The Use of Enzyme Catalysts in Organic Media for the Synthesis of Biodegradable Polyesters

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Doctor of Philosophy

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Summary

The enzyme catalysed polytransesterification of diesters with diols was investigated under various conditions. The most consistent results were obtained using crude porcine pancreatic lipase (PPL) suspended in anhydrous diethyl ether. Addition of molecular sieve to the above system gave higher molecular weight products. The PPL catalysed reaction of bis(2,2,2-trichloroethyl) adipate and glutarate with butane-1,4-diol in anhydrous ether with and without molecular sieve was investigated over a range of times from 8 to 240 hours. The 72 hour adipate reaction with molecular sieve gave the highest molecular weight polymer (Mn 6,500 and Mw 9,400). The glutarate gave the maximum molecular weight polyester after 24 hours (Mn 5,700 and Mw 9,500). Occasionally the glutarate reaction produced very high molecular weight polyester-enzyme complexes.

Toluene generally gave lower molecular weight products than diethyl ether. Dichloromethane and tetrahydrofuran gave mainly dimers and trimers. Alternative enzyme and diol systems were also investigated. These yielded no polymeric products.
The molecular weights of the polyesters were determined by ^{1}H NMR end-group analysis and by GPC. The molecular weights determined by NMR were on average about twice as great as those determined by GPC.
The synthesis of the following diesters is described:

i)Bis(2,2,2-trichloroethyl) succinate, glutarate, adipate, *trans*-3-hexenedioate, and *trans*-3,4-epoxyadipate. ii) Diphenyl glutarate and adipate.
iii) Bis(2,2,2-fluororoethyl) glutarate and *trans*-3-hexenedioate.
iv) Divinyl glutarate. v) N,N'Glutaryl dicyclohexanone oxime.

The polytransesterification of all the above esters with diols was investigated. The easily synthesised bis(2,2,2-trichloroethyl) glutarate and adipate gave the best results and the work was concentrated on these two esters.

Keywords: polytransesterification, diesters, diols, organic media, enzyme catalysis

Dedication

To Par, my Big brother

Acknowledgements

Firstly, I would like to thank my supervisor, Ann Jarvie for her excellent super
good friendship and incredible patience over the years.

Next, I would like to thank my colleagues and friends in labs. 208 and 209, for fon
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Finally, I would like to thank my special friend, Colm, for things too many to menti

Quotation

The great tragedy of Science: the slaying of a beautiful hypothesis by an ugly fact.

Thomas Henry Huxley

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List of Abbreviations

Adip, Adipate	Bis (2,2,2-trichloroethyl) adipate
Alkene ester	Bis (2,2,2-trichloroethyl) *trans*-3-hexenedioate
b.p.	Boiling point
Butanediol, Diol	Butane-1,4-diol
CCL	*Candida cylindracea* lipase
m-CPBA	*m*-Chloroperoxybenzoic acid
DCC	N,N'-Diccylcohexylcarbodiimide
DHU	N,N'-Dicyclohexylurea
DMAP	4-(Dimethylamino) pyridine
DP	Degree of polymerisation
Epoxy ester	Bis (2,2,2-trichloroethyl) (±)-3,4-epoxyadipate
Ether	Diethyl ether
FTIR	Fourier transform infra-red spectroscopy
Glut, Glutarate	Bis (2,2,2-trichloroethyl) glutarate
GPC	Gel permeation chromatography
HB	3-hydroxybutyrate
Hexanediol	Hexane-1,6-diol
HV	3-hydroxyvalerate
NMR	Nuclear magnetic resonance spectroscopy
PBT	Poly(butylene terephthalate)
PCL	Poly(caprolactone)
PEG	Poly(ethyleneglycol)
PET	Poly(ethylene terephthalate)
P(3HB-co-3HV)	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PPL	Porcine pancreatic lipase
Succinate	Bis (2,2,2-trichloroethyl) succinate

THF	Tetrahydrofuran
TRIC	2,2,2-Trichloroethanol
TRIF	2,2,2-Trifluoroethanol
TRIF-Glut, Trifluoroglutarate	Bis (2,2,2-trifluoroethyl) glutarate

1) Introduction

1.1) Introduction

The use of enzymes as catalysts is not a new idea. Enzymes have been used traditionally as catalysts for many years in the laboratory and for many millions of years in nature. However, their role as catalysts *in vitro* was limited since, apparently, it was restricted to use in aqueous media. During the last 15 years or so, significant developments have been made in the modification of these enzyme systems which has enabled them to be used as catalysts in organic media. Paradoxically, the enzymes work well in nearly anhydrous solvents and in fact, are rendered more stable[1,2,3].

This new development offers tremendous potential for a chemist since it opens up a wealth of possibilities previously impossible in a reaction medium such as water. Many reaction substrates and products are far less soluble in water than in an organic solvent. In addition, some reaction products may be labile in an aqueous environment giving rise to undesirable side reactions. For example, transesterification reactions are virtually impossible in aqueous media due to the dominating hydrolysis side reaction. Also, in water the thermodynamic equilibrium is unfavourable for reactions such as esterifications. Often in aqueous systems the product is difficult and hence, expensive to isolate. In contrast, product isolation in organic media is usually a relatively simple operation. In the majority of organic solvents the enzyme is insoluble and therefore can be simply filtered off and even re-used. This would be a very attractive approach on large scale industrial terms but in the research laboratory the ready availability and inexpense of enzyme catalysts means that from a financial point of view, even this regeneration of the catalyst is not essential. Another implication arising from the insolubility of the enzyme in organic media is that immobilisation of the enzyme on a solid support is unnecessary, again because the enzyme can just be filtered off. If however, immobilisation is desired, then the method required is relatively facile compared to the complicated covalent bonding or entrapment techniques required for aqueous systems. This results from the insolubility of the enzyme in organic media and

hence the reluctance of the enzyme to desorb from the surface in question. As mentioned earlier, the additional advantage of using enzymes in organic media are that they are in fact made more stable, both thermodynamically and with respect to denaturing. Their insolubility stabilises them to much higher temperatures than in aqueous solution because in organic media the enzyme is locked in its active conformation in much in the same way as when it is immobilised on a solid support. For example, porcine pancreatic lipase in boiling water is almost instantly inactivated, whereas in organic solvent at the same temperature, the half-life is in excess of 12 hours[4]. Consequently, this also makes them far more robust in terms of actual physical distortion and so they are able to be employed where possibly more vigorous reaction conditions are needed. This also means that their shelf-life is much improved and they can be stored at room temperature for months without loss in catalytic activity. Enzyme denaturing by microbial contamination as a result of the release of proteases is also far less pronounced in organic solvents.

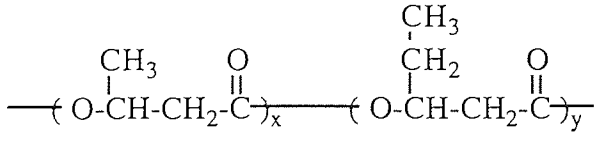
However, it must be stressed that the use of enzymes in non-aqueous systems is also not a new concept. Many enzymes including lipases function in natural hydrophobic environments (usually immobilised) where the water concentration is much less than the 55 M concentration of an aqueous solution. It is true however, that the use of enzyme catalysts in synthetic applications is a relatively new and potentially very exciting area of chemistry[5,6].

The use of biotransformations for the manufacture of speciality materials is rapidly increasing. One area in particular in which these biotransformations are being used, is in the flavour and fragrance industries[7,8]. Here, there is a growing consumer demand for products using natural and environmentally friendly ingredients. Consequently, this is an ideal area for enzyme use. This is especially true in view of the fact that enzyme processes are stereospecific, which is very often essential in these syntheses.

The direction in which this research work has been aimed, has been towards the

synthesis of biodegradable polyesters using enzyme catalysis. The rationale behind this being that if polyesterification, a reversible reaction, can be effected using enzymes then they can also be degraded by enzymes into their harmless constituents, carboxylic acid, carbon dioxide and water. Dordick *et al* used enzyme catalysts in conjunction with conventional chemical catalysts to make and to modify existing biodegradable as well as biocompatible polyesters[9]. Their work involved using enzyme catalysts to modify sugar molecules and then conventional catalysts for subsequent polymerisation. The introduction of sugar molecules renders the polymer more hydrophilic. An example of one of these applications is in the modification of hydrogels. Hydrogels are lightly cross-linked polymers that are able to absorb large amounts of water. On the introduction of sugar moieties, a hydrogel is capable of vastly increasing the quantity of water it can hold; typically, from 10 to several hundred times its own dry weight[10]. Further examples of polymer synthesis using enzyme catalysis by other workers in the field and the approach taken for the synthesis of polyesters in this research project, will be discussed in more detail in a later section.

To date, one truly biodegradable polymer has been produced commercially under the trade name BIOPOL, by Holmes *et al* of ICI[11]. This polymer, however, is not produced using conventional synthetic laboratory techniques. It is produced naturally by bacteria under imbalanced growth conditions. For commercial large-scale production purposes, a 2-stage fermentation process was developed in 1981, in which bacterial monocultures were fed with the carbon substrates, propionic acid and glucose, to produce a copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate, P(3HB-co-3HV):



3HB 3HV

There are a number of techniques used for the extraction of the resulting polymers from the fermentation broth. A diagram of one patented procedure is shown in diagram 1. After centrifugation to remove the spent nutrients, the polyester-containing cells are washed sequentially with acetone and then a mixture of refluxing dichloromethane and ethanol. The mixture is then filtered to remove any waste cellular material and finally the product polyester is precipitated using diethyl ether.

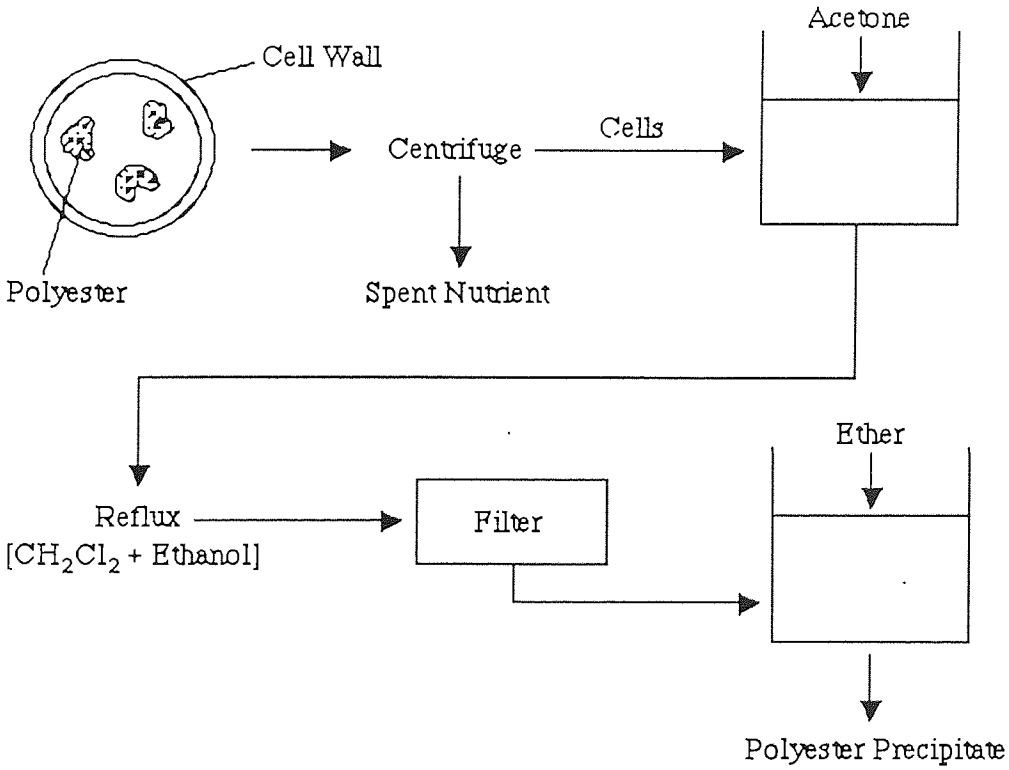


Figure 1. Flow diagram for producing polyhydroxybutyrate from cells[12]

It was first discovered in 1925, that some bacteria naturally produced polyesters, when Lemoigne isolated the polymer poly(3HB) from Bacillus megaterium[12A]. For many bacteria P(3HB) functions either as a carbon and/or energy reserve and is accumulated in bacterial cells in conditions when the cells have a limited supply of an essential nutrient (e.g. ammonium chloride) but are exposed to an excess of carbon (e.g. glucose). Unlike other biological polymers, such as proteins and polysaccharides, however, P(3HB)

isolated from bacterial cells (molecular weight approximately 10^5-10^6 Da) is a thermoplastic with a melting temperature around 180°C. The biosynthesis and degradation of P(3HB) in bacterial cells occur via a cyclic metabolic pathway. Hence, these polymers are ideal biodegradable materials as they can be degraded in soil, sludge or seawater on the secretion of P(3HB) depolymerases (enzymes) by some bacteria and fungi which are present in abundance in these environments.

Clearly these poly(hydroxyalkanoates) seem very useful in that they are fully biodegradable. However, their are drawbacks to this process. Firstly, only a relatively narrow range of substrates can be incorporated into the polymer. Secondly, the resulting polyesterification always incorporates a certain amount of hydroxybutyrate monomers. Finally, the polymer backbone is always a four-atom chain with a different group in the carbon-3 position. In addition, the process is carried out in the aqueous media of a fermentation broth which means that polymer isolation is a relatively complex operation compared with extraction from organic media.

A lot of work has been done in a relatively short period of time in the field of enzyme catalysis, but relatively little research has been aimed at polymer synthesis. It was, therefore, the aim of this research project to exploit the attractive mild reaction conditions offered by enzyme catalysis for polyester synthesis and to attempt to find a more versatile process for biodegradable polymer synthesis. This is to enable the drawbacks of the fermentation process to be overcome and to establish the necessary conditions for an alternative, more versatile method of biodegradable polyester production.

1.2) Uses & Advantages of Enzyme Catalysts Over Conventional Catalysts

Enzymes can catalyse a wide variety of reaction types. At first, most of the work done

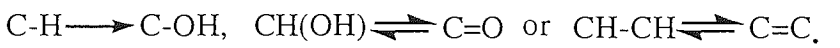
using enzyme catalysts was concentrated in the pioneering laboratories of Klibanov, and Dordick etc., but currently, due to ease of use, ready availability and inexpense of these enzymes, chemists in all areas are exploiting the advantages offered by enzymes[13,14,15]. There are several immediately obvious advantages of using enzyme catalysts over conventional catalysts such as acids and bases. The first of these is that enzymes require much milder reactions conditions in terms of the reaction temperature, pH and pressure. Secondly, they are chiral catalysts and consequently are extremely useful for and are widely used in the syntheses of chiral compounds. Obviously this ability gives them great potential in pharmaceutical applications and is in fact, one of the major uses for enzyme catalysts. Finally, enzymes are often able to catalyse reactions that are not always feasible using conventional catalysts. For example, they can be used in regiospecific roles where it might be required that only a particular hydroxy function amongst many others, undergoes reaction[16,17]. Examples of this type of enzyme usage has been with the esterification of sugars. The polymerisation of sugars for the purpose of making biodegradable materials, such as drug-delivery systems or disposable sanitary products, is potentially a very problematic synthesis. Sugars possess multiple hydroxy functionalities and if polyesterified in a conventional way using diacids, lead to the formation of highly crosslinked polymers. A possible way to get around such a problem would be to first protect all of the hydroxy moieties not required for the polyesterification reaction. Subsequently, the desired polymerisation could be carried out, followed by the removal of the protecting groups. However, this protecting-deprotecting procedure is usually a lengthy and costly process. An alternative, much simpler and inexpensive approach is to employ the use of enzyme catalysts to selectively polyesterify the desired functionalities. Work undertaken by Patil *et al*, demonstrated the linear polyesterification of sucrose with bis (2,2,2-trifluoroethyl) adipate[18]. The reaction catalyst used was Proleather, an alkaline protease. Another example where a sugar was used for polymerisation purposes used a similar procedure but instead used an alternative esterification reagent, vinyl acrylate[19]. The advantages of using vinyl systems is that the vinyl group is a good leaving group and forms an unstable vinyl alcohol on

transesterification. The vinyl alcohol then rearranges to form the more stable ketone. The overall effect of this is to push the reaction equilibrium to favour product synthesis. A more detailed discussion of this is presented in section 3.5.2.

There are a great many (more than 2,000) known enzymes available for numerous transformations, although 'only' several hundred of these are available commercially. As a result, it is usually possible to find a suitable enzyme catalyst for most reaction types. Enzymes have been classified into six main groups by the International Union of Biochemistry[20]:

(i) Oxidoreductases

These are as the name suggests, used to catalyse oxidation or reduction reactions such as



(ii) Transferases

These are used in the transfer of functional groups from one molecule to another. The types of moieties which can be transferred are acyl, aldehyde, ketone, sugar and phosphoryl.

(iii) Hydrolases

These are able to hydrolyse a broad range of functional groups which include anhydrides, esters, glycosides, amides peptides and other C-N containing groups.

(iv) Lyases

These catalyse addition and elimination reactions usually of HX with double bonds such as C=C, C=O and C=N.

(v) Isomerases

Examples of the isomerisations which can be carried out include C=C bond migration, *cis-trans* isomerisation and racemisation.

(vi) Ligases

These are used for the formation of C-C, C-O, C-N, C-S and phosphate ester bonds.

1.3) Choice of Enzyme

Although there are a great number of lipases, the enzyme which has been most extensively used for esterifications is *Candida cylindraea* lipase. For transesterifications, however, porcine pancreatic lipase works most effectively. Both of these enzymes are readily obtainable commercially and are relatively inexpensive. Also, they are available with a range of catalytic activities from as low as a few units per mg of protein up to several million units per mg. The pH and temperature activity curves for the two enzymes used, are shown below.

