) because of the poor solubility of the polymers in the aqueous reaction medium. If this lack of solubility can be overcome this procedure would have great appeal as unsaturated polymers could be halogenated under extremely mild conditions.

Chapter 6.

Conclusions

6.0. Conclusions and Further Work

The aim of this project was to investigate the use of enzymes as catalysts for the modification of synthetic polymers. In the only reports of work in this area, Ritter found that enzymes could be used to modify hydroxyl and carboxyl groups distant from the polymer chain.[9, 86] It was therefore of interest to investigate the use of enzymes in catalysing the modification of groups close to the polymer backbone or even functional groups within the polymer main chain itself.

In the first example of a reaction of this kind, it was found that enzymes could indeed be used as catalysts for the modification of the backbone of synthetic polymers. An immobilised lipase from *Candida antartica* (Novozyme 435), in the presence of hydrogen peroxide and catalytic amounts of acetic acid, was found to catalyse the selective epoxidation of poly(butadiene). The *cis* and *trans* double bonds of the polymer backbone were converted to the corresponding epoxides in yields of up to 59 %. In contrast, the pendent vinyl groups were left unreacted. The effect of varying a number of parameters on the reaction was investigated but higher yields of epoxide could not be obtained. The effect of varying even a single parameter did not lead to a readily predictable outcome. This is probably due to the complex nature of this three phase system and the subtle interplay of the various processes involved. However, these results do suggest that the conformations adopted by the partially epoxidised polymer chain may preclude further reaction.

Applications of this reaction to the Baeyer-Villiger oxidation of poly(vinyl phenyl ketone) and poly(vinyl methyl ketone) were unsuccessful. Whilst the latter polymer was recovered unchanged, the former showed some modification *via* a non-enzymatic process. Since the same results were obtained in the conventional chemical reaction with peracetic acid, it appears that it is the nature of the polymers which prevent reaction from occurring rather than the enzyme catalysed system.

The enzyme catalysed esterification of poly(vinyl alcohol), poly(vinyl alcohol-co-ethylene) and poly(2-hydroxyethyl methacrylate) with benzoic acid, vinyl benzoate, vinyl pivalate and benzoic anhydride, in the presence of either porcine pancreatic lipase or *Candida rugosa* lipase were unsuccessful. The transglycosidation reactions of these polymers with sucrose, β-D-glucose, D-lactose and the activated sugar 2-nitrophenyl-β-D-galactopyranoside, in the presence of almond meal from bitter almonds, were also unsuccessful.

No modified product was obtained from the reactions of poly(2,2,2-trichloroethyl methacrylate), poly(2,2,2-trifluoroethyl methacrylate), and poly(tert-butyl acrylate) with a variety of alcohol, amine and thiol nucleophiles in the presence of porcine pancreatic lipase or *Candida rugosa* lipase. Although poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) was similarly unreactive, reaction with 2-phenylethylamine was found to occur but in a non-enzymatic process.

In contrast to the enzyme catalysed epoxidation of poly(butadiene) and Baeyer-Villiger oxidation of poly(vinyl phenyl ketone), the reactions of poly(vinyl alcohol), poly(vinyl alcohol-co-ethylene), poly(2-hydroxyethyl methacrylate), poly(2,2,2-trichloroethyl methacrylate), poly(2,2,2-trifluoroethyl methacrylate), poly(*tert*-butyl acrylate) and poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) all required an intimate interaction between the enzyme and the polymer. Polymers have been found to be suitable substrates in the enzyme catalysed synthesis of polyesters, whilst Ritter found that groups distant from the polymer backbone could be modified by enzyme catalysed reactions and that spacer groups in the main chain resulted in more extensive modification. In light of this, our results suggest that steric hindrance is one of the most important factors in the modification of groups close to the polymer backbone. Further work in this area should include an investigation of the use of alternative enzymes to those in this study, the effect of spacers in the main chain and a systematic study of side-chain length on the yield of modified groups.

The results presented here suggest that at the current time enzymes may be used successfully to catalyse the modification of synthetic polymers if the enzyme is used to catalyse the formation a reagent *in situ* and this reagent then reacts with the polymer in a conventional chemical process. No interaction between the polymer and the enzyme is required in such systems. The use of chloroperoxidase from *Caldariomyces fumago* to catalyse the *in situ* synthesis of HOBr would appear to be such a reaction and has great potential given the mild conditions under which halogenation takes place. Unfortunately it was found that the poor solubility of poly(butadiene) and poly(hexaethylene glycol fumarate) in the aqueous reaction medium did not allow any bromohydroxylation reaction to occur. Future work in this potentially promising area should involve the use of the more robust chloroperoxidase immobilised on talc which may tolerate higher levels of organic solvents.[124] An alternative answer to the solubility problem would be to carry out the reactions in emulsions.[124]

Chapter 7.

Experimental Details

7.0. Experimental Details

In this chapter the reactions of each polymer are grouped together and are not arranged according to the discussion chapters.

7.1. Reagents

All reagents and solvents were used as supplied unless otherwise stated.

Compound	RMM	Mp. / Bp. °C	Supplier
α,α'-Azobisisobutyronitrile	164	-	Aldrich
β-D-glucose	180.16	153-156	Aldrich
Sucrose	342.30	185-187	Aldrich
β-D-lactose	342.30	-	Aldrich
1-Butanol	74.12	117.7	Aldrich
1-Butylamine	73.14	78	Aldrich
2,2,2-Trichloroethanol	149.40	151	Aldrich
2,2,2-Trifluoroethanol	100.04	77-80	Aldrich
27.5 wt % Hydrogen peroxide	34	-	Aldrich
2-Hydroxyethymethacrylate	130.14	67 / 3.5 mm Hg	Aldrich
2-Nitrophenyl-β-D-Galactopyranoside	301.25	195	Aldrich
2-Phenylethylalcohol	122.17	219-221	Aldrich
2-Phenylethylamine	121.18	197-200	Aldrich
32 wt % Peroxyacetic acid	76	-	Aldrich

Acetic acid	60.05	117.9	Aldrich
Acetic anhydride	102.09	138-140	Aldrich
Almond meal	-	-	Sigma
Aniline	93.13	184	Aldrich
Benzoic acid	122.12	122-123	Aldrich
Benzoic anhydride	226.23	38-42	Aldrich
Benzyl alcohol	108.14	205	Aldrich
Benzylamine	107.16	184-185	Aldrich
Benzyl mercaptan	124.21	194-195	Aldrich
1-Butanethiol	90.19	98	Aldrich
Butyric acid	88.11	162	Aldrich
Candida antartica lipase (Novozyme 435)	-	-	Novonordisk
Candida rugosa lipase	-	-	Aldrich
Chloroperoxidase from *Caldariomyces fumago*	-	-	Sigma
Citric acid	192.12	152-154	Aldrich
Dichloromethane	84.93	40	Fisher
Diethylamine	73.14	55	Aldrich
Diethyl ether	74.12	34.6	Fisher

N,N-dimethyl formamide	73.10	153	Fisher
Dimethyl sulfoxide	78.13	189	Aldrich
Fumaroyl dichloride	152.96	161-164	Aldrich
Hexaethylene glycol	282.34	217 / 4 mm Hg	Aldrich
Hexane	86.18	69	Fisher
L-leucine methyl ester hydrochloride	181.66	148-150	Aldrich
Magnesium sulfate	120.37	-	Aldrich
Methacrylic acid	86.09	163	Aldrich
Methacryloyl chloride	104.54	95-96	Aldrich
Methanol	32.04	64.7	Fisher
Methyl vinyl ketone	70.09	36.5-36.8 / 145 mm Hg	Aldrich
Palmitic acid	256.43	61-64	Aldrich
p-Toluenesulphonic acid monohydrate	190.22	103-106	Aldrich
Phenothiazine	199.28	182-187	Aldrich
Poly(butadiene), phenyl terminated (45 % vinyl, 35 % *trans*, 25 % *cis*)	Mn 1300	-	Aldrich
Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid)	Mn 12500	-	Aldrich
Poly(vinyl alcohol), 99% hydrolysed	Mw 50000	-	Aldrich

Poly(vinyl alcohol-co-ethylene) (44 mole % ethylene)	Mn 41400	-	Aldrich
Poly(vinyl phenyl ketone)	Mn 750	-	Aldrich
Porcine pancreatic lipase	-	-	Aldrich
Potassium acetate	98.15	-	Aldrich
Potassium bromide	119.01	734	Aldrich
Potassium phosphate	212.28	-	Aldrich
Pyridine	79.1	115	Aldrich
Silica gel	-	-	Aldrich
Sodium bicarbonate	84.01	-	Aldrich
Sodium chloride	58.44	801	Aldrich
tert-Butyl acrylate	128.17	61-63 / 60 mm Hg	Aldrich
Triethylamine	101.19	88.8	Aldrich
Trifluoroacetic acid	114.02	72.4	Aldrich
Vinyl acetate	86.09	72-73	Aldrich
Vinyl benzoate	148.16	95-96 / 20 mm Hg	Aldrich
Vinyl pivalate	128.17	110	Aldrich

7.2. Methods of Analysis

Infrared spectra were recorded on a Perkin Elmer 1710 Fourier Transform infrared Spectrometer. Solid samples were prepared as KBr discs, whilst polymers were cast as thin films on sodium chloride plates. NMR spectra were recorded on a Bruker AC300 spectrometer. ^{13}C NMR spectra were recorded as Pendent spectra. GPC analysis was performed by RAPRA Technology Ltd., Shawbury, Shrewsbury, Shropshire, SY4 4NR, U.K. GPC analysis was carried out with:

Column: PLgel 2 x mixed bed-B, 30 cm, 10 microns.

Solvent: Tetrahydrofuran, with antioxidant.

Flow-rate: 1.0 ml / min (nominal).

Temperature: 30 °C.

Detector: Refractive index.

The GPC system was calibrated with poly(styrene) standards and all results are expressed as the "poly(styrene) equivalent" molecular mass.

Elemental analysis was carried out in duplicate by Medac Ltd., Department of Chemistry, Brunel University, Uxbridge, Middlesex, UB8 3PH.

7.3. Synthesis of Monomers and Substrates

7.3.1. Synthesis of 2,2,2-trichloroethyl Methacrylate

Methacrylic acid (100.0 cm^3, 1.14 mol), 2,2,2-trichloroethyl alcohol (96.0 cm^3, 1.00 mol), p-toluenesulphonic acid monohydrate (5.0 g, 0.02 mol), phenothiazine (0.1 g, 0.5 mmol) and dry toluene (350 cm^3) were placed in a 2 neck round-bottomed flask and refluxed for 6 hours. The water was collected in a Dean-Stark trap. No more water collected in the trap after this time. After allowing the reaction to cool, the solution was washed with a saturated aqueous solution of $NaHCO_3$ (100 cm^3 portions) until no more effervescence was observed. The reaction mixture was then washed with a saturated aqueous brine solution (100 cm^3) and dried over anhydrous magnesium sulfate. Toluene was removed on a rotary evaporator and the product distilled under vacuum (46 °C 0.5mm Hg) (lit.,[125] 55 °C 1.0mm Hg) to yield a light yellow oil (195.8 g, 90 %), ν_{max} / cm^{-1} 1735 (ester carbonyl), 1637 (H_2C=C); δ_H (300 MHz; DMSO-d_6) 1.95 (3H, br m, -CH_3), 4.93 (2H, s, -CH_2-), 5.83 (1H, br m, *cis* to Me, =CH_2); 6.20 (1H, br m, *trans* to Me, =CH_2); δ_C (75 MHz, DMSO-d_6) 17.8 (-CH_3), 73.5 (-CH_2-), 95.3 (-O-CH_2-CCl_3), 127.6 (=CH_2), 134.8 (=$C(CH_3)$-CO_2-), 164.8 (-CO_2-).

7.3.2. Synthesis of 2,2,2-trifluoroethyl Methacrylate

2,2,2-Trifluoroethyl alcohol (17.4 cm^3, 0.24 mol), dichloromethane (50 cm^3) and triethylamine (42.1 cm^3, 0.30 mol) were placed in a 2 neck round-bottomed flask fitted with a water cooled condenser and an argon inlet. Methacryloyl chloride (23.4 cm^3, 0.24 mol) was added from a dropping funnel, at such a rate that a gentle reflux was maintained. During the addition a white solid was formed. Once the addition was complete, the reaction was refluxed for 2 hours. After allowing to cool, the mixture was filtered and the filtrate washed with a saturated aqueous solution of $NaHCO_3$ (50 cm^3 portions) until no more effervescence was observed. The organic phase was then washed with saturated aqueous brine solution (50 cm^3) and dried over anhydrous magnesium sulfate. The solvent was removed on a rotary evaporator to yield a light yellow oil (33.6 g, 83 %). This product was then used without further work up in polymerisation 7.4.4. See analysis of polymer (section 7.4.4.).

7.3.3. Synthesis of Sucrose Octaacetate

Sucrose (17.1 g, 0.05 mol) was ground with potassium acetate (13.7 g, 0.17 mol) in a pestle and mortar. The resulting powder was added to a 2 neck round-bottomed flask fitted with a water cooled condenser. Acetic anhydride (77.0 cm^3, 0.8 mol) was added and the reaction heated on a boiling water bath until a clear solution developed and then heating continued for a further 2 hours. The product mixture was poured over crushed ice (750 cm^3) and allowed to stand for 1 hour with occasional stirring to break up the solid lumps. The mixture was filtered and washed with cold water. Recrystallisation from methanol to give sucrose octaacetate as a white solid (33.3 g, 98 %), Mp 88-90 °C (lit.,[126] 88-90 °C); δ_H (300 MHz; $CDCl_3$)[126] 1.89 (3H, s, -CH_3), 1.91 (3H, s, -CH_3), 1.97 (6H, br s, 2 -CH_3), 1.99 (9H, br s, 3 -CH_3), 2.05 (3H, s, -CH_3) 3.98-4.23 (8H, m, 3 -CH_2-, 2 -CH), 4.74 (1H, dd, J 3.7, 10.2, -CH); 4.94 (1H, t, *J* 9.8, -CH), 5.24 (1H, t, *J* 5.8, -CH), 5.31 (1H, s, CH), 5.33(1H, t, J 5.5, -CH), 5.56 (1H, d, *J* 3.7, -CH); δ_C (75 MHz, $CDCl_3$) 20.50 (-CH_3), 61.6, 62.7, 63.5 (-CH_2), 68.8, 68.3, 69.4, 70.1, 74.8, 75.5, 79.0 (-CH), 89.9, 103.9 (C), 169.4, 169.5, 169.8, 169.9, 170.0, 170.3, 170.5 (-CO_2-); m/z 679.