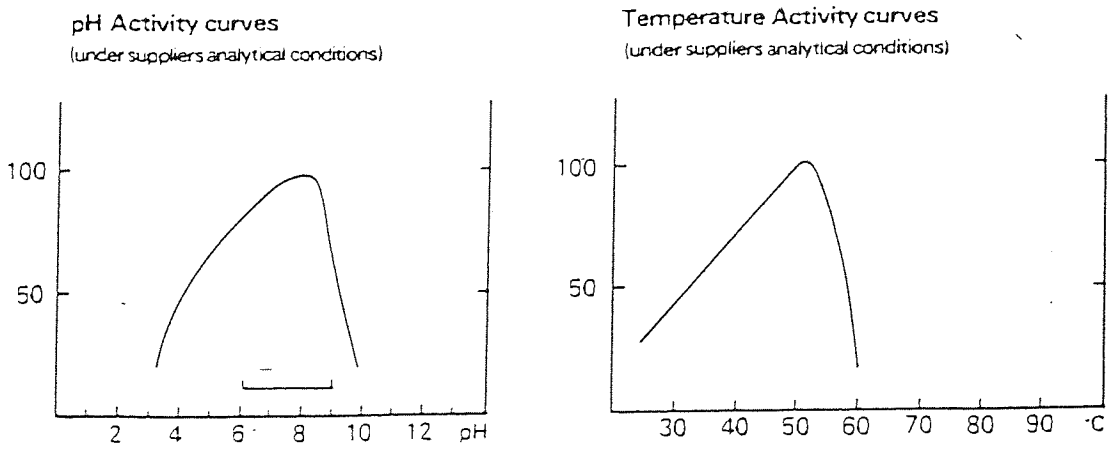


Figure 2. Temperature and pH activity curves for porcine pancreatic lipase

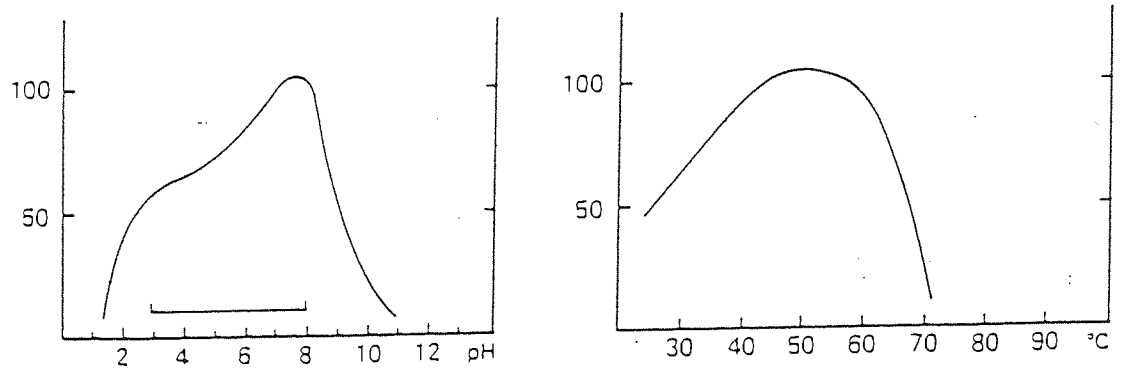


Figure 3. Temperature and pH activity curves for *Candida cylindraea* lipase

For porcine pancreatic lipase optimum enzyme activity is achieved in the pH range of 7.0-8.5 and temperature range of 45-55°C. *Candida cylindraea* lipase is somewhat more robust with an optimum working pH range of 6.5-8.5 and temperature range of 40-60°C[21]. At higher temperatures the enzyme activity may be increased, but it should be noted that it is usually at the expense of a loss in enzyme stability. Consequently, most of the reactions relating to this project were carried out at ambient temperature.

1.4) The Role of Water

When it is said that enzymes can act as catalysts in anhydrous solvents, it does not mean that enzymes can function without water. In a truly anhydrous media, the structure of the enzyme molecule would be distorted and so consequently, the enzyme activity would be destroyed. Enzymes need a certain amount of water for them to be held in the correct orientation for reaction. It has been shown[22] that the actual amount of water necessary for correct function is only very small. In fact, all that is required, is for the enzyme to be surrounded by a few layers of water. This has the same overall effect as does a bulk water solution. This is because the enzyme actually only 'sees' the few monolayers of water with which it is surrounded, whether it is an aqueous or an organic phase. It is believed that this layer acts as a buffer between the enzyme and the bulk phase so that the enzyme is not affected beyond this hydration layer[3]. Mechanistically, the water is

thought to be able to act as an unlocking device for the enzyme. This is due to its ability to form hydrogen bonds with the functional groups of the enzyme which are otherwise bonded to each other[23]. The precise amount of water necessary for the enzyme to function properly is dependent on the enzyme itself, with certain enzymes needing more water than others. For example chymotrypsin in octane is reported to need as much as 50 molecules of water per enzyme molecule, whereas some hydrolytic enzymes such as the lipases, require only a fraction of this amount. There can be quite a large variation of water requirements for the different enzymes and it has been suggested that a more accurate correlation concerning enzyme activity and water content may be obtained by measuring the quantity of water bound to the enzyme itself, rather than the water content of the organic solvent.

An interesting and convenient aspect concerning enzyme catalysis is how it is affected by reaction pH. Since in an organic phase there is no aqueous phase, this also means that there is no hydrogen ion content and consequently pH cannot be measured. However, enzymes are sensitive to pH and every enzyme has an optimum pH at which it operates. In an aqueous buffer, the enzymes ionogenic groups acquire an ionisation state which is dependent on the pH of the solution[24]. In an organic solvent there is no interaction between the water layer buffer of the enzyme and the organic solvent and so the chemical state of the enzyme remains unaffected in going from the aqueous phase to the organic. This phenomenom can be exploited well, by first lyophilising the enzyme from a suitably buffered aqueous solution with a pH which is at an optimum for the enzyme in question.

1.5) Effect of Temperature

Each enzyme has an optimum temperature range at which it works best. As the temperature is increased beyond this range, the enzyme will eventually reach a point at which it is deactivated. This thermal deactivation can be reversible or irreversible

depending on the duration of time for which the enzyme is subjected to raised temperatures[25]. Obviously, the longer the time the lesser the likelihood of recovery for the enzyme.

Zaks and Klibanov, investigated the effect of heating porcine pancreatic lipase to 100°C[4]. They found that relatively dry enzymes in organic solvents were far more thermostable than the same enzyme in aqueous solution.

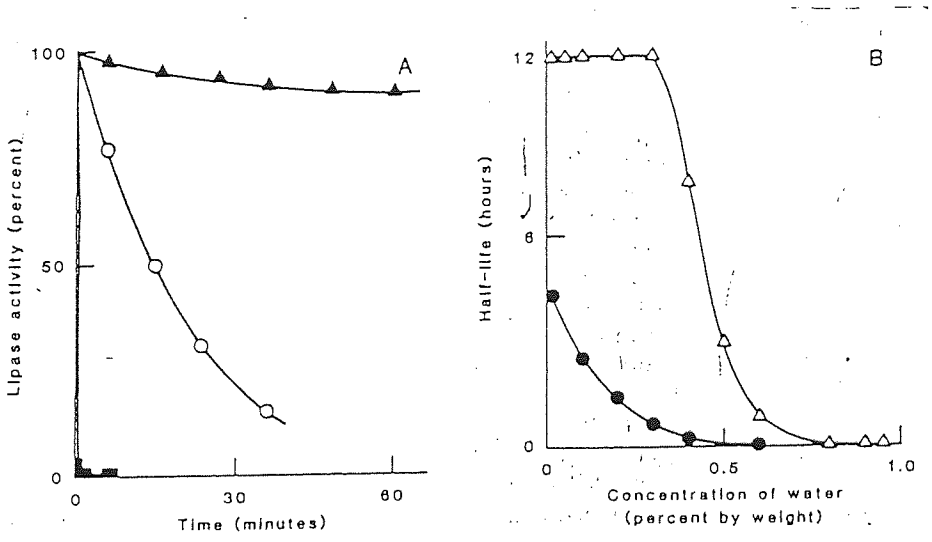


Figure 4. (A) Thermal inactivation at 100°C, of porcine pancreatic lipase placed in water or 0.1M phosphate buffer (pH 8.0) ([]) and in 2M solution of heptanol in tributyrin containing 0.8% water.(O) or 0.015% water (Δ). (B) The half-life at 100°C of wet (O) and dried (Δ) porcine pancreatic lipase placed in 2M solution of heptanol in tributyrin at different concentrations of water.

Additionally, it was found that whereas water activated the enzyme at 20°C, the addition of even very small amounts of water at 100°C, had a substantial deactivating effect on the enzyme.

1.6) Effect of Pressure

Although pressure has been one of the lesser studied effects compared to other effects such as temperature, pH and reaction solvent, recent developments have led to some interesting early findings. Kim and Dordick[26] studied the effects of pressure on a simple enzyme-catalysed esterification reaction. They found that an increase in pressure caused the essential enzyme-bound water to be stripped away more easily. This, in turn led to a decrease in the activity of the enzyme. They found also, that the effect of pressure on an enzyme's activity was dependent on the flexibility of an enzyme in a solvent. The more polar a solvent, the greater was the flexibility of the enzyme, making the water relatively easy to remove. In summary, it was found that the enzyme's reduction in activity due to the water-stripping effect, was more pronounced in more polar solvents.

Work undergone by Chaudhary *et al*, demonstrated how pressure could be used to control the molecular weight of polymers[27]. The reaction they concentrated on was the polytransesterification reaction of bis (2,2,2-trichloroethyl) adipate and butane-1,4-diol, catalysed by porcine pancreatic lipase. The reaction media used were two supercritical fluids, fluoroform and carbon dioxide, which allowed substantial pressure increases. It was found that as the pressure was increased, higher molecular weight materials were precipitated out of solution. Thus, this served as a control mechanism to narrow the polydispersity of the resulting polymers and also to limit the molecular weight of the polymer.

1.7) Solvent Effect

The solvent is thought to affect enzyme catalysis in three ways; firstly, by direct interaction with the enzyme or more specifically with the hydrogen bonds and hydrophobic interactions within the enzyme structure. This effect is more pronounced in more polar solvents and in biphasic systems. The implications of this type of interaction

are that the structure of the enzyme becomes distorted and hence, is deactivated. With less polar solvents and in monophasic systems, however, the enzyme interaction with the solvent is minimal and the observed effect is increased enzyme stabilisation and it does in fact, become far more robust even to mechanical damage *viz* vigorous stirring. This effect is similar to that observed with immobilised enzyme systems which are rendered more stable when used at higher temperatures. Both these effects are due to the fact that the enzyme (not solubilised by the organic solvent) becomes locked in its correct conformation for activity.

Another way that solvents affect enzyme catalysis is in their interaction with the water layer surrounding the enzyme. Highly polar solvents are capable of solubilising quite large amounts of water and can even strip the water layer away from the enzyme and hence, denature it. With polar solvents which are water-miscible, reaction cannot usually be carried out unless the solvent is first saturated with water before use. With hydrophobic solvents however, this water stripping effect does not occur and so they are generally thought to be more suitable solvents for enzyme catalysis. However, there are some exceptions to this rule, notably the lipases. These particular enzymes are capable in some instances, of supporting catalysis even in a water-miscible solvent (such as pyridine) which has not first been saturated with water[28,29]. This demonstrates the capability of some enzymes to hold on to their bound water layer very strongly, even in quite harsh conditions. The inherent ability of the lipases to bind the water so tightly, is most probably due to their having to operate in such hydrophobic environments in nature. An even more dramatic example of this kind, is that of the enzyme subtilisin Carlsberg[16]. It is capable of retaining its catalytic activity by binding the water layer extremely tightly to itself even in dimethylformamide, a solvent which is actually able to dissolve the enzyme.

Finally the importance of the solvent is in how it interacts with the reaction substrates and the products themselves. Obviously, since the enzyme itself is not soluble in the majority

of organic solvents, it is necessary that the solvent for the reaction is at least able to dissolve the reactants. At the same time it must not interfere with the reaction, unless of course this is desired, as in the case of an esterification reaction where the solvent may be an alcohol taking part in the reaction and driving the reaction to completion. There are other examples, however, of how the solvent can be used to affect the reaction without actually taking part in the reaction directly; such as changing the regioselectivity of the enzyme by employing an alternative solvent[30]. One such example was illustrated by Fitzpatrick and Klibanov who showed that the solvent can affect enantioselectivity[31]. They found that a solvent with a low dielectric constant such as dioxane increased the enantioselectivity of an enzyme. In a solvent with a low dielectric constant, the rigidity of the enzyme is increased and so puts more steric constraints on the substrates. In a high dielectric constant solvent such as water where the enzyme is more flexible, it may be easy for one stereoisomer to bind preferentially to the enzyme's active site and for the other isomer to only bind with some difficulty. If then, the solvent is replaced for one with a lower dielectric constant, such as hexane, the enzyme's flexibility is diminished, which results in the non-preferred isomer finding it even more difficult to bind to the enzyme.

For the products, it is usually desirable that they also have an affinity for the solvent, otherwise problems can occur. If the product is quite polar it may tend to have an affinity for the aqueous environment around the enzyme and so be involved in unwanted side reactions such as hydrolysis. Otherwise, it may interfere with the reaction equilibrium by crowding the enzyme and hence inhibiting the formation of further product. Concerning the solvent interaction with the substrates, there are examples where sometimes the solvent can indirectly reduce the overall effectiveness of the catalysis by its association with the reaction substrate. In the oxidation of phenols with horseradish peroxidase the reaction is catalysed by the enzymic formation of phenoxy radicals. However, if used in chloroform, the phenoxy radicals are quenched, thus greatly reducing the catalytic activity[32].

1.8) Choice of Solvent

Considering all of these factors, the choice of a suitable reaction solvent may appear a straightforward decision, no different than for any other organic reaction. However, it appears that enzymes are quite sensitive to the solvent used. This is demonstrated by the fact that their activity can vary greatly from one solvent to another. In fact, their activity is also affected by different water content in the same given solvent. In much of the work done to date, the choice of solvent seems to be an arbitrary event. Obviously, however, the single most important factor for solvent choice is for the solvent to be able to support the catalytic activity of the enzyme. Some attempts have been made by various workers to try to establish general rules for the selection of a suitable solvent for a particular reaction type. One model suggested by Brink and Tramper, makes use of the Hildebrand solubility parameter, δ, combined with the molecular weight of a solvent[29]. They concluded that high catalytic activity was expected from solvents which had a δ value of greater than ~8 and a molecular weight of below 150. This model, however, gave somewhat poor correlations between enzyme activity and solvent polarity. Subsequently, an alternative model was proposed by Laane *et al* , who preferred to make use of the logarithm of the partition coefficient, log P as an indication of the solvent polarity, where P is the solubility ratio of a solvent partitioned between octanol and water[28]:

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

They argued that log P is a better indicator of the solvent polarity since it gives a direct measurement of polarity, rather than an indirect one as in the case of δ. Values for the log P can quite easily be determined, either by calculation or by standard experimentation. Additionally, they are sensitive to a broad range of polarity differences, which is not the case for δ values. Finally, they contested the inclusion of the molecular weight for the solvent, since they found that high activities were found with solvents having a log P value >4 regardless of their molecular weight, which was invariably >150. They found

that solvents with a log P value of greater than 4 work best, whereas those with a value of less than 2 (e.g., short chain alcohols and esters, ethers and water-miscible solvents) are unsuitable. As well as solvent polarity values, they found that enzyme catalysis reactions could be further optimised when the polarities of the enzyme microenvironment (the water-layer surrounding the enzyme) and the bulk organic phase were correlated with the polarities of both the reaction substrate and product. The polarities of the enzyme microenvironment and the substrate should have similar values so that the substrate density around the enzyme is at a maximum hence promoting the reaction. Similarly, the polarity of the bulk phase should be comparable to that of the product and dissimilar to that of the substrate (and enzyme microenvironment). This means that following reaction, the product favours the environment of the bulk phase and so does not remain in the vicinity of the enzyme, thereby inhibiting further reaction. Also since the bulk phase polarity is dissimilar to the enzyme environment there is less likelihood of the solvent interacting with the enzyme and hence denaturing it.

This hypothesis gives very good correlations for the ability of a solvent to strip away the essential water-layer from an enzyme but still does not account for the examples given earlier of the good catalytic activities of lipase in pyridine and horseradish peroxidase in chloroform. An alternative model proposed by (Adlercreutz[89]) deals with this, to some extent, by including a term for the water-solubilising power of the solvent :

$$\log P_{corr} = (1 - x) \log P_{solvent} + x \log P_{water}$$

(x = water solubility as a mole fraction)

This revised model works quite well for solvents such as ether and ethyl acetate but still does not work for water-miscible solvents.

It was felt by Dordick, that the major flaw in these models was that the log P values give

an indication of the stability rather than the activity of the enzymes[2]. It is certainly true that so far there have been too many results which contradict these findings for any satisfactory conclusions to be drawn. For instance, in determining the best solvent type for stereoselective synthesis, there are contradicting views. Klibanov *et al* found that for the transesterification reactions they carried out with subtilisin, enantioselectivity decreased with increasingly hydrophobic solvents[33]. However, Wong *et al* , for their transesterification of glycerol acetonide reported that enantioselectivity was unaffected by solvent type[34]. Furthermore, Dordick *et al* showed that for the esterification of α-hydroxyacids, enantioselectivity was greatest with more hydrophobic solvents[35].

More recently, Narayan and Klibanov carried out further studies to determine whether solvent polarity and water-immiscibility were relevant to enzyme activity[36]. Firstly, they examined the effect of water-miscibility. They investigated the transesterification reactions of four different enzymes, three of which were lipases, and a series of nine solvents which had similar hydrophobicities. The hydrophobicity measure used for each solvent was calculated in terms of its log P value. Five of the solvents used were water-miscible and the remainder were not. It was anticipated that if enzyme activity was improved with more water-immiscible solvents, then an increase in its activity would be observed in going from a water miscible solvent to a water-immiscible one. However, no significant change in enzyme activity was detected. This suggested that water-miscibility alone, did not affect enzyme activity. Next, they investigated the effect of solvent polarity. A polar solvent is defined as one having a permanent dipole moment. Three groups of solvents were chosen where the solvents within a group were geometric isomers. One solvent in a group was chosen so that it was completely symmetrical and so had a zero dipole moment. The other solvent was unsymmetrical and hence, polar. Similarly as for the water-miscibilty test, it was anticipated that an enzyme activity increase in going from a polar to a non-polar solvent would mean that the activity was enhanced by a decrease in solvent polarity, **as a single factor**. However, this effect was also not observed. From this work it was concluded that neither water-immiscibility

nor solvent apolarity as single factors, improve enzyme activity.