7.4. Synthesis of Polymers

The general procedure for the free radical polymerisation of acrylates is illustrated by the synthesis of poly(methyl vinyl ketone).

7.4.1. Synthesis of Poly(methyl vinyl ketone)

Methyl vinyl ketone (41.6 cm^3, 0.50 mol) was dissolved in methanol (100 cm^3) in a 2 neck round-bottomed flask fitted with a reflux condenser. The solution was de-gassed for 30 minutes by bubbling argon though the mixture. α, α'-Azobisisobutyronitrile (0.82 g, 5.0 mmol) was added and the reaction mixture refluxed for 24 hours under an argon blanket. The reaction mixture was allowed to cool and poured into water (500 cm^3) giving a white solid. The majority of the solvent was decanted off and the solid filtered off and washed with methanol. The resultant poly(methyl vinyl ketone) was dried over anhydrous silica gel in a vacuum oven at 40°C / 1 mm Hg for 24 hours giving the polymer as a white solid (31.2 g, 89 %); Mn 6270, Mw 13700; δ_H (300 MHz; $CDCl_3$) 0.91-2.50 (br m); δ_C (75 MHz, $CDCl_3$) 29.2 (-CH_3), 32.6 (-CH_2-), 47.8 (-CH-), 210.5 (C=O).

7.4.2. Synthesis of Poly(2-hydroxyethyl methacrylate)

The method described in section 7.4.1. was repeated using 2-hydroxyethyl methacrylate (20.0 cm^3, 0.14 mol) and α, α′-azobisisobutyronitrile (0.23 g, 1.4 mmol) in methanol (100 cm^3) giving poly(2-hydroxyethyl methacrylate) as a white solid (17.8 g, 96 %); Mn 64600, Mw 428000; δ_H (300 MHz, DMSO-d_6) 0.75-0.92 (3H, br m, -C(C**H**$_3$)-), 1.22-2.50 (2H, br, -C**H**$_2$-), 3.56 (2H, br s, -CH$_2$-C**H**$_2$-OH), 3.87 (2H, br s, -C**H**$_2$-CH$_2$-OH), 4.80 (1H, br s, O**H**); δ_C (75 MHz, DMSO-d_6) 16.1, 18.1 (-**C**H$_3$), 44.2, 44.6 (-**C**H$_2$-), 58.5 (-CH$_2$-**C**H$_2$-OH), 66.24 (-**C**H$_2$-CH$_2$-OH), 176.5, 177.2, 177.5 (-**C**O$_2$-).

7.4.3. Synthesis of Poly(2,2,2-trichloroethyl methacrylate)

The method described in section 7.4.1. was repeated with 2,2,2-trichloroethyl methacrylate (28.8 g, 0.13 mol) and α, α′-azobisisobutyronitrile (0.21 g, 1.3 mmol) in methanol (100 cm^3) giving poly(2,2,2-trichloroethyl methacrylate) as a white solid (28.1 g, 98 %), Mn 23000, Mw 287000, δ_H (300 MHz, $CDCl_3$)[125] 1.07-2.15 (5H, br m, -C**H**$_3$, -C**H**$_2$-), 4.49 (2H, br s, -C**H**$_2$-CCl_3); δ_C (75 MHz, $CDCl_3$) 16.8, 18.8 (-**C**H$_3$), 44.8, 45.1 (-**C**H$_2$-), 52.3, 54.1 (-**C**(CH$_3$)-), 75.4, 75.7 (-**C**H$_2$-CCl_3), , 94.1, 94.3 (-**C**Cl_3), 174.6, 175.4, 176.3 (-**C**O$_2$-).

7.4.4. Synthesis of Poly(2,2,2-trifluoroethyl methacrylate)

The method described in section 7.4.1. was repeated with 2,2,2-trifluoroethyl methacrylate (40.6 g, 0.24 mol) and α, α′-azobisisobutyronitrile (0.4 g, 2.4 mmol) in methanol (100 cm^3) giving poly(2,2,2-trifluoroethyl methacrylate) as an off white solid (20.1 g, 50 %), Mn 2570, Mw 6070, δ_H (300 MHz, $CDCl_3$)[127] 0.86-1.91 (5H, br m, -C(C**H**$_3$)-, -C**H**$_2$-), 4.27-4.53 (2H, br m, -C**H**$_2$-CF_3); δ_C (75 MHz, $CDCl_3$) 10.1, 16.6, 18.4, 20.4 (-C(**C**H$_3$)-), 40.1, 44.5, 44.8, 53.1, 53.7 (-**C**H$_2$-), 61.0 (q, $^2J_{CF}$ 36.0, -**C**H$_2$-CF_3), 122.8 (q, $^1J_{CF}$ 277, -**C**F_3) 174.5, 174.7, 175.1, 175.5, 175.8, (-**C**O$_2$-).

7.4.5. Synthesis of Poly(*tert*-butyl acrylate)

The method described in section 7.4.1. was repeated using *tert*-butyl acrylate (100.0 cm^3, 0.68 mol), and α, α'-azobisisobutyronitrile (1.1 g, 6.8 mmol) in methanol (100 cm^3) giving poly(*tert*-butyl acrylate) as a white solid (85.1 g, 97 %), Mn 19900, Mw 271000, δ_H (300 MHz, $CDCl_3$)[128, 129] 1.39-1.50 (11H, br m, -C(**CH**$_3$)$_3$, -**CH**$_2$-), 2.18 (1H, br s, -**CH**-); δ_C (75 MHz, $CDCl_3$) 27.9 (-C(**C**H_3)$_3$), 35.7, 37.3 (-**C**H_2-), 41.7, 42.2 (-**C**H-), 80.2 (-**C**$(CH_3)_3$), 172.2, 173.8 (-**C**O_2-).

7.4.6. Synthesis of Poly(hexaethylene glycol fumarate)

Hexaethylene glycol (17.0 cm^3, 0.068 mol), dichloromethane (50 cm^3) and triethylamine (40.2 cm^3, 0.3 mol) were placed in a 2 neck round-bottomed flask fitted with a water cooled condenser and an argon inlet. Fumaroyl dichloride (7.4 cm^3, 0.068 mol) was added from a dropping funnel at such a rate that a gentle reflux was maintained. During the addition, a white solid was formed. Once the addition was complete, the reaction was refluxed for 2 hours. After allowing to cool, the reaction mixture was filtered and the filtrate washed with a saturated aqueous solution of $NaHCO_3$ (50 cm^3 portions) until no more effervescence was observed. The organic phase was then washed with saturated aqueous brine solution (50 cm^3) and dried over anhydrous magnesium sulfate. The solvent was removed on a rotary evaporator giving poly(hexaethylene glycol fumarate) as a viscous brown liquid (16.3g, 66 %), Mn 9770, Mw 20400, δ_H (300 MHz, $CDCl_3$) 3.53 (16H, br s, -O-C**H**$_2$-C**H**$_2$-O-), 3.63 (4H, br t, *J* 4.5, -O-C**H**$_2$-CH_2-O_2C-), 4.23 (4H, br t, *J* 4.5, -O-CH_2-C**H**$_2$-O_2C-), 6.77 (2H, s, -**H**C=C**H**-); δ_C (75 MHz, $CDCl_3$) 64.2 (-O-**C**H_2-**C**H_2-O-), 68.6 (-O-**C**H_2-CH_2-O-), 70.29 (-O-CH_2-**C**H_2-O-), 133.4 (-H**C**=**C**H-), 164.5 (-**C**O_2).

7.5. Modification of Poly(butadiene)

The following procedure describes the standard procedure adopted for the epoxidation of poly(butadiene) and the Baeyer-Villiger oxidation of poly ketones.

7.5.1. Epoxidation of Poly(butadiene)

Phenyl terminated poly(butadiene) (Mn 1300) (2.5 g, 0.046 mol of double bonds) was dissolved in dichloromethane (50.0 cm^3). Acetic acid (0.27 cm^3, 4.6 mmol), a 27.5 wt % aqueous solution of hydrogen peroxide (11.1 g, 0.09 mol) added in one portion and immobilised *Candida antartica* lipase (Novozyme 435) (0.25 g) were added and the reaction mixture stirred rapidly for 24 hours at 25 °C in the dark. The mixture was filtered and the filtrate washed with a saturated aqueous solution of $NaHCO_3$ (50 cm^3 portions) until no effervescence was observed. After washing with a saturated aqueous brine solution (50 cm^3) and drying over anhydrous magnesium sulfate the solvent was removed on a rotary evaporator. The product was dried over anhydrous silica gel in a vacuum oven at 40 °C and 1mm Hg for 24 hours giving an opaque viscous polymer (2.2 g), ν_{max} / cm^{-1} 1639m (CH=CH), 1265m (epoxide), 806m (epoxide); δ_H (300 MHz, $CDCl_3$) 1.11, 2.10 (br m, -CH_2-), 2.66 (br s, *trans* epoxide –HCOCH-), 2.86 (br s, *cis* epoxide –HCOCH-), 4.93 (br s, -CH=CH_2), 5.25-5.52 (br m, vinyl –CH=CH_2, *cis* & *trans* –CH=CH-), 7.10-7.15 (aromatic CH); δ_C (75 MHz, $CDCl_3$) 23.7-37.5 (m, -CH_2-), 40.3-42.9 (m, CH), 51.9-58.2 (m, epoxide –CHOCH-), 114.0-114.7 (m, -CH=CH_2), 124.9-130.6 (m, aromatic CH and –CH=CH-), 140.8-141.6 (m, -CH=CH_2).

7.5.1.1. The Effect of Different Acids on the Epoxidation of Poly(butadiene)

The procedure described in section 7.5.1. was repeated on the same scale, whilst the acid was varied as detailed in the following experiments:

Palmitic acid (2.3 g, 9.0 mmol) was utilised and yielded an opaque viscous epoxide polymer product (2.0 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.7.

Octanoic acid (1.3 g, 9.0 mmol) was utilised and yielded an opaque viscous epoxide polymer product (2.0 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.7.

Trifluoroacetic acid (0.53 g, 4.6 mmol) was utilised and yielded an opaque viscous epoxide polymer product (1.9 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.7.

Acetic acid (0.54 cm^3, 9.2 mmol) was utilised and yielded an opaque viscous product (2.0 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.7.

Acetic acid (0.14 cm^3, 2.3 mmol) was utilised and yielded an opaque viscous product (2.2 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.7.

7.5.1.2. The Effect of Time on the Epoxidation of Poly(butadiene)

The procedure described in section 7.5.1. was repeated on the same scale, whilst the time was varied as detailed in the following experiments:

The reaction was run for 2 hours and yielded an opaque viscous product (2.2 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.6.

The reaction was run for 6 hours and yielded an opaque viscous product (1.7 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.6.

The reaction was run for 4 days and yielded an opaque viscous product (2.2 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.6.

7.5.1.3. The Effect of Hydrogen Peroxide Concentration and the Method of its Addition on the Epoxidation of Poly(butadiene)

The procedure described in section 7.5.1. was repeated on the same scale, whilst the hydrogen peroxide concentration and method of addition was varied as detailed in the following experiments:

A 27.5 wt % aqueous solution of hydrogen peroxide (11.1 cm^3, 0.09 mol) was added drop wise *via* a syringe pump over 24 hours and yielded an opaque viscous product (2.4 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.8.

A 15 wt % aqueous solution hydrogen peroxide (20.4 cm^3, 0.09 mol) was added in one portion and yielded an opaque viscous product (2.3 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.8.

A 15 wt % aqueous solution of hydrogen peroxide (20.4 cm^3, 0.09 mol) was added drop wise *via* a syringe pump over 24 hours and yielded an opaque viscous product (2.0 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.8.

7.5.1.4. The Effect of Solvent on the Epoxidation of Poly(butadiene)

The procedure described in section 7.5.1. was repeated on the same scale, whilst the solvent used was varied as described in the following experiments:

Toluene (50 cm^3) was utilised and yielded an opaque viscous product (1.3 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.9.

Toluene (50 cm^3) was utilised with a 27.5 wt % aqueous solution of hydrogen peroxide (11.1 g, 0.09 mol) added drop wise *via* a syringe pump over 24 hours and yielded an opaque viscous product (1.3 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.9.

Hexane (50 cm^3) was utilised and yielded an opaque viscous product (2.0 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.9.

Hexane (50 cm^3) was utilised with a 27.5 wt % aqueous solution of hydrogen peroxide (11.1 g, 0.09 mol) added drop wise *via* a syringe pump over 24 hours and yielded an opaque viscous product (2.2 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.9.

7.5.1.5. The Effect of Temperature on the Epoxidation of Poly(butadiene)

The procedure described in section 7.5.1. was repeated on the same scale with the following variations whilst the temperature was raised to 37 °C.

The general procedure repeated at 37 °C yielded an opaque viscous product (2.2 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.10.