It appears, with reports such as this, that solvent choice may be particular to the system under investigation. However, it still seems evident by the multitude of examples in the literature, that the solvent of most popular choice for a variety of uses, is diethyl ether. Consequently, many workers still find that when it comes to choosing a suitable solvent for reaction, 'trial and error' seems to work best.

1.9) Types of Systems

There are a number of different ways in which enzyme catalysed reactions can be carried out:

1.9.1) Biphasic Mixtures

These contain a stirred aqueous phase in which the enzyme is dissolved, along with an immiscible organic solvent[37,38]. In these systems reaction occurs at the interface of the two solvents, with the resulting products being cast into the organic phase. Enzymes such as the lipases work particularly well in these interfacial systems since, they are inherently designed for such purposes.

The drawback with this type of system, however, is the large volume of water present, since the disadvantages of aqueous media are the primary reasons for the use of enzyme catalysis in organic media. Additionally, since reaction only occurs at the interface, quite vigorous stirring is required which can cause denaturing of the enzyme. Consequently, although this type of sytem was predominantly used in very early years, it is now rapidly declining in popularity as a method for catalysis.

1.9.2) Monophasic Co-Solvent Systems

Here a water miscible solvent such as methanol is used with water at a concentration of typically, 10% by volume[39]. Whilst a large volume of a co-solvent is known to have a denaturing effect on the enzyme, a small amount is actually beneficial and in fact serves to stabilise the enzyme[40]. These systems are employed to increase the solubility of water-insoluble lipophilic substrates in mainly aqueous media[41]. Dordick, Marletta and Klibanov used horseradish-peroxidase enzyme for catalysing polymerisation reactions of various phenols[42]. The monophasic co-solvent systems employed contained around 95% of the solvents dioxane, acetone, dimethylformamide and methyl formate. These systems were used to make phenol-formaldehyde resins with relative molecular weights ranging from 400 to 26,000 Da.

1.9.3) Microemulsion-Based Reverse Micelles

This is a variation of the biphasic system in that a relatively large amount of water is used. The difference, however, is that the two phases are separated by a surfactant intermediate. The surfactant, typically *bis*(2-ethylhexyl) sodium sulphosuccinate (AOT), suspended in the organic phase, forms micelles which contain the enzyme dissolved in water[43]. Conventionally, in aqueous solution micelles are orientated such that the polar hydrophilic heads are on the outside in contact with water, with the hydrophobic tails pointing inwards towards the centre of the micelle. In an organic solvent the micelle is turned completely inside out with the heads pointing inwards and hence, is termed a **reverse** micelle. Over a narrow concentration range, termed the critical micelle concentration, the surfactant molecules immediately form micelles which are 15-20 Å in diameter and are comparable in size to the enzyme molecules[44,45]. The amount of water contained in the micelles is very small, typically between 50 and 500 molecules of water per enzyme molecule[1]. The optimum enzyme activity observed for porcine pancreatic

lipase is at a water-to-AOT molar ratio of about 12[46].

The enzyme in its microenvironment behaves in much the same way as it does in aqueous solution with the advantage that it is additionally protected from the water distorting influence of the organic phase. Other features of these microemulsions are that the enzyme is able to be dispersed evenly throughout the mixture for maximum efficiency, which also means that the resulting mixture is transparent, so is useful for spectrophotometric type assays. Also, since they are thermodynamically stable, they do not require stirring as for the biphasic systems so there is less of a likelihood of denaturisation in this manner. Marangoni studied the effects of the interaction between porcine pancreatic lipase and AOT reverse micelles and found that the enzyme interacted with the surfactant membrane[46]. Enzymes solubilised in reverse micelles fall into two categories; those which interact with the micellar membrane and those which are solubilsed within the aqueous phase and do not interact with the membrane. With porcine pancreatic lipase, and other surfactant-interacting enzymes, an increase in the AOT concentration relative to the enzyme, causes a decrease in catalytic activity.

Robinson *et al* used a modified microemulsion-based system in which gelatin was solubilised in the microemulsion to give a transparent gel[47]. These enzyme immobilised gels can be made in a large batch and conveniently stored in a freezer until required. The reproducibilty of results is allegedly comparable to those of enzyme-containing microemulsions. They can also be reused quite easily as well as making product isolation a relatively simple operation.

The Robinson technique was employed for a substantial amount of time, in our exploratory attempts to effect polymer synthesis. Initially, the solvent used was heptane, as was stated in the literature. This was chosen as it was relatively non-polar and was believed to be the best solvent type to be used in conjunction with enzyme reactions. Since no success was achieved with systems using this solvent, it was decided to move

to other solvents which were better solvents for the reactants, but at the same time did not interfere with the reaction. For example, when used with the gel experiments, solvents such as THF and dichloromethane were able to dissolve the reactants quite well without affecting the gel. Diethyl ether, however, tended to cause dissolution of the gel and hence, was considered unsuitable.

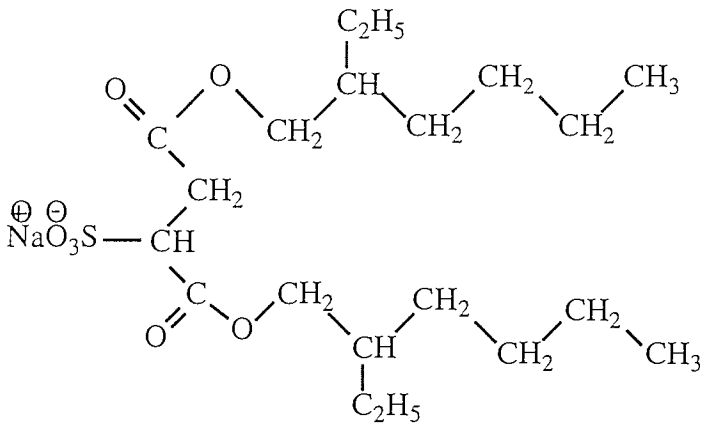


Figure 5a. Sodium bis-2-ethylhexylsulphosuccinate (Aerosol-OT)

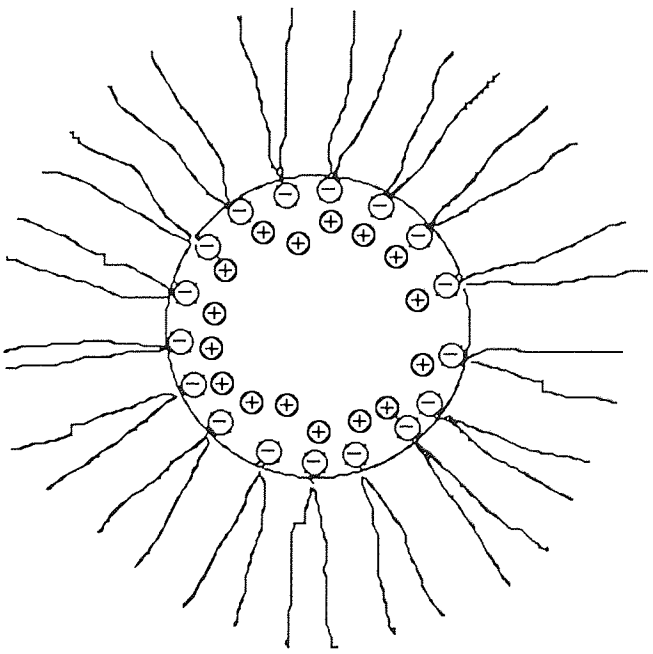


Figure 5b. Structure of a reverse micelle[48]

1.9.4) Enzyme Immobilisation

Enzyme immobilisation is reported to markedly stabilise enzymes against denaturisation[41]. Here the enzymes are adsorbed onto inert solid supports with a large surface area such as Celite or glass and then used as slurries in water-immiscible organic solvents[49]. The supports provide the enzymes with a more stable base, reducing their tendency to unfold into a non-active conformation. This type of simple enzyme adsorption is different to the covalently bonded entrapment techniques which have to be employed in aqueous or biphasic solutions. For the covalently bonded enzymes in aqueous or biphasic systems, the enzyme is first washed with a buffered solution, then a support such as Chromosorb or Sepharose is allowed to absorb the enzyme solution resulting in a swollen gel containing enzyme[50]. For monophasic organic solvents, the necessity of chemically binding the enzymes onto the solid supports in this way is eradicated since, the enzyme has no inclination to desorb from the surface into the organic solvent. The technique is much simpler, with the buffered enzyme solution being mixed with the solid support and then being allowed to dry.

1.9.5) Modified Enzymes

Relatively recently there has been substantial progress in trying to increase the stability of enzymes to denaturisation. Two alternative reports showing promise have both involved the incorporation of another material with the enzyme during lyophilisation.

The first method, reported by Dabulis and Klibanov involved the lyophilisation of three different lipases with the ligand, N-acetyl-L-phenylalanine-2-chloroethyl ester[51]. The ligand is thought to enhance the resistance of the enzyme to reversible denaturisation during the lyophilisation stage. It is believed that most lyophilised enzymes exist in a reversible denatured state which greatly reduces the catalytic activity of the enzyme. In

water, the enzyme is more flexible and is able to resume its active conformation. In organic media, however, the enzyme is held more rigidly and is effectively locked in a denatured state to a much greater extent than in aqueous solution. Hence, the ligand is thought to aid enzyme activity by acting in a preventative way.

The second method, proposed by Dordick *et al*, is similar in its approach; that is, the enzyme is lyophilised with another material from an aqueous solution[52]. It was found that the addition of simple potassium salts to the enzyme solution, prior to lyophilisation, improved considerably, the activity of the enzyme. The mechanism of action was slightly different, however. Here, it was thought that the salts aided enzyme activity by protection from the organic solvent against denaturisation, rather than protection during the lyophilisation stage.

1.9.6) Monophasic Enzyme Slurries

This method is probably the simplest and most versatile of all the approaches mentioned. Here, the dry enzyme is added to the organic solvent in which it is insoluble and the resulting slurry is stirred gently. The 'dry' enzyme still has enough water surrounding it as supplied by the manufacturer, to be catalytically active. For example, the following lipases, porcine pancreatic, *Candida cylindracea* and *Mucor* were calculated as containing 3.6%, 6.1% and 4.8% water respectively[53].

Although the solvent is in direct contact with the enzyme, the latter is still able to function as a catalyst. This is due to the ability of the enzyme to bind the water layer very tightly to itself and hence be surrounded by enough water to retain the correct orientation for activity. With fairly non-polar solvents the enzyme is easily able to retain catalytic activity but with more polar or water miscible solvents they may require saturation with water prior to the addition of the enzyme. As mentioned earlier, this is so that any disruptive solvent interaction with the aqueous layer bound to the enzyme is minimised.

This approach is the one most often employed for numerous tranformations in organic media and for the majority of enzyme-catalysed syntheses, these are quite often stereoselective transformations[54]. For example, for the resolution of racemic mixtures of alcohols and carboxlic acids, enzymes can be employed for the asymmetric hydrolyses of the corresponding esters[55,56]. Conversely, asymmetric esterifications or transesterifications can be achieved by using enzymes to catalyse reactions using racemic mixtures of alcohols and carboxlic acids or esters, respectively[57,58,59,60,61,62].

Enzyme use in this field is particularly desirable due to the mild reaction conditions offered in this sensitive area of chemistry. Stereoselective syntheses of polymers and oligomers has also been attempted, with the formation of optically active pentamers[63]. These have a range of uses, for example as chiral adsorbents for the separation of racemic mixtures. Kobayashi *et al* demonstrated the use of enzymes catalysts for the non-biosynthetic polymerisation of cellulose, which involved high degrees of both regio and stereoselectivity[64]. Other polymerisation attempts have included the formation of large macrocyclic lactones starting from a dicarboxylic acid and a diol[65]. The reverse of this, i.e. ring-opening polymerisation, starting with a lactone, was attempted in this research project (see section 3.6.2). A different approach to polymer synthesis is one starting with an hydroxyacid. All of the reactions mentioned so far have been of the heteropolymerisation-type. That is, they have involved condensation reactions between diester and diol molecules. A homopolymerisation-type condensation is one where initially, all the molecules are identical and possess both acid and alcohol functionalities. The advantage of such a system is that a precise equimolar mix of alcohol and ester moieties is present. Ajima and co-workers used this approach in the attempted polyesterification of 10-hydroxydecanoic acid using a polyethylene glycol-modified lipase[66]. The molecular weight achieved was not reported, although they believed that some degree of polymerisation had occurred. A more successful polyesterification was reported using this approach, by O'Hagan *et al* (see section 3.3.3), who made a polyester with Mn and Mw values of 9,300 and 12,100, respectively[67]. Knani *et al* also used

hydroxy esters in attempts to make polyesters[68]. Methyl 6-hydroxyhexanoate and methyl 5-hydroxypentanoate were polyesterified in the presence of porcine pancreatic lipase and using hexane as the solvent. The largest polyester synthesised by this method was reported to have a Mw of 12,000, determined by NMR. Additionally, they also investigated macrolactone synthesis by ring-opening polymerisation.

As well as actual polymer synthesis, enzymes can also be very beneficially used in other polymer-related work. For example, Ritter and Pavel used lipase to catalyse the esterification of 11-methacryloylaminoundecanoic acid[69]. The ester was subsequently polymerised using a conventional radical polymersiation process[70]. Additionally, they used lipase to catalyse further reactions on another polymer. Lipase was used to catalyse acylation of the pendant groups of a methacrylic comb-like polymer. Various acyl groups ranging from a simple acetyl group to the much longer heptadecoyl group were used to acylate chiral hydroxy functions in the pendant side chains of the polymer.

Surprisingly, it is also the most recently developed method to have wide usage, especially in consideration of the time since it was first reported to have been used. Bourquelot and Bridel demonstrated in 1913, the stereospecific synthesis of a range of alkyl glucosides from glucose and alcohol, using a dry emulsin catalyst, in good yield (80-85%)[71]. They also suggested that this approach could have a wide applicability with the employment of other enzymes.

1.10) Mode of Action of the Lipases

1.10.1) Overview

The catalytic action of an enzyme macromolecule is a complex operation not completely understood or defined. However, some sort of picture can usually be formed and basic

mechanisms found which provide a limited understanding of how a particular enzyme works. Firstly, it is necessary for the enzyme to be able to bind to the substrate. Every enzyme has a distinct structure which is able to bind to substrates in a highly specific way and thereby aiding in their transformation to yield the products[72]. It was previously thought that lipases contained a higher proportion of hydrophobic amino groups to hydrophilic groups, which enabled them to bind closely with hydrophobic materials. Later, however, this was discovered to be untrue and it is now known that lipases as a group are not especially hydrophobic[73]. The ability of the lipases to interact strongly with hydrophobic substrates is believed to be due to the presence of a lid which is activated at an interface.

The common catalytic feature of the lipases (except for the cutinase lipases) is the involvement of a lipid-water interface. Normally a hydrophobic lid covers the active site. During interfacial activation or substrate binding, the lid is displaced to reveal the active site[74]. This unique characteristic of the lipases gives them an inherent affinity for hydrophobic environments and makes them the most suited for use in organic media. The principles regarding interfacial enzyme catalysis are shown below[75].

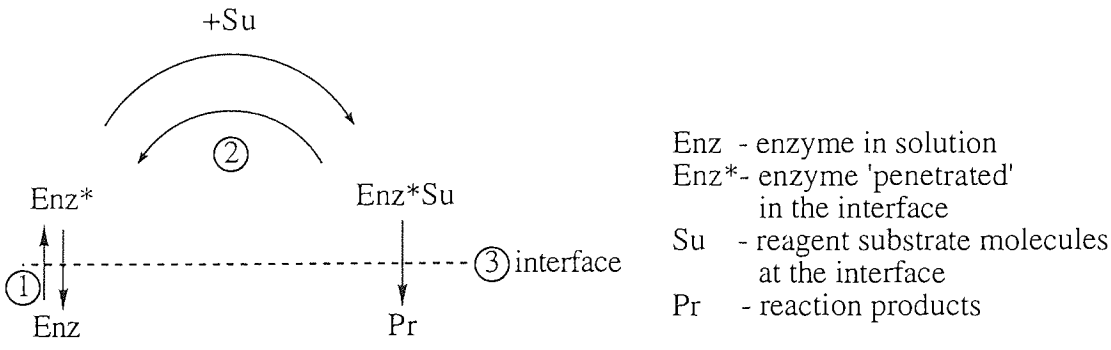


Figure 6. Lipase at the water/organic solvent interface

Steps

1) The reversible binding of the enzyme to the interface (penetration step) which causes it to assume its new conformation, **Enz***.

2) The reversible binding of the 'penetrated' enzyme with the substrate molecule to form **Enz*Su**.

3) The substrate is catalysed to form the products, with the regeneration of **Enz***.

It was postulated by Haldane (1930) and Pauling (1946, 1948) that the enzyme was favourable to the structure of the activated substrate i.e. the transition state of the reaction rather than the original substrate structure. Since that time this theory has been confirmed by X-ray diffraction studies. The transition state of the substrate is thought to make better contacts and interactions with the enzyme than the unactivated form, which in turn favours the formation of the transition state[76].

1.10.2) Mechanism of Action

Van Tilbeurgh *et al* made a more detailed exploration of enzyme activity[77]. They showed, through the use of X-ray crystallography, the action of lipases and of the lid, a surface helix, which covers the active site of the enzyme.

The lipases have in common a well hidden β-sheet with an active site in the centre consisting of the three amino acid residues (catalytic triad) Ser, His, and Asp or Glu. The serine residue is embedded in a tight turn at the top of the β-sheet between a β-strand and an α-helix. The histidine is in a similar arrangement with loops on one side of the serine and the oxyanion hole is formed on the other side[78]. The active site is hidden from the bulk solvent by a lid, or flap. The lid is made up of one or two helices, which on activation, rolls back onto the body of the enzyme molecule thus, both exposing the active site and increasing the hydrophobicity around it. It is thought that this mechanism

with the lid is activated by the lipid-water interface. Pancreatic lipase is different to the other lipases in that it requires a co-enzyme (procolipase) to aid in the activation process. This is due to the presence of bile salts from the intestine which prevent the lipase from binding to the lipid-water interface. The procolipase binds to the β-sheet of the lipase and so exposes the opposite hydrophobic site of the enzyme. This hydrophobic region helps in bringing the active site of the enzyme into close contact with the interface.