A 27.5 wt % aqueous solution of hydrogen peroxide (11.1 g, 0.09 mol) added drop wise *via* a syringe pump over 24 hours yielding an opaque viscous product (2.0 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.10.

A 27.5 wt % aqueous solution of hydrogen peroxide (11.1 g, 0.09 mol) was added drop wise *via* a syringe pump over 24 hours and Novozyme 435 (0.5 g) was used to yield an opaque viscous product (2.3 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.10.

A 27.5 wt % aqueous solution of hydrogen peroxide (11.1 g, 0.09 mol) was added drop wise *via* a syringe pump over 24 hours to a reaction using toluene (50 cm^3) as solvent yielding an opaque viscous product (1.3 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.10.

A 27.5 wt % aqueous solution of hydrogen peroxide (11.1 g, 0.09 mol) was added drop wise *via* a syringe pump over 24 hours to a reaction using hexane (50 cm^3) as solvent yielding an opaque viscous product (1.9 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.10.

7.5.1.6. The Effect of the Quantity of Enzyme on the Epoxidation of Poly(butadiene)

The procedure described in section 7.5.1. was repeated on the same scale with the enzyme being varied as detailed below:

Using Novozyme 435 (0.5 g) yielded an opaque viscous product (2.4 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.11.

Using Novozyme 435 (0.5 g) at 37 °C yielded an opaque viscous product (2.3 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.11.

Using Novozyme 435 (0.125 g) yielded an opaque viscous product (2.0 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.11.

A 27.5 wt % aqueous solution of hydrogen peroxide (11.1 g, 0.09 mol) was added drop wise *via* a syringe pump over 24 hours with Novozyme 435 (0.5 g) yielding an opaque viscous product (2.3 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.11.

After 24 hours of the general reaction a second portion of enzyme was added and the reaction continued for a further 24 hours to yield an opaque viscous product (2.4 g). Analysis was as described in section 7.5.1. The analysis of the spectra is discussed in section 2.1.11.

Previously used Novozyme 435 (0.25 g) was utilised. Unmodified poly(butadiene) was recovered.

7.5.1.7. Epoxidation of Poly(butadiene) Using Peroxyacetic Acid

The procedure described in section 7.5.1. was repeated on the same scale with peroxyacetic acid used in place of acetic acid and hydrogen peroxide:

A 32 wt % solution of peroxyacetic acid in acetic acid (16.4 cm^3, 0.078 mol) yielded an opaque viscous product (5.3 g), ν_{max} / cm^{-1} 3445s (OH), 1730s (ester C=O); δ_H (300 MHz, $CDCl_3$) 1.0-2.7 (br m, -CH_2- & -CH_3), 3.0-4.6 (br m, -CH-), 7.0-7.2 (aromatic CH); δ_C (75 MHz, $CDCl_3$) 20.9 (-CH_3), 29.6 (-CH_2-), 81.6 (-CH-), 111.0 (-CH=), 125.7, 128.3 (aromatic CH).

A 32 wt % solution of peroxyacetic acid in acetic acid (16.4 cm^3, 0.078 mol) with no enzyme yielded an opaque viscous product (4.5 g). Analysis was as described in the above experiment.

7.5.2. Bromohydroxylation of Poly(butadiene)

A citrate / phosphate pH 3.0 buffer solution (80.3 cm^3 citric acid solution (0.1 ML^{-1}) and 19.7 cm^3 potassium phosphate solution (0.2 ML^{-1})) was placed in a jacketed round-bottomed flask, ethanol ($30cm^3$) added and the flask wrapped in aluminium foil. Poly(butadiene) (2.7 g, 5.0 mmol), potassium bromide (2.98 g, 25.0 mmol) and chloroperoxidase (3000 units) were added to the buffer solution and the mixture stirred vigorously at 25 °C to disperse the poly(butadiene). To this reaction mixture was added a 27.5 wt % aqueous solution of hydrogen peroxide (2.0 cm^3) which resulted in a brief colour change from colourless to light yellow. A further portion of 27.5 wt % aqueous solution of hydrogen peroxide (1.0 cm^3) was added after 15 minutes. After 24 hours the reaction mixture was filtered and extracted with dichloromethane (3 x 100 cm^3). The dichloromethane phase was dried over anhydrous magnesium sulfate and the solvent removed on a rotary evaporator. Poly(butadiene) starting material (2.5 g) was recovered unchanged.

7.6. Modification of Poly(vinyl phenyl ketone)

7.6.1. Baeyer-Villiger Oxidation of Poly(vinyl phenyl ketone)

The successful oxidation procedure for epoxidation of poly(butadiene) described in section 7.5.1 was repeated using poly(vinyl phenyl ketone) (2.5 g, 0.019 mol), acetic acid (0.11 cm^3, 1.9 mmol), 27.5 wt % aqueous solution of hydrogen peroxide (4.6 g, 0.037 mol) and Novozyme 435 (0.25 g) in dichloromethane (50 cm^3) giving the product as a white solid (2.2 g), ν_{max} / cm^{-1} 3059w (aromatic CH), 1677s (aromatic ketone, C=O), 1595m (aromatic), 1579m (aromatic); δ_H (300 MHz, $CDCl_3$) 1.46-3.90 (br m, -**CH**-, -**CH$_2$**-), 7.40-8.00 (br m, aromatic **CH**); δ_C (75 MHz, $CDCl_3$) 30.2-35.2 (m, -**CH$_2$**-), 40.5-42.7 (br m, -**CH**-), 127.9-128.7 (aromatic **CH**), 132.9 (br, aromatic **CH**), 201.2-202.8 (br m, -**CO$_2$**-).

7.6.1.1. The Effect of Time on the Baeyer-Villiger Oxidation of Poly(vinyl phenyl ketone)

The procedure described in section 7.6.1. was repeated on the same scale whilst the reaction time was varied as detailed in the following experiments:

The general procedure described in section 7.6.1. was repeated for 2 hours giving polymer as a white solid (2.2 g). Analysis was as described in section 7.6.1.

The general procedure described in section 7.6.1. was repeated for 6 hours giving polymer as a white solid (2.1 g). Analysis was as described in section 7.6.1.

The general procedure described in section 7.6.1. was repeated for 4 days giving polymer as a white solid (2.2 g). Analysis was as described in section 7.6.1.

The general procedure described in section 7.6.1. was repeated for 7 days giving polymer as a white solid (2.2 g). Analysis was as described in section 7.6.1.

7.6.1.2. The Effect of Hydrogen Peroxide Concentration on the Baeyer-Villiger Oxidation of Poly(vinyl phenyl ketone)

The procedure described in section 7.6.1. was repeated on the same scale whilst the concentration of hydrogen peroxide is halved. A 14 wt % aqueous solution of hydrogen peroxide (9.0 g, 0.037 mol) yielded polymer as a white solid (2.2 g). Analysis was as described in section 7.6.1.

7.6.1.3. The Effect of the Quantity of Enzyme on the Baeyer-Villiger Oxidation of Poly(vinyl phenyl ketone)

The procedure described in section 7.6.1. was repeated on the same scale whilst the quantity of enzyme was doubled. Novozyme 435 (0.5 g) yielded polymer as a white solid (2.2 g). Analysis was as described in section 7.6.1.

7.6.1.4. The Effect of Acid on the Baeyer-Villiger Oxidation of Poly(vinyl phenyl ketone)

The procedure described in section 7.6.1. was repeated on the same scale whilst the more powerful acid, trifluoroacetic acid was used. Trifluoroacetic acid (0.15 cm^3, 1.9 mmol) yielded polymer as a white solid (2.2 g). Analysis was as described in section 7.6.1.

7.6.1.5. Baeyer-Villiger Oxidation of Poly(vinyl phenyl ketone) with Peroxyacetic Acid

The procedure described in section 7.6.1. was repeated on the same scale with peroxyacetic acid. 32 wt % solution in acetic acid peroxyacetic acid (9.0 cm^3, 0.038 mol) yielded polymer as a white solid (2.2 g). Analysis was as described in section 7.6.1.

7.6.2.1. Transcyanation of Poly(vinyl phenyl ketone) with Acetone Cyanohydrin and Almond Meal

Poly(vinyl phenyl ketone) (5.0 g, 0.038 mol), acetone cyanohydrin (9.1 cm^3, 0.1 moles) and catalytic quantity of acetic acid (1 drop) was dissolved in dichloromethane (100 cm^3). De-fatted almond meal (4.0 g) was added and the reaction mixture stirred vigorously at 25 °C for 24 hours. The product mixture was then filtered with the dichloromethane solvent and acetone cyanohydrin being removed on a rotary evaporator. The resultant polymer was then dissolved in the minimum quantity of dichloromethane (40 cm^3) and precipitated from methanol (400 cm^3). After drying in a vacuum oven (50 °C) over silica gel a white solid (4.0 g) was recovered. Analysis was as described in section 7.6.1.

7.6.2.2 Repeat of Reaction 7.6.2.1. at 35 °C

Reaction 7.6.2.1. was repeated at a temperature of 35 °C to yield a white solid product (4.3 g). Analysis was as described in section 7.6.1.

7.6.2.3. Repeat of Reaction 7.6.2.2. Without Almond Meal Present

Reaction 7.6.2.2. was repeated without de-fatted almond meal and a white solid (3.8 g) of unaltered polymer recovered. Analysis was as described in section 7.6.1.

7.7. Modification of Poly(methyl vinyl ketone)

7.7.1. Baeyer-Villiger Oxidation of Poly(methyl vinyl ketone)

The successful oxidation procedure described in section 7.5.1. was repeated with poly(methyl vinyl ketone) (2.5 g, 0.036 mol), acetic acid (0.21 cm^3, 3.6 mmol), a 27.5 wt % aqueous solution of hydrogen peroxide (8.7 g, 0.070 mol) and Novozyme 435 (0.25 g) in dichloromethane (50 cm^3) resulting in the isolation of a brown solid product (2.2 g). Unaltered starting materials were recovered.

7.8. Modification of Poly(vinyl alcohol)

The following procedure describes the standard method of modification of poly(vinyl alcohol), poly(vinyl alcohol-co-ethylene) and was also employed in poly(2-hydroxyethyl methacrylate) experiments described latter.

7.8.1. Esterification of Poly(vinyl alcohol) with Benzoic Acid

Poly(vinyl alcohol) (2.5 g, 0.057 mol) was dissolved in dimethyl sulfoxide (50 cm^3) at 80 °C in a heated water jacketed round-bottomed flask. The reaction mixture was then cooled to 35 °C. Benzoic acid (3.47 g, 0.028 mol) and porcine pancreatic lipase (1.0 g) were added and the reaction mixture stirred vigorously for 24 hours. After filtering off the lipase, the polymer was precipitated from methanol (250 cm^3). Excess solvent was decanted off and the solid filtered off and washed with methanol (50 cm^3). The resultant white solid was dried over silica gel in a vacuum oven at 40 °C and 1mm Hg for 24 hours. Benzoic acid was recovered.

7.8.2. Glycosidation of Poly(vinyl alcohol) with β-D-Glucose

The procedure described in section 7.8.1. was repeated with poly(vinyl alcohol) (10.0 g, 0.23 mol), β-D-Glucose (5.1 g, 0.028 mol) and almond meal (3.0 g) in dimethyl sulfoxide (100 cm^3). A mixture of unaltered starting materials was recovered (7.6 g).

7.8.3. Transglycosidation of Poly(vinyl alcohol) with β-D-Lactose

The procedure described in section 7.8.1. was repeated with poly(vinyl alcohol) (10.0 g, 0.23 mol), β-D-lactose (9.6 g, 0.028 mol) and almond meal (3.0 g) in dimethyl sulfoxide (100 cm^3). A mixture of unaltered poly(vinyl alcohol) and epimerised sugar residues was recovered (12.1 g).

7.9. Modification of Poly(vinyl alcohol-co-ethylene)

7.9.1. Esterification of Poly(vinyl alcohol-co-ethylene) with Benzoic Acid in DMSO

The procedure described in section 7.8.1. was repeated with poly(vinyl alcohol-co-ethylene) (5.0 g, 0.076 mol of OH groups), benzoic acid (4.63 g, 0.038 mol) and porcine pancreatic lipase (1.0 g) in dimethyl sulfoxide (50 cm^3). A mixture of unaltered starting materials was recovered.

7.9.2. Esterification of Poly(vinyl alcohol-co-ethylene) with Benzoic Acid in DMF

The procedure described above in section 7.9.1. was repeated with N,N-dimethylformamide in place of dimethyl sulfoxide. A mixture of unaltered starting materials was recovered.

7.9.3. Esterification of Poly(vinyl alcohol-co-ethylene) with Benzoic Acid in DMF at 35°C for 4 Days

The experiment detailed in 7.9.2. was repeated for 4 days. A mixture of unaltered starting materials was recovered.

7.9.4. Transesterification of Poly(vinyl alcohol-co-ethylene) with Vinyl benzoate in DMF

The experiment detailed in 7.9.2. was repeated with vinyl benzoate (5.3 cm^3, 0.038 mol). A mixture of unaltered starting materials was recovered.

7.9.5. Repeat of Reaction 7.9.4. with *Candida rugosa* Lipase

The experiment detailed in 7.9.4. was repeated with *Candida rugosa* lipase (1.0 g). A mixture of unaltered starting materials was recovered.

7.10. Modification of Poly(2-hydroxyethyl methacrylate)

7.10.1. Transesterification of Poly(2-hydroxyethyl methacrylate) with Vinyl Benzoate

The procedure described in section 7.8.1. was repeated with poly(2-hydroxyethyl methacrylate) (2.0 g, 0.015 mol) being dissolved in pyridine (50 cm^3). Vinyl benzoate (3.2 cm^3, 0.023 mol) and porcine pancreatic lipase (0.5 g) were added to the reaction and the mixture stirred rapidly at 35 °C. Unaltered starting materials were recovered.