When the lid is opened and the enzyme becomes bound to the lipid-water interface, there is a change in the conformation of the enzyme. The lid contains α-helices in both the closed and open forms but they are formed by different residues.
The consequences of this change in the lid structure are:

(i) The substrate has access to the active site.

(ii) Another loop (β-5 loop) which usually leans against the lid when it is closed, changes its conformation to a more stable one when the lid is open. Normally, this β-5 loop is minimally associated with the bulk of the lipase. When the lid is open its conformation changes forming new polar interactions with amino acid residues both from the core of the enzyme and from the lid. This change in the β-5 loops conformation, although has no significant effect on the position and conformation of the catalytic triad, does induce a considerable change in the environment surrounding the active site. The serine in the active site becomes accessible to solvents and its action is assisted by the creation of an oxyanion hole. This hole is an electrophilic region which stabilises the negative charge of the tetrahedral intermediate generated during the nucleophilic attack of the serine oxygen on the carbonyl carbon of the reacting ester.

(iii) A considerable hydrophobic region is formed around the entrance to the active site. This hydrophobic 'plateau' is formed by a combination of the hydrophobic side

chains being exposed and the hydrophilic side chains being buried. This hydrophobic region surrounding the catalytic site plays a crucial role in binding the enzyme to the lipid-water interface.

(iv) In the case of pancreatic lipase, the lipase interacts strongly with the procolipase through interaction with the open lid. In the closed form the lids interaction with the main body of the enzyme is virtually all made up of non-polar interactions. In the open form, the amphipathic lid rolls back onto the core of the enzyme, opening out its hydrophobic face around the entrance to the active site. In this conformation the lid is stabilised by the formation of new hydrogen bonds and salt bridges. The open lid has a similar effect on the enzyme's catalytic site, as does the conformational change of the β-loop, in that it greatly alters the environment of the catalytic triad making it possible for catalysis to occur.

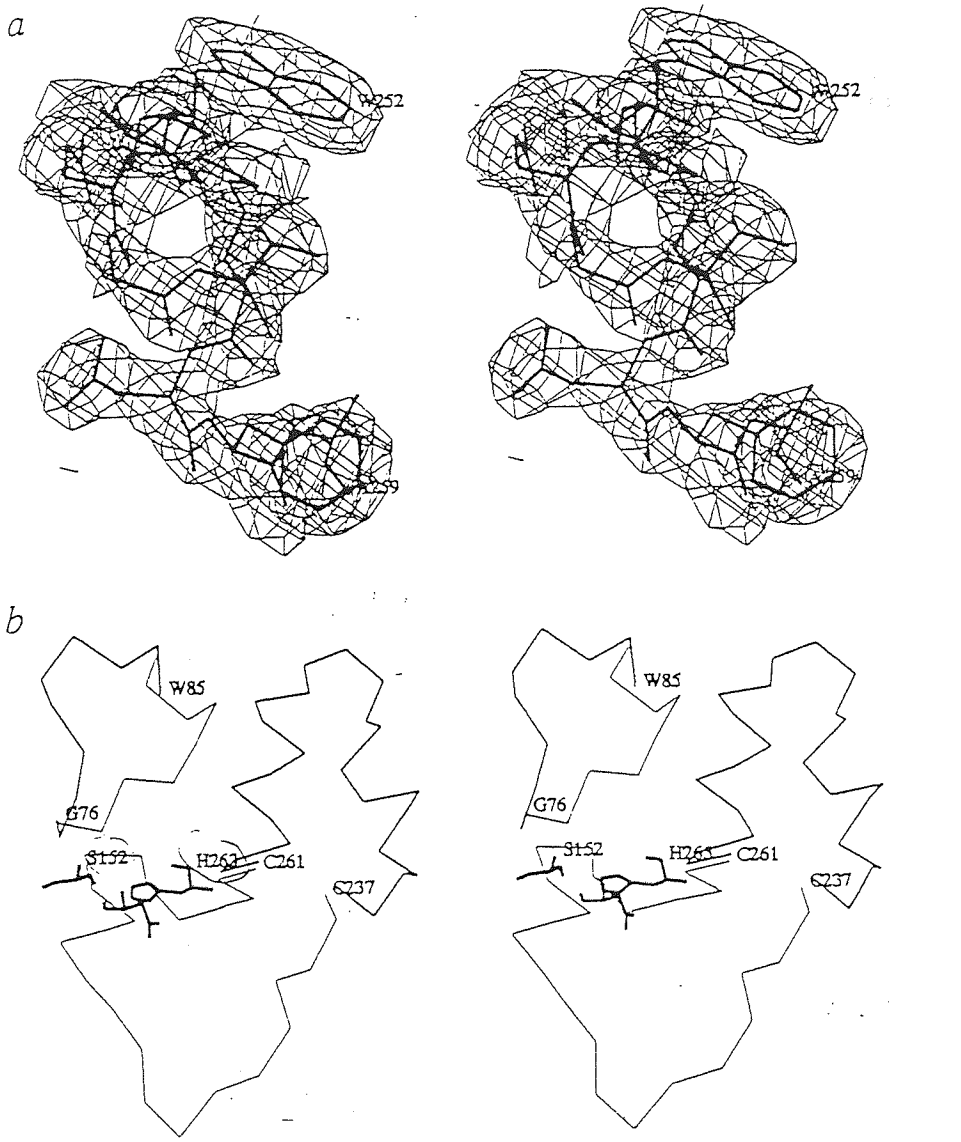
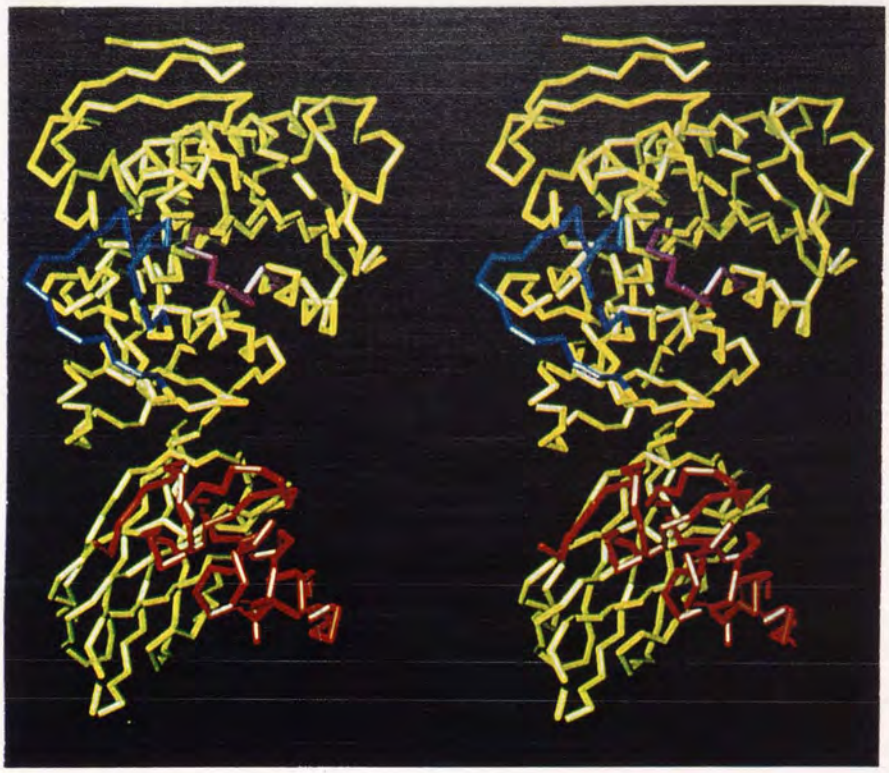


Figure 7. (**a**)-Final electron density map from the refined model of the open structure of the pancreatic lipase-procolipase complex. The main helix (residues 252-259) of the rearranged lid is represented. (**b**)-Detailed stereographic view of the conformational change of the β-5 loop (residues 76-85) and the lid (residues 237-261) (Cα atoms). The closed and open structures are represented by thin and thick lines, respectively. The catalytic triad residues are also shown: Ser 152, His 263 and Asp 176 (no label). The disulphide bridge (Cys 237-Cys 261) remains fixed and anchors the lid to the protein core.

a



b

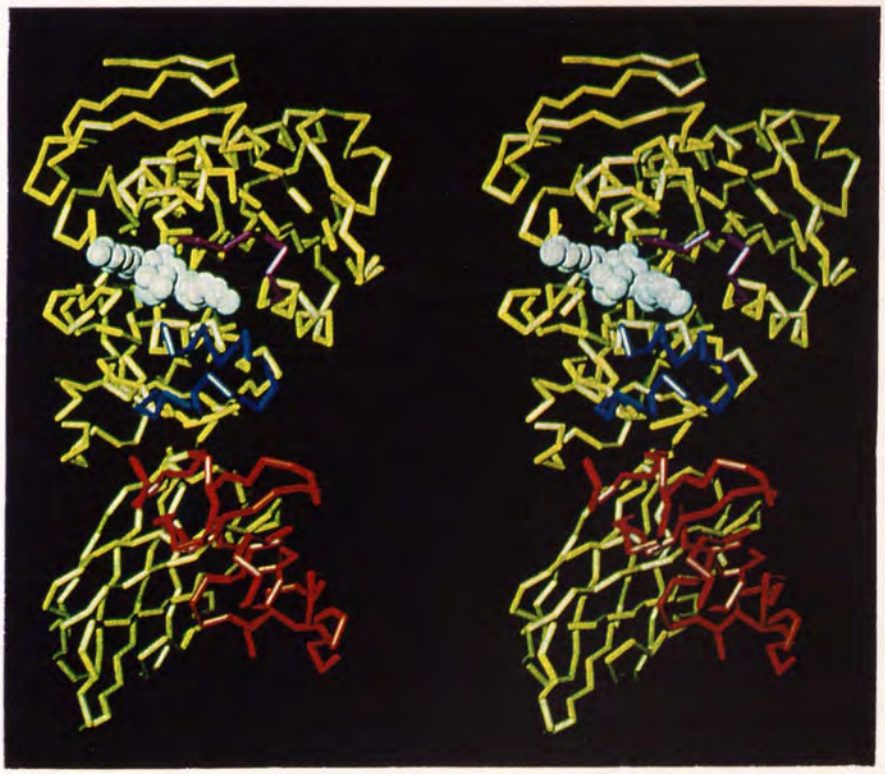


Figure 8. a & b

C

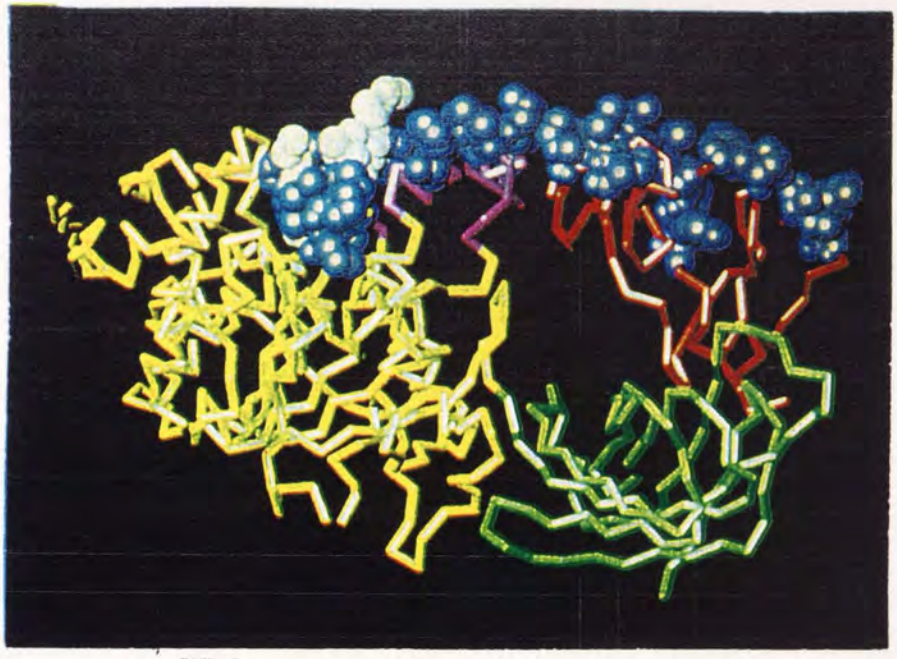


Figure 8. Stereo pictures of the comparison of the closed (**a**) and open (**b**) forms of the lipase-procolipase complex (Cα atoms). Pancreatic lipase is represented in yellow. Thge lid (residues 237-261) is shown in blue and the β5 loop (residues 76-85) in mauve; colipase is in red. The open form has also a bound phospholipid in the active site (white spheres). The picture shows how the active site, hidden under these two surface loops opens up like the petals of a flower. The conformational transitions in the lid and in the β5 loop are characterised by a profound change in secondary and tertiary structure.

(**c**)-Hypothetical lipid-binding surface of the lipase-procolipase complex. The lipase catalytic N-terminal domain is in yellow, the C-terminal domain in green and colipase in red. Hydrophobic residues are represented as blue spheres and the model of the bound phospholipid is in white. The change in conformation of the lid brings its own hydrophobic residues to the surface and uncovers many others around the active site.

1.11) Aims of the Project

The aims of the research project are:

1) To find a suitable system for the production of oligoesters/polyesters.

2) To determine the optimum conditions for enzyme-catalysed polyester production.

The starting point of this research work was to try to find any system in which polyesterification or even oligoesterification could be effected. Initially, the work was based on that done by Robinson *et al,* where the enzyme was dissolved in reverse micelles contained in a gel matrix[47]. Other systems were also investigated. These included immobilising the enzyme on various solid supports and also just using the enzyme as a dry powder to form a slurry. The two systems containing relatively large amounts of water, the biphasic enzyme system and the monophasic co-solvent system, were not investigated as these were not considered to be of worthwhile pursuit, for reasons mentioned earlier. Most of the work done in the second phase of the research was inspired by the findings of Wallace and Morrow[79].

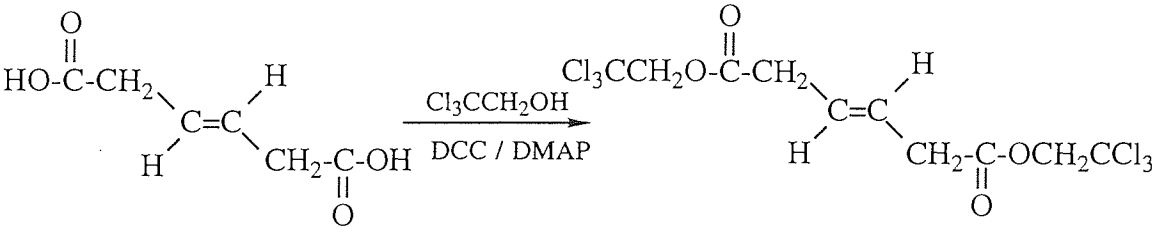
1.11.1) Wallace and Morrow Polymerisation

The work that was of particular interest and the starting point to the major part of this research project was the lipase-catalysed synthesis of an optically active, epoxy-substituted polyester.

In this work, the three-stage synthesis of a polyester having a number average molecular weight (Mn) of 5,300 daltons was demonstrated, starting from a commercially available dicarboxylic acid and diol.

1.11.1.1) First Stage

The fundamental strategy behind the polymerisation was in the first stage of the synthesis. This was the incorporation of a poor nucleophile or good leaving group into the diacid molecule so that subsequent displacement by the competing nucleophile, *viz* 1,4-butanediol, was a facile operation resulting in dimerisation and ultimately, polymerisation.

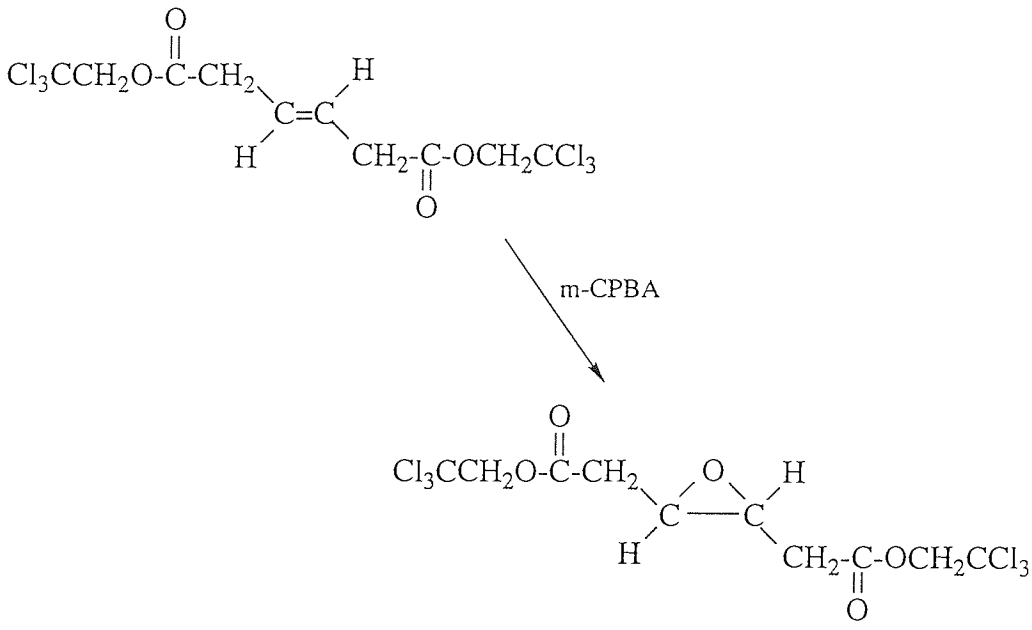


Scheme 1. Synthesis of bis (2,2,2-trichloroethyl) *trans*-3-hexenedioate (**2**)

The formation of bis(2,2,2-trichloroethyl) *trans*-3-hexenedioate was effected by the reaction of *trans*-3-hexendioic acid with 2,2,2-trichloroethanol, in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC)[80] and a catalytic amount of 4-(dimethylamino)pyridine (DMAP)[81,82].