7.10.1.1. Repeat of Reaction 7.10.1. with *Candida rugosa* Lipase

The procedure described in section 7.10.1. was repeated with *Candida rugosa* lipase (0.5 g). Unaltered starting materials were recovered.

7.10.1.2. Transesterification of Poly(2-hydroxyethyl methacrylate) with Vinyl Acetate

The procedure described in section 7.10.1. was repeated with vinyl acetate (2.1 cm^3, 0.023 mol) and *Candida rugosa* lipase (0.5 g). Unaltered starting materials were recovered.

7.10.1.3. Transesterification of Poly(2-hydroxyethyl methacrylate) with Vinyl Pivalate

The procedure described in section 7.10.1. was repeated with vinyl pivalate (3.4 cm^3, 0.023 mol) and *Candida rugosa* lipase (0.5 g). Unaltered starting materials were recovered.

7.10.1.4. Transesterification of Poly(2-hydroxyethyl methacrylate) with Benzoic Anhydride

The procedure described in section 7.10.1. was repeated with benzoic anhydride (4.3 cm^3, 0.023 mol) and *Candida rugosa* lipase (0.5 g). Benzoic acid and unaltered polymer were recovered.

7.10.1.5. Esterification of Poly(2-hydroxyethyl methacrylate) with Butyric Acid

The procedure described in section 7.10.1. was repeated with butyric acid (2.1 cm^3, 0.023 mol) and *Candida rugosa* lipase (0.5 g). Unaltered starting materials were recovered.

7.10.2.1. Transglycosidation of Poly(2-hydroxyethyl methacrylate) with Sucrose

The procedure described in section 7.10.1. was repeated with sucrose (5.1 g, 0.015 mol) and almond meal (1.0 g). Unaltered starting materials were recovered.

7.10.2.2. Transglycosidation of Poly(2-hydroxyethyl methacrylate) with Sucrose Octaacetate

The procedure described in section 7.10.1. was repeated with sucrose octaacetate (10.2 g, 0.015 mol) and almond meal (1.0 g). Starting materials were recovered unchanged.

7.10.2.3. Transglycosidation of Poly(2-hydroxyethyl methacrylate) with 2-Nitrophenyl-β-D-Galactopyranoside

The procedure described in section 7.10.1. was repeated with 2-Nitrophenyl-β-D-Galactopyranoside (1.0 g, 0.0033 mol) and almond meal (1.0 g) and yielded unaltered poly(2-hydroxyethyl methacrylate).

7.11. Modification of Poly(2,2,2-trichloroethyl methacrylate)

7.11.1. Transesterification of Poly(2,2,2-trichloroethyl methacrylate) with Benzyl Alcohol

The procedure described in section 7.10.1. was repeated with poly(2,2,2-trichloroethyl methacrylate) (2.5 g, 0.011 mol), benzyl alcohol (2.4 cm^3, 0.023 mol) and porcine pancreatic lipase (0.5 g) in dichloromethane (100 cm^3). Unaltered starting materials were recovered.

7.11.2. Repeat of Reaction 7.11.1. with *Candida rugosa* Lipase

The procedure described in section 7.11.1. was repeated with *Candida rugosa* lipase (0.5 g). Unaltered starting materials were recovered.

7.11.3. Transesterification of Poly(2,2,2-trichloroethyl methacrylate) with 2-Phenylethyl Alcohol

The procedure described in section 7.11.1. was repeated with 2-phenylethyl alcohol (2.8 cm^3, 0.023 mol). Unaltered starting materials were recovered.

7.11.4. Repeat of Reaction 7.11.3. with *Candida rugosa* Lipase

The procedure described in section 7.11.3. was repeated with *Candida rugosa* lipase (0.5 g). Unaltered starting materials were recovered.

7.11.5. Amination of Poly(2,2,2-trichloroethyl methacrylate) with Aniline

The procedure described in section 7.11.1. was repeated with aniline (2.1 cm^3, 0.023 mol). Unaltered starting materials were recovered.

7.11.6. Repeat of Reaction 7.11.5. with *Candida rugosa* Lipase

The procedure described in section 7.11.5. was repeated with *Candida rugosa* lipase (0.5 g). Unaltered starting materials were recovered.

7.11.7. Amination of Poly(2,2,2-trichloroethyl methacrylate) with 2-Phenylethylamine

The procedure described in section 7.11.1. was repeated with 2-phenylethylamine (3.0 cm^3, 0.023 mol). Unaltered starting materials were recovered.

7.11.8. Transesterification of Poly(2,2,2-trichloroethyl methacrylate) with β-D-Glucose

The procedure described in section 7.11.1. was repeated with β-D-glucose (2.5 g, 0.013 mol). Unaltered starting materials were recovered.

7.11.9. Repeat of Reaction 7.11.8. with *Candida rugosa* Lipase

The procedure described in section 7.10.1. was repeated with *Candida rugosa* lipase (0.5 g). Unaltered starting materials were recovered.

7.11.10. Hydrolysis of Poly(2,2,2-trichloroethyl methacrylate)

The procedure described in section 7.10.1. was repeated with poly(2,2,2-trichloroethyl methacrylate) (2.5 g, 0.011 mol), water (1.0 cm^3, 0.18 mol) and porcine pancreatic lipase (0.5 g) in tetrahydrofuran (100 cm^3). Unaltered starting materials were recovered.

7.12. Modification of Poly(2,2,2-trifluoroethyl methacrylate)

7.12.1. Transesterification of Poly(2,2,2-trifluoroethyl methacrylate) with 2-Phenylethyl Alcohol

The procedure described in section 7.11.1. was repeated with poly(2,2,2-trifluoroethyl methacrylate) (2.5 g, 0.016 mol), 2-phenylethyl alcohol (3.93 cm^3, 0.033 mol) and porcine pancreatic lipase (1.0 g) in dichloromethane (100 cm^3). Unaltered starting materials were recovered.

7.12.2. Amination of Poly(2,2,2-trifluoroethyl methacrylate) with 2-Phenylethylamine

The procedure described in section 7.12.1. was repeated with 2-phenylethylamine (3.8 cm^3, 0.033 mol). Unaltered starting materials were recovered.

7.13. Modification of Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid)

7.13.1. Transesterification of Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) with Benzyl Alcohol

The procedure described in section 7.10.1. was repeated with poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) (5.0 g), benzyl alcohol (9.6 cm^3, 0.092 mol) and porcine pancreatic lipase (1.0 g) in dichloromethane (100 cm^3). Unaltered starting materials were recovered.

7.13.2. Amination of Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) with Aniline

The procedure described in section 7.13.1. was repeated with aniline (9.1 cm^3, 0.1 mol). Unaltered starting materials were recovered.