1.11.1.2) Second Stage

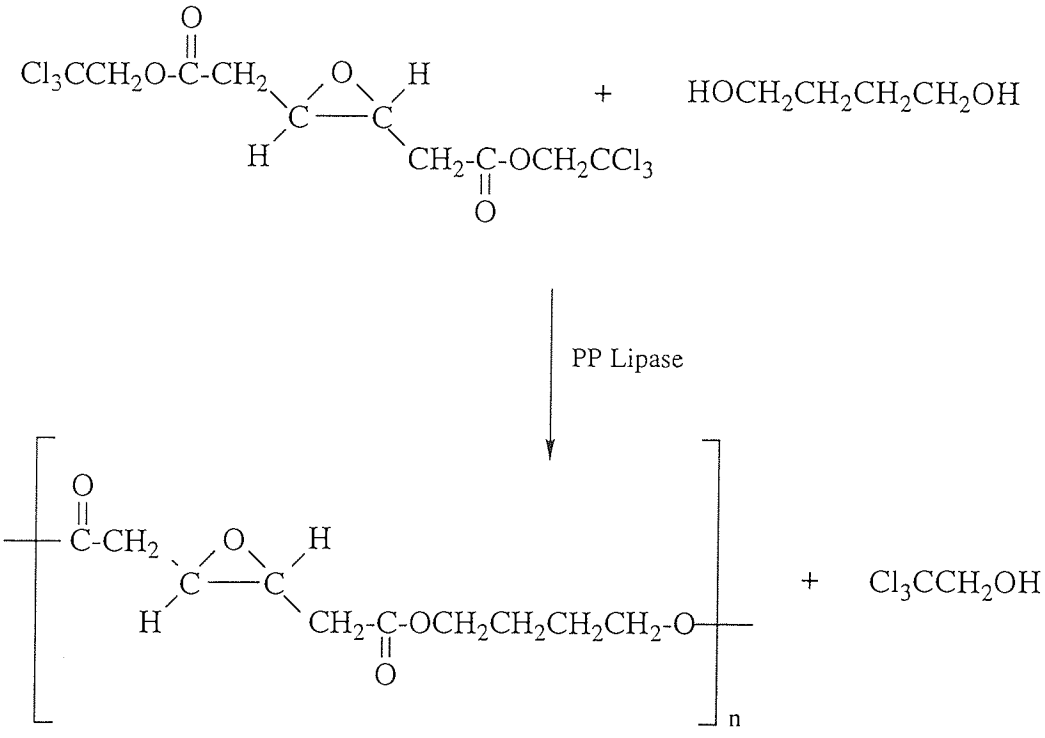
Bis(2,2,2-trichloroethyl) *trans*-3-hexendioate was epoxidised to bis(2,2,2-trichloroethyl) (±)-3,4-epoxyadipate using m-chloroperoxybenzoic acid (m-CPBA). Alternatively, the epoxidation step could also be carried out after the polymerisation step, but this resulted in lower yields.



Scheme 2. Synthesis of Bis(2,2,2-trichloroethyl) (±)-3,4-epoxyadipate (**1**)

1.11.1.3) Third Stage

The final stage of the sequence was the polymerisation step. This was carried out using the enzyme catalyst, porcine pancreatic lipase, as a dry powder slurry in anhydrous diethyl ether. The resulting polyester was found by nuclear magnetic resonance (NMR) end-group analysis, to have a Mn of 5,300 daltons.



Scheme 3. Polymerisation of Bis(2,2,2-trichloroethyl) (±)-3,4-epoxyadipate

Morrow (*et al*)[83], in a later publication reported making higher molecular weight polymers, with the largest one being synthesised in the high boiling solvent, 1,2-dimethoxybenzene and having a Mn of 31,000 Da. A more detailed account of this work is discussed in chapter 3.

Wallace and Morrow repeated this work using the diesters bis(2,2,2-trichloroethyl) glutarate and bis(2,2,2-trichloroethyl) adipate, which in some instances gave higher molecular weights than the bis(2,2,2-trichloroethyl) (±)-3,4-epoxyadipate. In addition to using alternative diesters, longer diols such as 1,6-hexanediol, 1,10-decanediol and 1,4-cyclohexanedimethanol were used. The systems which gave the highest molecular weight values are shown in the table below. The Mn values were determined by NMR and Mw values, by GPC.

Diester	Diol	Reaction Time/hours	Mn (NMR)	Mw (GPC)
Glutarate	Butanediol	122	8,200	11,800
Glutarate	Cyclohexane-dimethanol	74	2,000	14,900
Adipate	Butanediol	120	4,900	5,200
Adipate	Hexanediol	120	6,500	-

Table 1. Results of some polymerisation reactions carried out by Wallace & Morrow

Since the Mn and Mw values were calculated using different techniques, the resulting polydispersity values cannot be regarded with confidence, as being accurate. In fact, for many of the experiments carried out during the course of this research project, extremely high Mw values were obtained in conjunction with low Mn values, both values having been determined by GPC. Using Mn and Mw values from two different sources can give misleading information about the nature and quality of the polymer in question. Nevertheless, the results obtained by Wallace and Morrow using the glutarate and adipate diesters appeared promising, and hence were the basis for the work carried out in the latter stages of the project.

2) Introductory Work

2.1) Introduction

The work reported here was based on the original investigations of Wallace and Morrow[79]. The initial approach taken was to attempt to repeat their work. Wallace and Morrow had considered the following factors in their choice of substrate for the synthesis of chiral polymers:

i) The synthesis of the substrate should produce no meso isomers. If a mesoform, a molecule having a mirror plane of symmetry within the same molecule was involved in the polymerisation stage, this would cause termination of the polymerisation, assuming of course that the enzyme catalyst was acting as expected, in an enantioselective way. A mesoform contains both R and S configurations. If, for instance, the enzyme selectively catalysed the R configuration only, then the growing polyester chain would become end-capped with the S configuration ester groups and the polymerisation reaction would effectively be terminated.

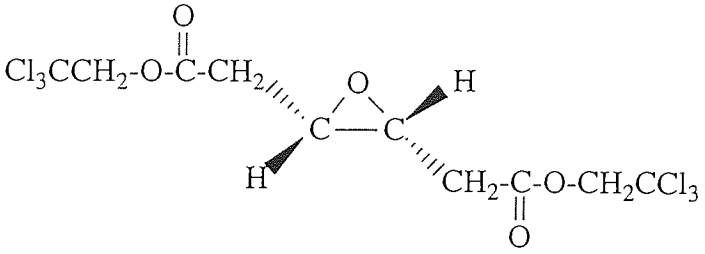
ii) The chirality should be present in the diacid rather than in the diol. If a chiral diol, was employed it would imply branching of the diol and would therefore, not be as reactive, thus resulting in a slower transesterification step.

iii) To make full use of the mild reaction conditions associated with enzyme catalysis, a substrate with a 'sensitive' functional group should be used to enable reactive functionality to be incorporated into the resulting polyester; in this case, an epoxide group.

iv) A good leaving group would also serve to activate the substrate towards polymerisation.

v) The substrate should be easily and inexpensively synthesised from a commercially available precursor.

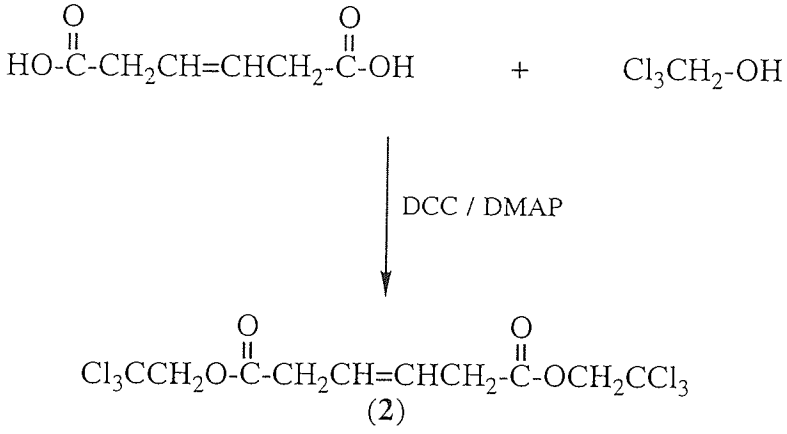
The substrate chosen which satisfied all of the above criteria was bis(2,2,2-trichloroethyl) (±)-3,4-epoxyadipate:



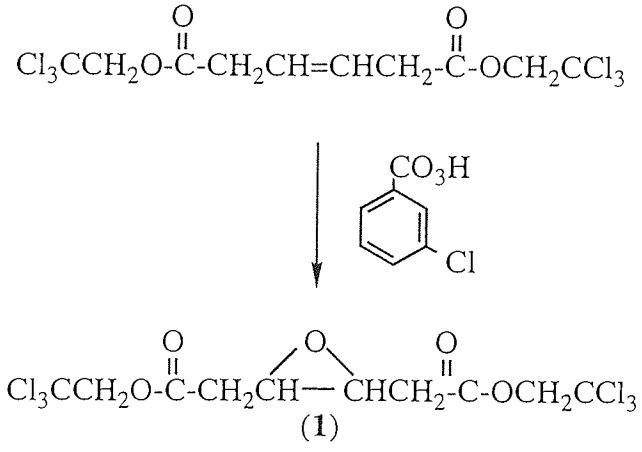
Bis(2,2,2-trichloroethyl) (±)-3,4-epoxyadipate (**1**)

2.2) Synthesis of Bis (2,2,2-trichloroethyl) (±)-3,4-epoxyadipate (1)

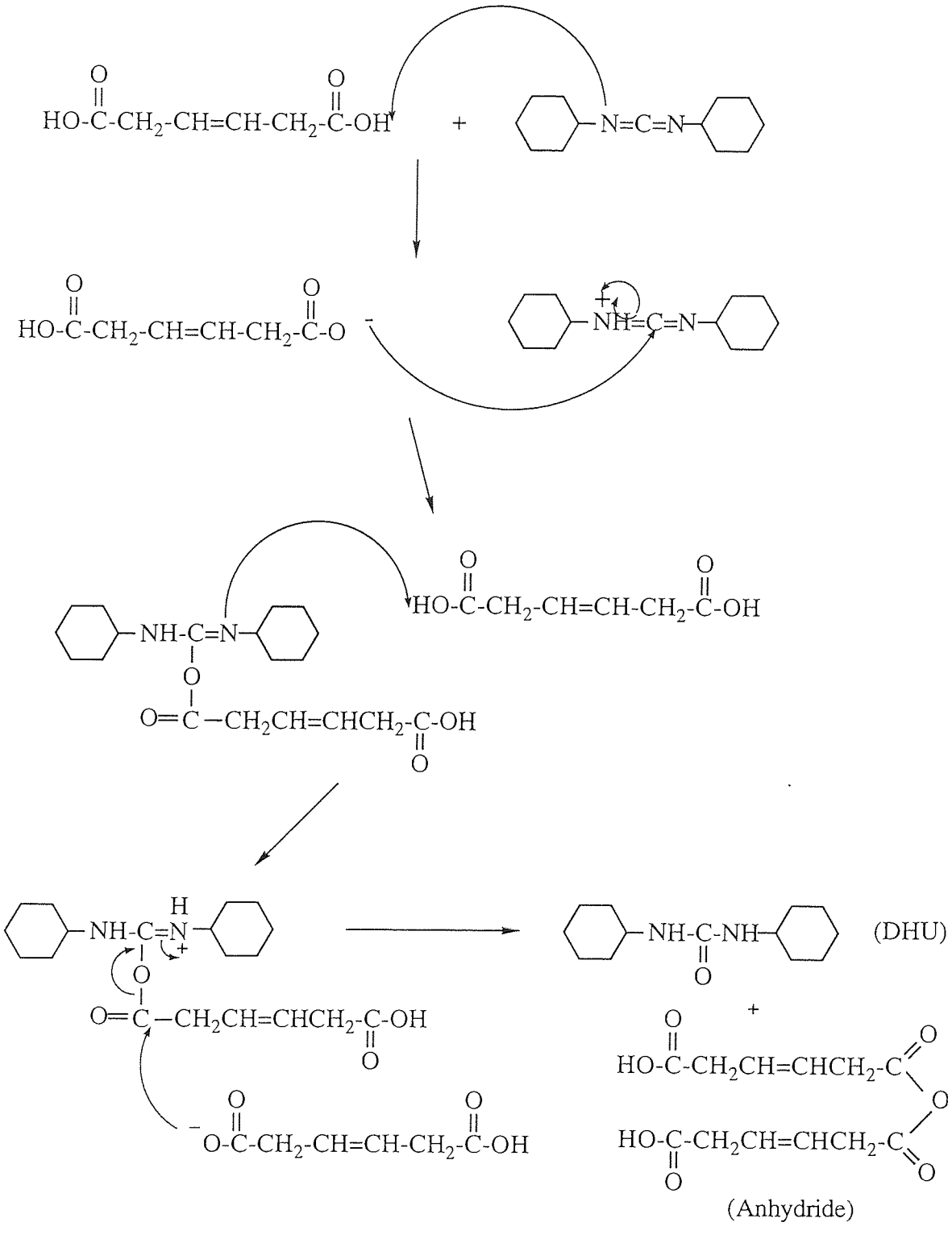
The target diester was synthesised in a two-stage process, via the bis (2,2,2-trichloroethyl) *trans*-3-hexenedioate, (**2**), with a quoted overall yield of 86%. The esterification and epoxidation stages are shown in schemes 4 and 5, respectively. The proposed mechanism for the esterification reaction, showing the action of the dehydrating agent, DCC and the catalyst, DMAP, is shown in scheme 6. After the esterification stage,the next stage of the synthesis was either the epoxidation step or else the alkene substrate could be polymerised as it was and then made to undergo epoxidation in the final stage of the synthesis. However, the overall yield quoted for this latter approach is far lower than if the substrate is epoxidised prior to being polymerised. Consequently, it was decided to adopt the former approach.



Scheme 4. Synthesis of bis (2,2,2-trichloroethyl) *trans*-3-hexenedioate (**2**)

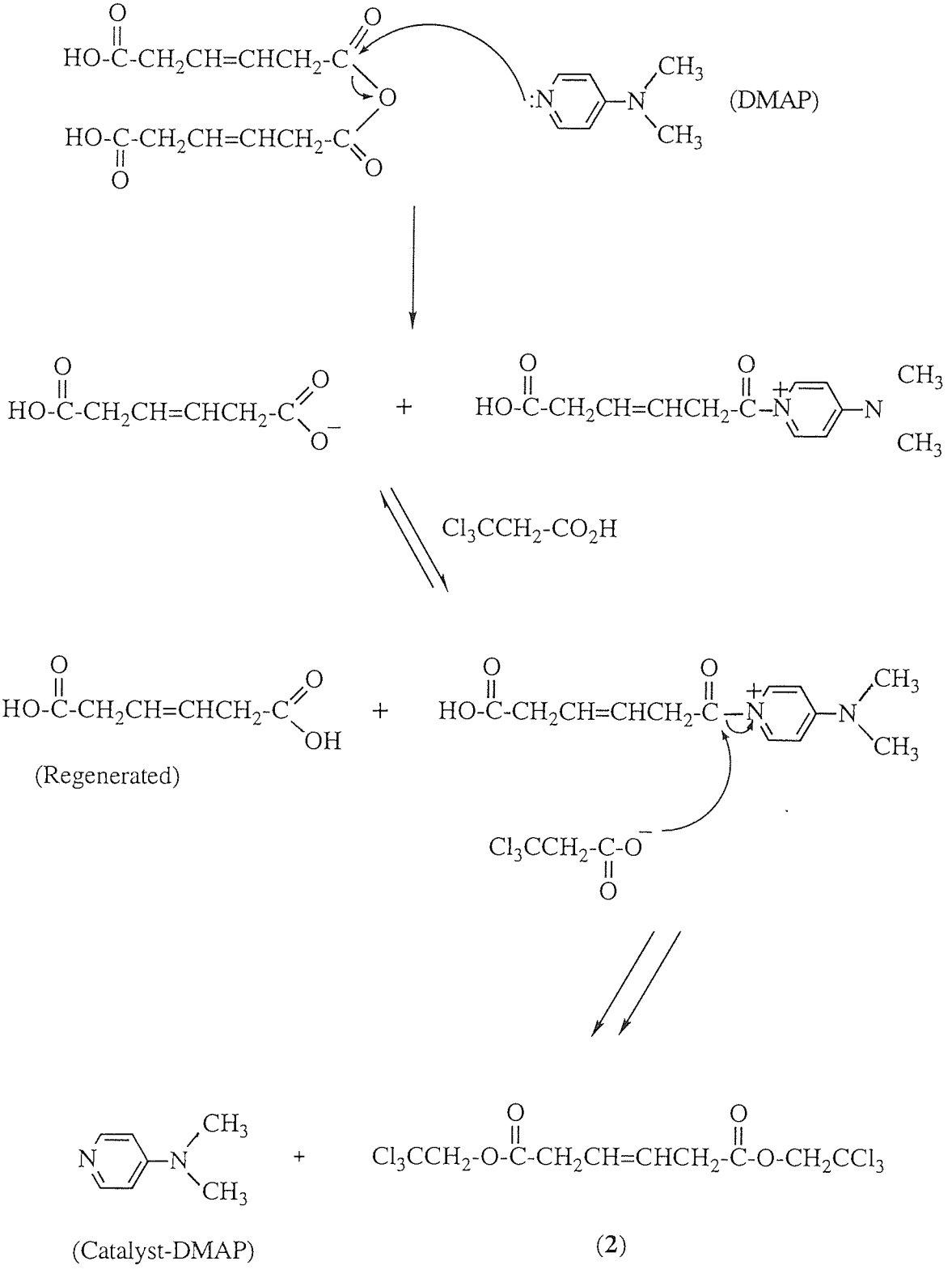


Scheme 5. Synthesis of bis (2,2,2-trichloroethyl) *t*(±)-3,4-epoxyadipate (**1**)



Scheme 6. Esterification mechanism

Then:



Scheme 6. (continued)

2.3) Polymerisation of Bis(2,2,2-trichloroethyl) (±) -3,4-epoxy adipate (1) with Butane-1,4-diol

After recrystallisation, twice, from 20:1 hexane / dichloromethane, the ester was dried in vacuo at 40°C for 48 hours. Porcine pancreatic lipase (Sigma, L 3126, activity = 35-70 units per mg of protein) was also dried prior to use, in vacuo over phosphorus pentoxide for 3 days. There are two slightly different methods used for the polymerisation. Both methods started off the same in that the ester. Bis(2,2,2-trichloroethyl) (±) -3,4-epoxy adipate was dissolved in anhydrous diethyl ether and 0.5 molar equivalents of butane-1,4-diol were added. This is because it was assumed that only one enantiomer of the ester would be polymerised. The enzyme was added and the mixture stirred mechanically under a nitrogen atmosphere at ambient temperature.