7.13.3. Amination of Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) with 2-Phenylethylamine

The procedure described in section 7.13.1. was repeated with a large excess of 2-phenylethyl amine (22.8 cm^3, 0.2 mol) yielding a brown viscous polymer (4.8 g), δ_H (300 MHz, DMSO-d_6) 1.0-1.20 (br m, -C**H**$_2$-), 1.64 (br s, -C**H**$_2$-), 1.86 (br s, -C**H**$_2$-), 2.48-3.50 (br m, -C**H**$_2$-C**H**$_2$-N**H**-), 7.21 (br s, aromatic); δ_C (75 MHz, DMSO-d_6) 18.0 (**C**H$_3$) 28.7 (-**C**(CH$_3$)$_3$), 36.8 (-**C**H$_2$-CH$_2$-NH-), 52.6 (-CH$_2$-**C**H$_2$-NH-), 125.9, 128.2, 128.8 (aromatic **C**H), 140.7 (aromatic **C**).

7.13.4. Control Experiment for Reaction 7.13.3. with no Enzyme Present

The procedure described in section 7.13.3. was repeated without enzyme, yielding a brown viscous polymer (4.3 g). Results were as described in Section 7.13.3.

7.13.5. Transesterification of Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) with 1-Butanol

The procedure described in section 7.13.1. was repeated with large excess of 1-butanol (11.0 cm^3, 0.12 mol). Unaltered starting materials were recovered.

7.13.6. Thiolation of Poly(*tert*-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) with Benzyl Mercaptan

The procedure described in section 7.13.1. was repeated with large excess of benzyl mercaptan (11.7 cm^3, 0.1 mol). Unaltered starting materials were recovered.

7.14. Modification of Poly(*tert*-butyl acrylate)

7.14.1. Transesterification of Poly(*tert*-butyl acrylate) with 2-Phenylethyl Alcohol

The procedure described in section 7.10.1. was repeated with poly(*tert*-butyl acrylate) (2.0 g, 0.016 mol), 2-phenylethyl alcohol (3.7 cm^3, 0.031 mol) and *Candida rugosa* lipase (1.0 g) in toluene (50 cm^3). Unaltered starting materials were recovered.

7.14.2. Transesterification of Poly(*tert*-butyl acrylate) with 1-Butanol

The procedure described in section 7.14.1. was repeated with 1-butanol (2.8 cm^3, 0.031 mol). Unaltered starting materials were recovered.

7.14.3. Amination of Poly(*tert*-butyl acrylate) with 2-Phenylethylamine

The procedure described in section 7.14.1. was repeated with 2-phenylethylamine (3.9 cm^3, 0.031 mol). A light brown solid (1.8 g) was recovered, δ_H (300 MHz, $CDCl_3$) 1.41 (-$C(CH_3)_3$), 2.19 (br s, -CH-), 2.76-2.80 (br m, -CH_2-CH_2-NH-), 2.91-2.93 (br m, -CH_2-NH-), 7.11-7.16 (Aromatic CH); δ_C (75 MHz, $CDCl_3$) 28.0 (-$C(CH_3)_3$), 35.7-37.4 (m, -CH_2-), 38.4 (-CH_2-CH_2-NH-), 41.8-42.3 (m, -CH-), 42.9 (-CH_2-CH_2-NH-), 80.3 (-$C(CH_3)_3$), 126.3, 128.5, 128.8 (Aromatic CH), 139.1 (Aromatic C), 171.9-173.9 (m, -CO_2-).

7.14.4. Amination of Poly(*tert*-butyl acrylate) with Butylamine

The procedure described in section 7.14.1. was repeated with butylamine (3.1 cm^3, 0.031 mol). Unaltered starting materials were recovered.

7.14.5. Amination of Poly(*tert*-butyl acrylate) with Diethylamine

The procedure described in section 7.14.1. was repeated with diethylamine (3.2 cm^3, 0.031 mol). Unaltered starting materials were recovered.

7.14.6. Thiolation of Poly(*tert*-butyl acrylate) with Benzyl Mercaptan

The procedure described in section 7.14.1. was repeated with benzyl mercaptan (3.7 cm^3, 0.031 mol). Unaltered starting materials were recovered.

7.14.7. Thiolation of Poly(*tert*-butyl acrylate) with 1-Butanethiol

The procedure described in section 7.14.1. was repeated with 1-butanethiol (3.3 cm^3, 0.031 mol). Unaltered starting materials were recovered.

7.14.8. Transesterification of Poly(*tert*-butyl acrylate) with β-D-Glucose

The procedure described in section 7.14.1. was repeated with β-D-glucose (2.9 g, 0.016 mol) and pyridine (50ml) yielding unaltered polymer and epimerised glucose.

7.14.9. Reaction of Poly(*tert*-butyl acrylate) with L-Leucine Methyl Ester Hydrochloride Salt

The procedure described in section 7.14.1. was repeated with L-leucine methyl ester hydrochloride (2.91 g, 0.016 mol) in pyridine (50 cm^3). Unaltered starting materials were recovered.

7.15. Modification of Epoxidised Poly(butadiene)

7.15.1. Ring Opening of Epoxidised Poly(butadiene) with 2-Phenylethylamine

Epoxidised poly(butadiene) (1.0 g) product produced in section 7.5.1. was dissolved in dichloromethane (50 cm^3). 2-Phenylethylamine (4.5 cm^3, 0.036 mol) and porcine pancreatic lipase (1.0 g) were added and the reaction was stirred at a temperature of 35 °C for 24 hours. The reaction was then filtered and dried over anhydrous magnesium sulfate. The solvent and unreacted benzylamine were removed on a rotary evaporator. The product was further treated in a vacuum oven, over anhydrous silica gel at 40 °C and 1mm Hg for 24 hours. Unaltered polymer was recovered.

7.15.2. Repeat of Reaction 7.15.1. with Diethyl Ether as Solvent

The procedure described in section 7.15.1. was repeated with diethyl ether (50 cm^3). Unaltered starting materials were recovered.

7.15.3. Ring Opening of Epoxidised Poly(butadiene) with 2-Phenylethyl Alcohol

The procedure described in section 7.15.1. was repeated with 2-phenylethyl alcohol (4.3 cm^3, 0.036 mol). Unaltered starting materials were recovered.

7.15.4. Repeat of Reaction 7.15.3. with Diethyl Ether as Solvent

The procedure described in section 7.15.3. was repeated with diethyl ether (50 cm^3). Unaltered starting materials were recovered.

7.15.5. Ring Opening of Epoxidised Poly(butadiene) with Benzyl Mercaptan

The procedure described in section 7.15.1. was repeated with benzyl mercaptan (4.3 cm^3, 0.036 mol). Unaltered starting materials were recovered.

7.15.6. Repeat of 7.15.5. with Diethyl Ether as Solvent

The procedure described in section 7.15.5. was repeated with diethyl ether (50 cm^3). Unaltered starting materials were recovered.

7.16. Modification of Poly(hexaethylene glycol fumarate)

7.16.1. Bromohydroxylation of Poly(hexaethylene glycol fumarate)

The procedure described in section 7.5.2. was repeated with poly(hexaethylene glycol fumarate) (1.81 g, 5.0 mmol) and ethanol (30.0 cm^3). Unaltered starting materials were recovered.

Chapter 8.

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