2.3.1) Method A

After stirring for 3.5 days the enzyme was filtered off and washed with dichloromethane. The solvent from the filtrate and washings was removed by evaporation to yield a viscous oil. This oil was washed twice to remove any non-polymeric material, by stirring the oil with diethyl ether for 1 hour, then decanting off the ether. The yield was quoted as being 93%.

2.3.2) Method B

After stirring for 1.5 days, a phase separation was observed between the ether and the polymer / enzyme phase. The ether phase was consequently removed by pipette and a fresh sample of anhydrous ether added. The mixture was then stirred for an additional 4 hours to remove the unreacted enantiomer of the ester and any 2,2,2-trichloroethanol

produced during the reaction. Again the ether layer was removed and this time was replaced with a 3:1 mixture of dichloromethane / ether. The viscous polymer dissolved in this solvent mixture and the resulting slurry was stirred at ambient temperature for a further 3.5 days. The polymer product was worked up as described for method A. A 96% yield of product was obtained.

2.4) Molecular Weight Determination

The method of molecular weight determination employed by Wallace and Morrow, was end group analysis, a method using mathematical ratios calculated from the nuclear magnetic resonance (N.M.R.) spectra[84]. Here, the area represented by the polymeric methylene groups was compared with the area represented by the unreacted end groups in order to establish the degree of polymerisation and hence the molecular weight of the polymer. A more detailed explanation of the molecular weight determination methods employed is given in chapter 4.

2.5) Repeat of the Wallace and Morrow Work

2.5.1) Synthesis of Bis(2,2,2-trichloroethyl)-*trans*-3-hexenedioate (2)

The reaction was carried out as described in the Materials and Methods chapter, (section 6.3.2.1, p169) using the apparatus shown in figure 9. Extremely thorough washing was essential in the product isolation stages to remove all traces of the by-product formed, N,N'-dicyclohexylurea (DHU). The presence of even quite small amounts of DHU caused significant problems when trying to purify the ester, as it inhibits the ester from crystallising out of solution.

2.5.2) Synthesis of Bis(2,2,2-trichloroethyl) (±)-3,4-epoxyadipate (1)

Bis(2,2,2-trichloroethyl)-*trans*-3-hexenedioate (**2**) was epoxidised as described in the Materials and Methods chapter, (section 6.3.2.2, p67).

2.5.3) Polymerisation of Bis (2,2,2-trichloroethyl) *trans*-3-hexenedioate

Several attempts were made to polymerise the ester (**2**) before epoxidation, using butane-1,4-diol. The solvent system employed was that used by Wallace and Morrow *viz* 5:1 anhydrous diisopropyl ether / THF. The quoted yield of polymer was 14%, although no value was given for the actual molecular weight. Nevertheless, it was decided to carry out the polymerisation so that at least, a comparison could be made between the proposed polymerisation of the epoxidised ester (**1**) and the polymerisation of the alkene ester. All of the attempts to polymerise the alkene ester (**2**) proved unsuccessful. Other solvents were also tried, these being laboratory grade 'wet' diethyl ether and 'wet' CH_2Cl_2. The solvents being 'wet' meant that they had not been dried prior to being used, but were used as supplied by the manufacturers. Different reactions times were also investigated to eliminate the possibility that the polymers were not in fact, being synthesised and then subsequently degraded (due to the long reaction times), before they were analysed. This situation could quite likely have occurred, since the reaction is a reversible one and with the presence of the enzyme, the back reaction (or polymer degradation) could also be catalysed.

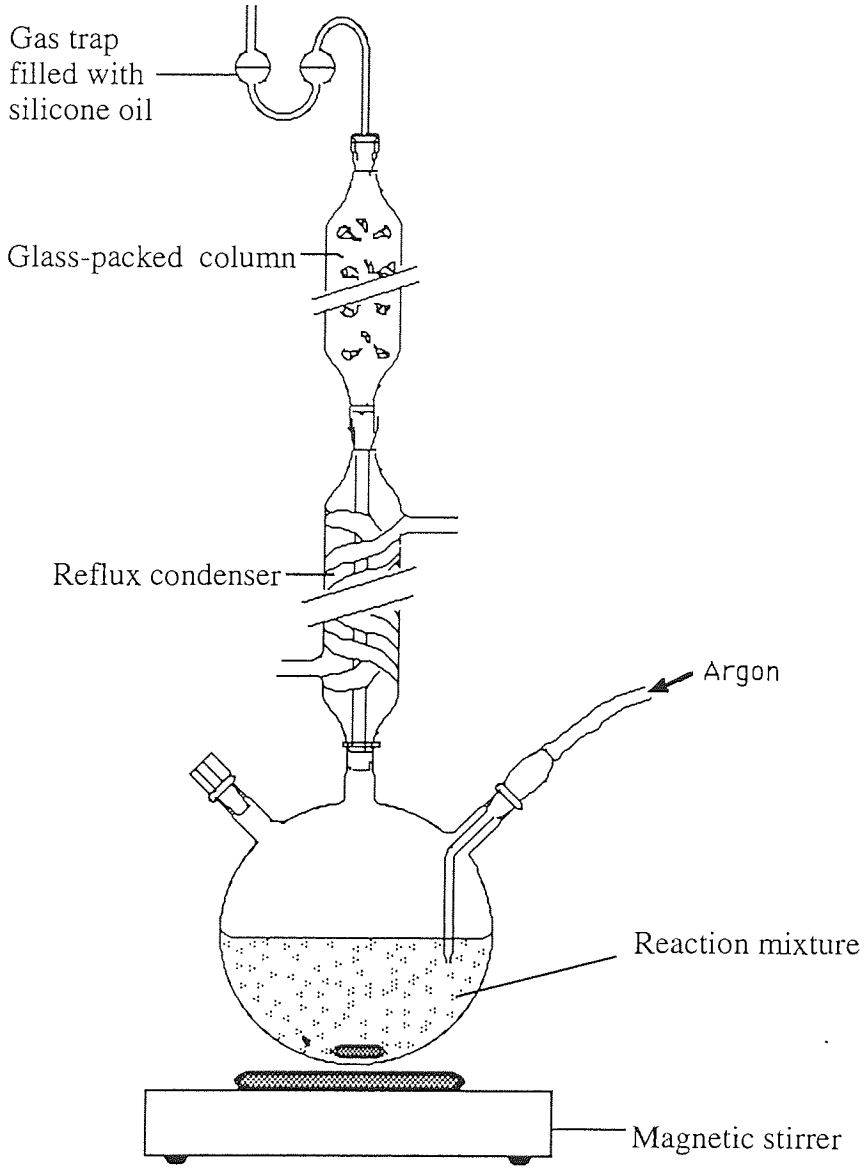


Figure 9. Apparatus for the esterification of dicarboxylic acids

2.5.4) Polymerisation of Bis(2,2,2-trichloroethyl) (±) -3,4-epoxy adipate

Since the alkene ester polymerisation reactions did not appear promising, the epoxidation was carried out as according to the literature procedure and the resulting ester, bis(2,2,2-trichloroethyl) (±) -3,4-epoxy adipate used for subsequent experiments. Various attempts were made to repeat the procedure carried out by Wallace and Morrow for the

polymerisation of bis(2,2,2-trichloroethyl) (±) -3,4-epoxy adipate (**1**). However, since these attempts failed, it was decided to make slight variations to the reaction conditions in an attempt to effect some sort of polymerisation.

2.5.5) Preliminary Variations on the Wallace & Morrow Polymerisation

Although the Wallace and Morrow method of polymerisation was repeated several times, all attempts to polymerise the ester (**1**) using the literature-stated conditions proved unsuccessful. Consequently, slight variations were made to the reaction conditions.

A series of experiments were carried out using different reaction times. Experiment 1, (**E-1**) was carried out using a reaction time of 1 day. Three other reactions, **E-2**, **E-3** and **E-4** were carried out using reaction times of 2, 5 and 6 days respectively. All four of the reactions were carried out using similar reaction conditions and substrate quantities as used by Wallace and Morrow.

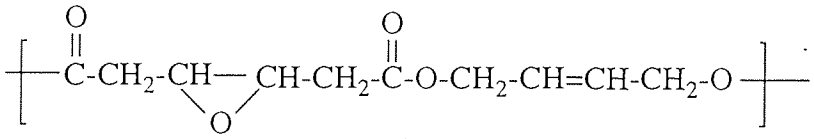
No obvious trend emerged from the time study experiments, **E-1** - **E-4**, inclusive. However, this result in itself indicated that further time study investigations were needed.

Expt No.	Time/days	Mn	Mw
E-1	1	1500	2400
E-2	2	900	1600
E-3	5	2200	3000
E-4	6	1200	2600

Table 2. Effect of time on the polytransesterification of ester (**1**) with butane-1,4-diol

The other three components of the reaction, the solvent, diol and enzyme were also varied. Since a relatively rigid ester (**1**) was being employed, it was decided to investigate also, a rigid diol. Hence, for experiment **E-5**, 2-Butene-1,4-diol was substituted for butane-1,4-diol. For experiment **E-6**, an immobilised lipase preparation from *Mucor Miehei* (a yeast lipase)[85], was used as an alternative catalyst to porcine pancreatic lipase. Experiment **E-7** was carried out using diethyl ether which had not been dried prior to use. Finally for experiment **E-8**, after 3 days of reaction (as carried out by Wallace and Morrow), no polymeric product was observed. The reaction mixture was worked up and then a fresh quantity of enzyme and a new solvent, dichloromethane, was added.

The results for the 2-butene-1,4-diol experiment (**E-5**) were not very promising. The products showed the presence of low oligomeric material with a Mn value of 1,600 and Mw = 2,000. The molecular weight value for the dimer or polymer repeat unit was calculated using the structure:



(**3**)

Repeat Unit Formula - $C_{10}H_{12}O_5 = 212$

and found to be 212 Da. This enabled the degree of polymerisation to be calculated as being approximately 8 dimer units long. For molecular weight calculation details see section 4.2.

For experiments **E-7** and **E-6** using 'wet' ether and an immobilised catalyst respectively, there was no apparent reaction.

Although the Wallace and Morrow synthesis was repeated several times, all of the attempts to polymerise the ester using the literature-stated conditions proved unsuccessful. It was only after the reaction was continued further, using fresh enzyme in

unsuccessful. It was only after the reaction was continued further, using fresh enzyme in a new solvent, CH_2Cl_2, that a polymer of extremely encouraging molecular weight was obtained (**E-8**). The resulting polyester, was shown by GPC to have a number average molecular weight (Mn) of 5,200 and weight average molecular weight (Mw) of 7,900.

Since it had not been possible to repeat the Wallace and Morrow work, it was decided to modify the conditions in the hope of obtaining polymeric products. The effects of changing the following variables on the polytransesterification of ester (**1**) was investigated.

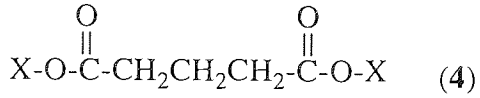
i) Ester

ii) Diol

iii) Enzyme

2.6) The Ester

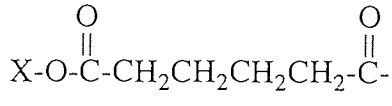
In a second publication by Morrow[86], it was reported that the number of carbons in the diester monomer chain was an important factor in the determination of the monomer's reactivity. It was noted that esters with an odd number of carbons in the chain were more reactive monomers, than those possessing an even number of carbons and that the optimum chain length was 5 carbons *viz* a glutarate diester:



(**4**)

(where X is a good leaving group)

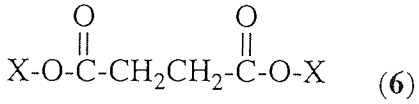
It was decided to investigate this observation by carrying out a series of parallel experiments using the glutarate ester shown above and the longer 6 carbon-containing (even number), adipate ester:



(5)

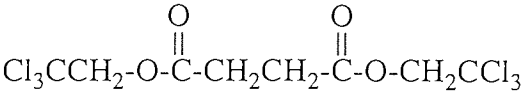
The two starting esters, glutarate and adipate were used in a series of experiments in an attempt to establish some basic reaction conditions which would provide a better understanding of the polyesterification process.

It was decided also, to investigate the reactivity of the shorter (even carbon number) succinic acid ester:

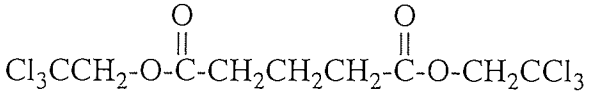


(6)

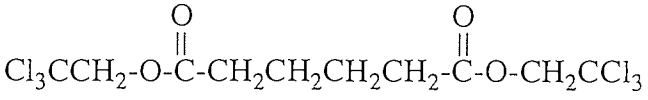
Before the experiments could be carried out the carboxylic acids chosen for the subsequent polymerisations (succinic, glutaric and adipic acids), were esterified with 2,2,2-trichloroethanol to give the corresponding trichloroethyl activated esters. These esters were then reacted under various reaction conditions. The reaction conditions quoted by Wallace and Morrow (Method A) were used as the 'standard' conditions[79]. Subsequently, various parameters were changed and the resulting effects upon polymerisation monitored.



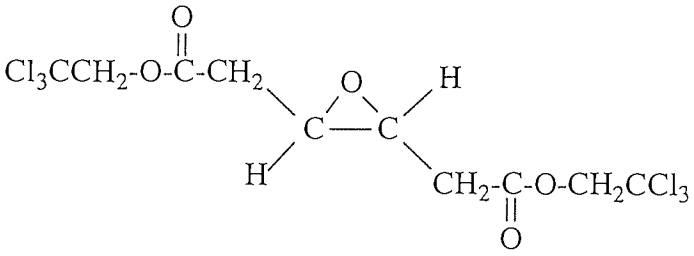
Bis (2,2,2-trichloroethyl) succinate (**7**)



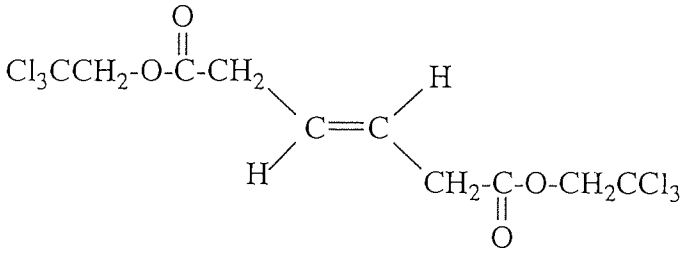
Bis (2,2,2-trichloroethyl) glutarate (**8**)



Bis (2,2,2-trichloroethyl) adipate (**9**)



Bis (2,2,2-trichloroethyl) (±) 3,4-epoxyadipate (**1**)



Bis (2,2,2-trichloroethyl) 1,6-hexene-3-dioate (**2**)

The approach that was taken was to consider the various parameters of the polymerisation reaction in order to establish a basic set of reaction conditions known to result in polymerisation or indeed oligomerisation. These parameters included the reaction

solvent, the diol, the ester or carboxylic acid, the enzyme and also a miscellany of other factors which transpired to be of significance during the course of the research work.

2.7) The Diol

For the majority of the work reported in the literature, the diol of choice was butane-1,4-diol. It was reported (Morrow) that increasing the diol chain length up to 6 carbons promoted polymerisation[86]. Beyond this, there was no improvement in the reaction. For comparison purposes the ideal diol for reaction was butane-1,4-diol and this was therefore used for most of the experiments. However, to determine whether butane-1,4-diol was the most suitable diol for use in our systems, other diols were also investigated.

2.7.1) Glycerol

Polymerisation of the succinate ester (7) with glycerol was attempted. The reaction solvent chosen was anhydrous THF. The glycerol was very viscous with a sticky nature which caused the enzyme to adhere quite strongly to the base of the reaction vessel.

No apparent reaction was observed by NMR or GPC.

2.7.2) Hexane-1,6-diol

The reactions of hexane-1,6-diol were compared directly with those using butane-1,4-diol. The two diols were each reacted with both the succinate and adipate esters in anhydrous diethyl ether, making a total of four experiments:

Experiment	Monomers	Mn
E-9	Adipate / Butane-1,4-diol	300
E-10	Adipate / Hexane-1,6-diol	250
E-11	Succinate / Butane-1,4-diol	300
E-12	Succinate / Hexane-1,6-diol	250

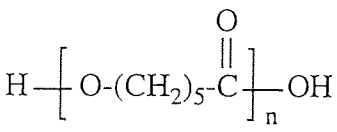
Table 3. The effect of varying ester and siol chain length in the polytransesterification reactions E-9 - E-12

GPC analysis showed that no significant reaction had occured with either the hexanediol or the butanediol reactions.

2.7.3) Reactions With Polyethylene Glycols

An alternative approach to synthesising polymers was to start off with quite large molecules. Various polyethylene gylcols (PEGs) were chosen for this work. The properties of any polymer resulting from such a synthesis were taken into consideration. It was considered likely that a polymer of this kind would be of limited biodegradability due to the large proportion of relatively unreactive hydrocarbon methylene groups. Nevertheless, the experiments were proceeded with, as the work was still in the explorative stages.

The succinate (**7**) and adipate esters (**9**) were used for these experiments, this time in anhydrous CH_2Cl_2, using PPL. The PEGs used were PEG 200 and PEG 1000. As well as these PEGs, alternative macromolecules with different end groups were investigated. These included polycaprolactone (PCL) diol 1250 and PCL triol 300:



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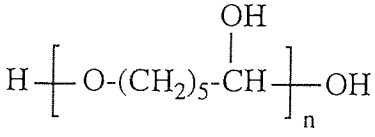


(11)

The reactions were as carried out as shown in Table 4.

Experiment	Ester	PEG
E-13	Adipate	PEG 200
E-14	Succinate	PEG 200
E-15	Adipate	PEG 1000
E-16	Adipate	PCL diol 1250
E-17	Adipate	PCL triol 300
E-18	Succinate	PEG 1000
E-19	Succinate	PCL diol 1250
E-20	Succinate	PCL triol 300

Table 4. The reactions of succinate (**7**) and adipate (**9**) with PEGs and other macromolecules (E-13 - E-20)

The reactions proved unsuccessful.

2.8) The Enzyme

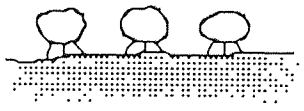
Yeast lipase (*Candida cylindracea*) and porcine pancreatic lipase are known to catalyse vigorously, solvolysis type reactions in organic media, in a highly stereospecific way. The yeast lipase is the most suitable for esterification reactions and for transesterification reactions, porcine pancreatic lipase is the enzyme of choice[57].

The range of activities that are commercially available for these enzymes is very large. For highly active enzyme samples where the activity could be several thousands of units/mg protein, theoretically, only a very small volume of enzyme would be required to bring about the desired transformation. However, it was felt that for interfacial reactions the greater bulk or volume would be an advantage. Hence, it was decided to use an enzyme with mid-range activity.

2.8.1) Enzyme Immobilisation

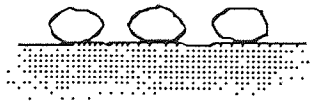
There have been a number of reports which establish that enzymes are stabilised by immobilisation on solid supports (Introduction-4). There are a number of different types of solid support[87]:

(i) Enzymes covalently bound to a solid support:



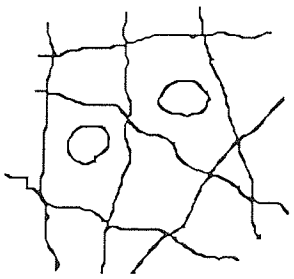
Examples of these supports include porous glass beads, ceramics, charcoal, cellulose and synthetic polymers. The support is first activated before the enzyme is added. The enzyme is usually bound through its amino or carboxyl groups.

(ii) Enzymes adsorbed on a solid surface:



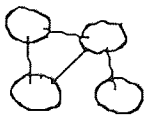
Most examples of this type are ion-exchange materials. Both the anion-exchange resin, Sephadex and the cation-exchange resin, CM-cellulose have been used. Their main attraction is the relative ease with which they complex with the enzyme.

(iii) Enzymes entrapped in polymeric gels:



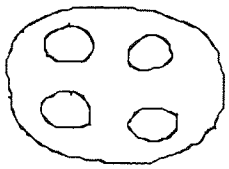
Here, the enzyme is first dissolved in solution. Then the gelling agent is introduced so that the enzyme becomes entrapped within the matrix of the gel. Examples of gels include calcium alginate and gelatin.

(iv) Enzymes cross-linked with bifunctional reagents:



These are cross-linking agents which usually, as for the covalent bonding supports, bind through either the carboxyl or the amino groups of the enzyme. Examples of bifunctional reagents used include glutaraldehyde, dimethyl adipimidate and aliphatic diamines. The cross-linking can either be intramolecular in nature or intermolecular. The enzyme can be linked either with itself or with a neighbouring enzyme molecule, respectively.

(v) Encapsulated Enzymes:



In this system, the enzymes are contained within a semi-permeable membrane. The membrane allows small molecules to pass, but does not allow the enzyme and other large macromolecules through. Examples include microcapsules (which are produced by, for example, interfacial polymerisation), liposomes and hollow fibres.

Three of these systems were investigated more closely. The simplest, experimentally, to carry out, *viz* the adsorption of the enzyme on a solid support, was chosen for further study, as was the enzyme entrapment method, using a polymeric gel and the encapsulation process, which is a variation of the entrapment method.

2.8.1.1) Enzyme Adsorption

The supports chosen for the adsorption were celite and glass beads. These two simple inert materials had been used previously by Cesti[88] and Reslow[89] respectively, to carry out enzyme catalysed esterifications.

The enzyme adsorption procedures described in the literature, all used the same basic technique. The enzyme and solid adsorbent were mixed together in a phosphate buffer solution. The mixture was then allowed to dry to yield the enzyme immobilised on the surface of the support (**E-21**). For the celite, the mixture was said to have been left on a watch glass at ambient temperature, with occasional mixing, until visibly dry. For the glass beads, the mixture was dried under reduced pressure for three hours.

Both celite and glass beads were mixed with a pH 7.2, phosphate buffer and then dried at ambient temperature on a watch glass. However, it was found that it took several weeks for the enzymes to dry. Experiments with the epoxyadipate (**1**), using the immobilised enzyme in both anhydrous and 'wet' ether, were compared to standard experiments using free enzyme. There was no apparent reaction with the immobilised systems.

The overall effect of immobilising an enzyme, is to increase its surface area. Hence, for comparison purposes, it was decided to simulate this effect of increased surface area, using free enzyme. An experiment using five times the usual amount of enzyme in both anhydrous and 'wet' ether was carried out. Again, there was no apparent reaction.

2.8.1.2) Enzyme Entrapment

This technique which involves entrapment of the enzyme in a polymeric gel was the very first approach that was adopted in this research work and was based on the findings of Rees *et al*[47]. To synthesise the polymeric gel, a concentrated solution of the enzyme was added to a solution of sodium *bis*-2-(ethylhexyl)sulphosuccinate (AOT) in heptane at 55°C. This mixture was then added to a solution of gelatin in water, also at 55°C, and shaken vigorously. The resulting mixture was then allowed to cool in air to yield a rigid gel. The gel offers some quite unique advantages. The enzyme is uniformly distributed throughout the gel, which can be cut into pieces and stored conveniently until required. Also, removal of the catalyst becomes a very simple operation, with one solid piece which can easily be isolated and even reused. However, the diversity of the reagents which can be made to undergo reaction using these gels appears to be severely limited.

The work carried out by Rees *et al*, used was a (*Chromobacterium viscosum*) lipase to catalyse simple monoesterification reactions between straight chain, aliphatic carboxylic acids and straight chain, primary, aliphatic alcohols in n-heptane.

The literature procedure was followed exactly as stated for the synthesis of the gel. However, great difficulty was encountered in the formation of a gel with uniform consistency. It was found that when trying to dissolve the gelatin in water, the best approach was to have the water, at 55°C, stirring very fast so that a strong vortex was created. The gelatin was then added rapidly, in one portion. Slow addition of the gelatin caused it to lump together with visibly dry pockets of gelatin contained in the aggregates. After the gel had been formed in this way, it became virtually impossible to try to obtain uniformity and subsequent mixing with the enzyme/hexane solution. The gel was also made at the lower temperature of 37°C to reduce the possibility of the enzyme being denatured by excessive heat.

Numerous attempts were made to esterify octanoic acid with ethanol or octanol. However, none of these simple esterifications were successful. Thereafter, the gel was made using a pH 7 phosphate buffer instead of water. A gel prepared by the latter procedure was used in the successful esterification of octanoic acid with ethanol (**E-22**).

2.8.1.3) Enzyme Encapsulation

This approach is a simpler variation of the previous, enzyme entrapment method. More accurately, the latter approach which been derived from this more straightforward way of trapping the enzyme in a reverse micelle.

Luthi and Luisi used AOT reverse micelles in isooctane to encapsulate the enzyme a-chymotrypsin[90]. The encapsulated enzyme was used to catalyse the formation of a peptide linkage. The enzyme was dissolved in water and the resulting solution held within the boundaries of the micelles as there is no propensity for migration beyond these confines. The substrates which are water soluble are able to enter into these micropools, undergo reaction and then migrate to the bulk organic phase.

For our experiments, the lipase was dissolved in pH 7 phosphate buffer. Then AOT surfactant dissolved in heptane was added to the enzyme solution and the whole mixture placed in an ultra-sound bath for several minutes to aid the formation of reverse micelles (E-23). This system was employed to catalyse two simple esterification reactions, *viz* octanoic acid with ethanol and octanol.

It was also used in the attempted polymerisation of three different hydroxyacids in diethyl ether. These included both a long and short aliphatic acid, 2-hydroxyhexanoic acid and 2-hydroxyisobutyric acid, as well as the aromatic *p*-hydroxybenzoic acid.

Both of the simple esterifications were successful. For the polymerisation of both aliphatic hydroxyacids there was no apparent reaction, but for *p*-hydroxy benzoic acid, it appeared as though polymer formation had occured. On further investigation however, it transpired that it was the surfactant, AOT which had undergone esterification.

The drying times for the enzyme absorptions were extremely long (usually several weeks). During this time the enzyme was left in an open container at ambient temperature. Even though the enzyme has a reasonable shelf-life at ambient temperatures, it was felt that this drying procedure could result in a loss of enzyme activity. It was thought that freeze-drying might be a better technique. For freeze-drying, the enzyme is frozen in solution and then the water or solvent is removed using a strong vacuum. This method was considered for future investigation. Since, however, the current adsorption method did not appear very promising, it was decided to pursue an alternative approach in the meantime.

It was thought that the rigidity of the gel might severely restrict the movement of substrates and also any possible products. This appeared highly likely, in light of the difficulty encountered when trying to wet the dry pockets of gelatin trapped within the gel aggregates. If the diffusion of water through the gel was being impeded, then it is

reasonable to assume that substrate and product movement would be restricted even more severely. It is even possible, that large substrate or product polymer diffusion would be prevented altogether.

The more simple enzyme encapsulation technique seemed to eradicate, to some extent, the restrictive conditions of the gel. This was shown by the fact that an esterification reaction was being catalysed in the *p*-hydoxybenzoic acid experiment, albeit of the wrong material. The AOT was esterified preferentially to the hydroxyacid since it was undergoing a **trans**esterification reaction. This occurs because an ester loses a poor nucleophile or 'good leaving group' instead of the strongly nucleophilic hydroxy group of a carboxylic acid. Nevertheless, it was decided, ultimately, that the whole concept of trapping or encapsulating the enzyme in small microenvironments would severely limit the extent of polymerisation. Hence, it seemed that what was needed was an alternative system where the enzyme was in a more open environment.

2.8.2) Enzyme Activation by Adsorption on Potassium Salts

It was shown by Dordick *et al,* that when an enzyme is lyophilised from a phosphate buffer containing simple potassium salts, the catalytic activity of the enzyme is greatly improved[52]. It was reported that in some cases, there was an almost 100 fold increase in reaction rate.

It is thought that there are two factors responsible for the increased stabilisation:

(i) It is said that the highly polar nature of the potassium salt serves to protect the enzyme from the denaturing effect of the solvent. The structure of the enzyme becomes more rigid as the salt content is increased and causes the enzyme to be

locked in the correct orientation for reaction. This effect is said to increase as the salt concentration is increased, to a maximum of about 98% salt content.

(ii) The salt helps to maintain the active structure of the enzyme during lyophilisation.

In order to determine which factor was predominating, a simple transesterification reaction was carried out in both liquid and gaseous media. The reactants in the gaseous medium, at 30°C, were said to have had a sufficient vapour pressure for them to be present in the gas phase. It was found that in the liquid medium, there was a 100-fold increase in the enzyme activity on going from a salt-free enzyme to one having been lyophilised with 95% (w/w) KCl. In the gaseous phase, however, the comparitive increase was only 10-fold. This result was, consequently, taken as an indication that effect (**i**) was the mechanism by which enzyme activity was being enhanced. If effect (**ii**) was in operation, i.e. enzyme activation was due to lyophilisation, then the increase in the gas phase would have been the same as for the increase in the liquid phase.

It is felt, however, that there is a flaw in this hypothesis. In the gas phase, it may be true that the reactants are also present as a gas, but the same is not true of the solid enzyme catalyst. In the liquid phase, both the reactants as well as the enzyme are able to move and interact freely. In the gas phase, however, the solid enzyme would be resting on the bottom of the reaction vessel, whilst the reactants move freely. This would mean that, in the gas phase, there would be reduced interaction between the reactants and the enzyme. Any potential increase in the activity of the enzyme could not fully be observed in these relatively restricted conditions.

Regardless of the precise mechanism for the increased activation, the substantial improvement in the enzymes activity, reported by Dordick *et al*, warranted further investigation.

2.8.2.1) Enzymes Stabilised with Potassium Salts

Three simple potassium salts (potassium acetate, chloride and nitrate) were added to porcine pancreatic lipase in a pH 7.2 phosphate buffer. The salts were added to the lipase in varying amounts from 25-100% (w/w). The buffer solution was added and the different enzyme/salt mixtures lyophilised for several weeks until they were visibly dry. The lyophilising process involved freezing the samples and then applying a high vacuum to them whilst they thawed. This process was repeated several times a day for numerous weeks. It was found that the enzyme samples took a considerably lengthy time to be dry enough to be used in the polyesterification reactions. Additionally, it was noted that the greater the salt content in an enzyme sample, the shorter time it took for the sample to dry. The drying time differed also for each salt used. The potassium nitrate was the fastest to dry and the potassium acetate, the slowest. Evidently, an increase in polarity has a strong influence on the affinity the enzyme has for water. An explanation for this may be that the polar groups of the enzyme normally have a strong affinity for the water, but when a salt is added, to some extent, they are able to bind with the enzyme in place of the water. Thus, the enzymes polar groups are 'satiated' and so are not as tightly bound to the water as a free enzyme.

The 12 enzyme/salt samples were used in the same way as a normal enzyme sample in the polyesterification reaction of the glutarate ester (**8**) and butane-1,4-diol in anhydrous diethyl ether (**E-24 - E-35**).

The results showed no evidence, by GPC analysis, of polymers being formed. It was felt that the reason for this was that the enzyme samples were not dry enough. These experiments were the last set to be carried out of the research work and regretably there was not enough time for them to be repeated using drier conditions.

2.8.3) Other Enzymes Used

So far this report has only discussed reactions carried out using porcine pancreatic lipase. This enzyme is used for transesterification reactions. However, for esterifications, the best enzyme is *Candida cylidracea*. During the early stages of the project work, esterifications using carboxylic acids were investigated.

2.8.3.1) Roberts Apparatus

Roberts *et al*, have reported the highest molecular weight polyesters made from a dicarboxylic acid and diol, to date[85]. The main advantages of esterifications over transesterifications, making them more desirable for large-scale industrial processes, are that they are relatively inexpensive and give less toxic products. For example, for a halogeno end-group ester the halogen-containing alcohol produced as a by-product is toxic and hence causes disposal problems. Also, activated esters are not usually commercially available and are generally more costly than the corresponding carboxylic acid.

The system employed by Roberts produced a polyester with a Mn value of 4,172 and Mw of 4,645. This was made using adipic acid and 1,4-butanediol in diisopropyl ether. One very interesting aspect of the reaction was the use of a two chambered reaction vessel. The main problems associated with esterification reactions is production of water as a by-product, which inhibits the polymerisation reaction due to the competing hydrolysis reaction. This problem was overcome, to some extent, by using a two chambered pot; one containing the enzyme and the other containing molecular sieves. The two chambers were separated by a grating through which both the reactants and products could pass. This set-up allowed the molecular sieves and hence the reaction mixture to be agitated, without damage to the enzyme. It was reported that porcine

pancreatic lipase worked well but was prone to mechanical damage and hence, deactivation. Consequently, a *Mucor miehei* lipase immobilised on an anion exchange resin was preferred.

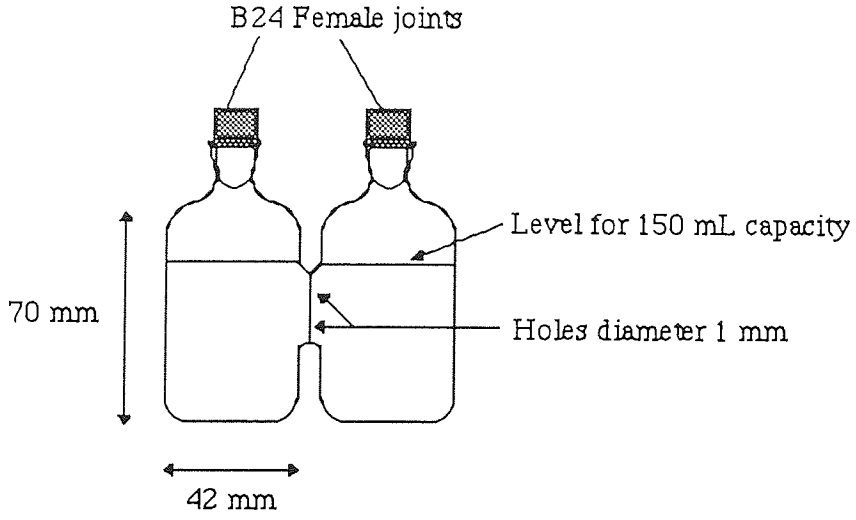


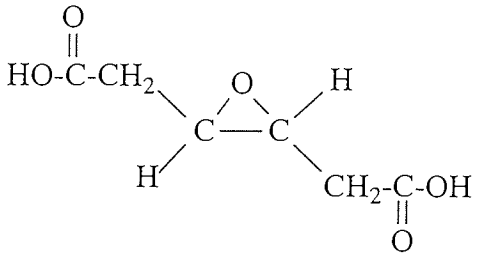
Figure 10. Horizontal two-chamber vessel

Another interesting and unconvential method was employed by Roberts during the extraction process. After 3 days of stirring at ~40°C in anhydrous diisopropyl ether, the reaction mixture was filtered and washed with THF to remove any unreacted reactants and low molecular weight oligomers. The residue was dissolved in ethyl acetate, and then washed with water to remove all the lower molecular weight material and dried. The resulting crystalline material was put on again for further reaction, using fresh samples of enzyme, solvent and molecular sieves and left to stir in a nitrogen atmosphere for 3 more days.

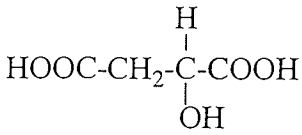
This method of washing the mid-point residue with water was reported to raise the overall molecular weight as well as to narrow the polydispersity.

2.8.3.2) Experiments Using the Roberts Apparatus

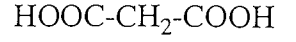
The following acids were reacted with butane-1,4-diol using the Roberts approach and slightly different conditions than stated above.



Wallace and Morrow acid (**1**)



Malic acid (**12**)



Malonic acid (**13**)

The Wallace and Morrow epoxy acid was reacted in both diisopropyl ether (**E-36**) and THF (**E-37**). The malic (**E-38**) and malonic acids (**E-39**) were used in THF only. The reactions were left for the much longer period of one month and were not washed with water at the half-way stage of the synthesis. None of the starting acids or the butanediol appeared to be very soluble in either of the solvents used. There was no evidence of polymeric product from any of these reactions.

2.8.3.3) Other Esterification Reactions

A number of esterifications were carried out without the Roberts apparatus. Adipic acid and the Wallace and Morrrow acid were reacted with butane-1,4-diol using both *Candida cylindracea* lipase (CCL) and the adsorbed *Mucor miehei* lipase as catalysts, in diisopropyl ether with 4Å molecular sieves. This time the reactions were left for ~4.5 days (**E-40 - E-43**).

All of these reactions were also unsuccessful.

2.9) Conclusions

The most significant result to emerge from this preliminary work was for experiment **E-8**. After modification of the reaction conditions stated by Wallace and Morrow, this experiment resulted in the successful polymerisation of the epoxy ester (**1**) to yield a polymer with Mn 5,200 and Mw 7,900. This established the fact that polymerisation reactions were possible using these enzyme slurry systems. Consequently, it was decided to investigate a wider range of systems to see if it was possible to achieve higher molecular weights using polytransesterification reactions in organic solvents.

No success was achieved using the succinate (**7**) and the alkene esters (**2**) which consequently were no longer employed. Subsequent experiments, carried out in the second phase of the research project (chapter 3), used butane-1,4-diol as no improvement was observed with alternative diols. The enzyme system used predominantly for the second phase of the work was porcine pancreatic lipase which was used in the form of a dry slurry in anhydrous solvents. All the solvents used were anhydrous, although it was considered unnecessary to use an inert atmosphere. It was observed that the experiments worked equally well in sealed air-tight reaction vessels as they did in conditions where

inert gas blankets were used, provided that the reaction vessel and substrates were thoroughly dried prior to carrying out the reaction.

Considering the substantial activity increase reported by Dordick *et al*, experiments **E-24 - E-35** should have been repeated. However, there are a few adjustments which it is felt should be made. Firstly, care should be taken not to leave the enzyme in an unfrozen state for any length of time during the course of the freeze-drying, lyophilisation process. This is just to minimise the possibility of denaturing the enzyme. Secondly, when the buffer is added initially to the enzyme, the very minimum amount required to wet the mixture, should be added. This should reduce the drying time quite substantially. It was felt that in the preparation of these enzyme/salt samples, the buffer solution was added in too great an excess.

No success was achieved in polymerising carboxylic acids or in using alternative enzymes to porcine pancreatic lipase. Additionally, the highest molecular weight quoted in the literature using this approach, was well below the range considered to be of any potential value in polymer chemistry. As a consequence, this approach was no longer considered worthwhile to pursue.

3) Optimisation of the Polytransesterification Reactions

3.1) Introduction

In this chapter, the work of the second phase of the research project is described. The bulk of this work was concerned with the optimisation of the polymerisation reaction and was concentrated, predominantly, on manipulations and modifications of the ester and the reaction solvent. During this stage of the work changes were made in a more structured manner, with a view to obtaining a better understanding of the polymerissation reaction. Other variables, such as atmosphere, temperature and ultrasound radiation etc., were also examined briefly. The results of all of these investigations are reported in this chapter.

3.1.1) The Solvents

For enzymic catalysis, the choice of solvent is critical. It can mean the difference between the synthesis of relatively large molecular weight polymers or no reaction whatsoever. Throughout the course of the research work, a range of solvents was used; from relatively non-polar ones such as hexane, to the more polar diethyl ether and dichloromethane.

3.1.2) The Esters

Most of the research work concentrated on the reactions of the following esters:

		Abbreviation
(i)	bis (2,2,2-trichloroethyl) succinate (**7**)	- Succinate
(ii)	bis (2,2,2-trichloroethyl) *trans* 3-hexenedioate (**2**)	- Alkene Ester
(iii)	bis (2,2,2-trichloroethyl) (±) 3,4-epoxyadipate (**1**)	- Epoxy Ester
(iv)	bis (2,2,2-trichloroethyl) glutarate (**8**)	- Glutarate
(v)	bis (2,2,2-trichloroethyl) adipate (**9**)	- Adipate

3.2) Reactions of Bis (2,2,2-trichloroethyl) Adipate, (±) 3,4-Epoxyadipate and 1,6-Hexene-3-dioate

3.2.1) Experiments Using Excess Enzyme

Experiments were performed using up to five times the amount of enzyme recommended by Wallace and Morrow. These were carried out to confirm that the enzymes were not in fact, being denatured to the extent that insufficient active enzyme remained for any reasonable polymerisation reaction to occur. Using a much larger enzyme quantity meant that if the same proportion of enzyme was being denatured, there would hopefully, still be sufficient remaining which was still active and could effect polymerisation.

One experiment was kept as the reference reaction (**E-44**), whilst for another two reactions a five-fold excess of enzyme was added. In one case, this excess was added at the start of the reaction (**E-45**) and for the other, the five-fold equivalent was added in batches, over the course of the reaction (**E-46**). The adipate ester was used for all three reactions which were carried out in anhydrous diethyl ether and had a reaction time of two weeks.

There did not appear to be any reasonably sized oligomer produced in any of these systems. However, the products from the reference experiment (**E-44**) and from the single addition of excess enyzme experiment (**E-45**), were pooled together for further reaction. The product mixture was then polymerised further using fresh quantities of both enzyme and butane-1,4-diol (**E-47**).

Another sample of adipate ester was used for further reactions using this batch-type of enzyme addition (**E-48** & **E-49**). This time, however, a smaller, three-fold excess of enzyme was added as a five-fold equivalent tended to clog the reaction vessel and hinder

stirring. Additionally, the reaction was carried out in both 'wet' (**E-45**) and anhydrous diethyl ether (**E-46**).

Again, there was no evidence of reaction using any of these systems. It can be said, with hindsight, that the reason for this was that the systems were not sufficiently dry.

3.2.2) Experiments Using 'Wet' Solvents and Longer Reaction Times

It had been reported to us by a more experienced worker in this area that higher molecular weight products could be obtained by using longer reaction times and solvents which had not been dried ('wet') prior to use.

Reactions were carried out using butane-1,4-diol, on the adipate (**9**) and alkene esters (**2**) in 'wet' diethyl ether, and in both anhydrous and 'wet' dichloromethane. They were left to react for 7 days and then worked up and samples retained for GPC analysis. The remaining products were put on again for a further 18 days with fresh enzyme and solvent. After this time they were worked up and analysed by GPC.

For two of the adipate reactions, one in dry CH_2Cl_2 (**E-52**) and the other in 'wet' ether (**E-51**), there was too little or no product for analysis after 18 days. The other adipate reaction, in 'wet' CH_2Cl_2 (**E-50**), showed no real improvement after the additional 18 days. However, for the remaining reactions, all of the samples showed an increase in the molecular weight after having been left for the additional 18 days. The results have been summarised in Table 5.

No	Ester	Solvent	7 day Reaction		7+18 day Reaction	
			Mn	Mw	Mn	Mw
E-50	Adipate	wet CH_2Cl_2	400	600	250	800
E-51	Adipate	wet ether	400	2,500	-	-
E-52	Adipate	dry CH_2Cl_2	400	500	-	-
E-53	Alkene ester	wet CH_2Cl_2	400	400	300	1,250
E-54	Alkene ester	wet ether	400	900	1,400	1,900

Table 5. The effect of time and solvent humidity on the PPL catalysed polytransesterification of the adipate (**9**) and alkene esters (**2**)

These proved to be interesting results in that the longer reaction times showed an improvement in the molecular weights. It was decided to investigate this more closely by performing another time study in a dry solvent. The esters chosen for this purpose were the alkene ester which had given higher molecular weight products after longer reaction times in the former experiments, as well as the epoxy ester, which Wallace and Morrow had found to polymerise more successfully than its alkene ester precursor. Finally, the behaviour of the adipate ester was also of interest, since it was the only ester that had not given improved products on changing to a longer reaction time.

It was thought, also, that the presence of the double bond in the alkene ester might have had an influence on the polymerisation reaction. Consequently, the alkene diol, 2-butene-1,4-diol was substituted for butane-1,4-diol. The reactions were carried out in anhydrous diethyl ether and sampled after approximately 4,5 and 11 days and analysed by GPC.

As can be seen from the results in Table 6, it appeared that only the alkene ester showed any increase in molecular weight, with time:

No	Ester	4 days		5 days		11 days	
		Mn	Mw	Mn	Mw	Mn	Mw
E-55	Adipate	1350	2050	1300	2050	1300	2000
E-56	Alkene ester	700	1200	850	1900	1000	2500
E-57	Epoxy ester	1650	2050	1250	1950	-	-

Table 6. The effect of time on the polytransesterification of **E-55 - E-57**, with butane-1,4-diol, in anhydrous diethyl ether

Additionally, it was observed that overall, slightly better results were achieved with the epoxy and adipate esters. It was noted that the results for the adipate ester were comparable to those for the epoxy ester. Hence, in view of the lengthy procedures involved with the synthesis of the epoxy ester, further research was concentrated on the adipate as a reference ester. The adipate ester could be synthesised relatively easily, in a one-stage procedure. Further work carried out on the adipate ester provided some promising results. From time to time, the most promising adipate experiments were repeated using the epoxy ester and confirmed that the adipate results could easily be compared to those of the epoxy ester.

It was decided to use the longer reaction times, as in most cases this brought about an increase in the molecular weight. In the worst case, as with the adipate reactions, the molecular weight remained apparently, unchanged over the whole time period. However, it can be said with the knowledge of hindsight, that this time study was misleading. The shortest reaction time used was 4 days. In later work, it was found that shorter times of around 3 days were more conducive to the production of higher molecular weight polyesters.

3.3) The Reference Reaction

As yet, suitable parameters had to be established for the synthesis of a reasonable sized polymer, so that the task of further 'fine-tuning' could be embarked upon in the future. Eventually, a set of reaction conditions which consistently brought about some degree of polymerisation was achieved. The solvent used was diethyl ether as this solvent consistently resulted, at least to some degree, in polymerisation and also provided higher molecular weights compared to dichloromethane. For example, the results shown in Table 5, show the highest Mw values for two adipate polymerisations to be 2,500 and 1,900, whereas in dichloromethane, the highest Mw value observed was only 1,250.

The quantities of reactants used were those used by Wallace and Morrow[79]. The diester and butane-1,4-diol were used in equimolar quantities of 10 mmoles. The porcine pancreatic lipase (having an activity of 50 units /mg of protein) was dried and stored in a vacuum dessicator over phosphorus pentoxide, at 5°C for at least two weeks prior to being used. The diester was synthesised and dried as described in section 2.2. It was further dried and stored in a vacuum dessicator over phosphorus pentoxide, at ambient temperature for at least two weeks prior to use. The solvent used was anhydrous diethyl ether. This was dried first over potassium hydroxide pellets for two days and then dried and stored over sodium wire for at least five days. When required, the ether was distilled off, discarding the first 10% and used immediately. The reaction mixture was stirred gently at ambient temperature for about 8-12 days. It was decided to use fairly long reaction times, as at this stage of the work, it was believed that long reaction times provided higher molecular weight polymers.

At the appropriate time the enzyme was filtered off and washed with dichloromethane. Dichloromethane was used to wash the polyester from the enzyme slurry, as it was found to be the best solvent for the polyesters. The filtrate and washings were combined and the solvent removed using a rotary evaporator. Then diethyl ether was added to the

resultant oil to precipitate out the product polyester. The solvent was decanted off, another portion of ether added and the washing/precipitation process repeated to yield the polyester product.

3.3.1) Reactions Using Adipate and Glutarate Esters

It was reported by Morrow, that rather high molecular weight polymers could be obtained with the glutarate than with the adipate ester. Subsequent experiments were, therefore, concentrated on both the bis (2,2,2-trichloroethyl) glutarate (**8**) and bis (2,2,2-trichloroethyl) adipate (**9**).

Eight reactions were set up; four using the adipate ester and four with the glutarate ester. Four different reaction conditions were employed in parallel for both the glutarate and adipate esters. The solvents used for the experiments were anhydrous dichloromethane and anhydrous diethyl ether. Half of the experiments were worked up after 3 days and then put on to react for a further 7 days, using fresh quantities of both solvent and enzyme. The latter approach was used since it had given such good results in the early stages of the project.

These experiments provided some very interesting results. A summary of these is shown in Table 7.

No	Ester	Solvent	Reaction time/days	Mn	Mw
E-58	Adipate	CH_2Cl_2	10	300	1,000
E-59	Adipate	CH_2Cl_2	3+7	-	-
E-60	Adipate	Ether	10	1,800	6,500
E-61	Adipate	Ether	3+7	2,100	6,100
E-62	Glutarate	CH_2Cl_2	10	-	-
E-63	Glutarate	CH_2Cl_2	3+7	-	-
E-64	Glutarate	Ether	10	-	-
E-65	Glutarate	Ether	3+7	900	56,000

Table 7. The effect of using different times and two reaction stages on the polytransesterifications of adipate and glutarate (**E-58** - **E-65**)

As can be seen from the results, some relatively high Mn and Mw values were obtained for the experiments carried out in ether. The reactions carried out in dichloromethane showed no significant reaction.

The most interesting results to emerge from these experiments, were for samples **E-61** and **E-65**. These parallel adipate and glutarate experiments, respectively were both carried out in ether and interrupted at the 3-day stage. Both showed high Mw values, with sample **E-65** having a far greater Mw value than has ever been reported previously, for a polyester. Consequently, these were very promising results indeed. Hence, it was decided that for subsequent work, it would be of interest to analyse reactions after a 3-day period.

All of the Mn and Mw values were obtained from GPC analyses. Molecular weights determined by ^1H NMR end-group analysis were also carried out, so that a direct comparison could be made with the results of Morrow *et al.* For some systems, the

ratios of polymer methylene groups to methylene, non-polymer end-groups was too high for any accurate measurements to be made.

3.3.2) Addition of Molecular Sieve

The use of molecular sieve was considered after favourable reports by a number of workers, that its addition had a beneficial effect on related reactions. Haken *et al* reported the use of 4Å molecular sieves to quench methanol liberated during the course of a transesterification reaction to resolve secondary alcohols[91]. O'Hagan and Zaidi[67] successfully polymerised 10-hydroxydecanoic acid, in the presence of 4Å molecular sieves to yield a polyester with a Mn value of 9,300 and Mw of 12,100. The molecular sieve in this case was used to mop up the water liberated during the course of the reaction.

At the time this work was being carried out, Morrow reported that the alcohol formed in the forward reaction promoted the release of enzyme-bound water[86]. Hence, if the alcohol by-product produced as a result of the forward polymerisation could be removed, this would serve to inhibit this detrimental effect. Molecular sieve might then serve the dual purpose of absorbing excess water and superfluous alcohol. It would be expected that the absorption of large or highly branched alcohols would be limited, but that smaller alcohols could be hindered to a lesser extent.

3.3.3) Adipate Reactions

A number of reactions were carried out in anhydrous dichloromethane, anhydrous diethyl ether and anhydrous tetrahydrofuran. This time for the dichloromethane reaction, it was decided to try the shorter reaction time of 3 days, to see if this would show any

improvement on the previous, 10-day dichloromethane reactions (**E-59** and **E-58**). Various reactions were carried out in ether, with range of reaction times as well as with the inclusion of molecular sieve. Finally, reactions using another ether, tetrahydrofuran were carried out, also using various reaction conditions. For reactions **E-72** and **E-68**, the reaction was worked up after 3 days and then both fresh solvent and enzyme added and the reaction allowed to continue for a further 7 days. For reaction **E-69**, the solvent alone was removed under reduced pressure, using a rotary evaporator, every 1-2 days.

A summary of the experiments and results is shown in Table 8.

No	Solvent	Reaction time/days	Reaction Conditions	Mn	Mw
E-66	CH_2Cl_2	3	Blank	-	-
E-67	THF	10	Blank	-	-
E-68	THF	3+7	Enz & solv changed	300	2,300
E-69	THF	10	Solvent changed frequently	2,100	8,250
E-70	Ether	3	Blank	3,300	6,100
E-71	Ether	3	Molecular sieve	6,000	9,400
E-72	Ether	3+7	Enz & solv changed Molecular sieve	2,300	8,900
E-73	Ether	10	Blank	1,450	4,800
E-74	Ether	10	Molecular sieve	1,600	6,700

Table 8. The effect of using different reaction conditions and solvents on adipate experiments (**E-66 - E-74**)

These experiments were very helpful as they gave the first reasonable results and formed the basis for further work carried out .

The adipate was reacted with butane-1,4-diol in diethyl ether with and without molecular sieve for 3, 3+7 and 10 days (Table 8). The results of these reactions were very encouraging. The three day reaction gave the highest Mn and Mw values so far observed. The longer reaction times both with and without molecular sievegave products with much lower molecular weights. It was rather disappointing to realise that if shorter reaction times had been used originally, much more might have been achieved.

A number of other modifications to the reference reaction were also looked at. Having obtained better results for the shorter reaction time in diethyl ether, it was decided to try a shorter reaction time in dichloromethane to see if this would show any improvement on the previous 10-day dichloromethane reaction (Table 7). In fact, no product was obtained from the 3-day dichloromethane reaction. So in this case, the shorter reaction time gave a poorer result than the longer reaction time.

Since diethyl ether had proved to be a relaatively successful solvent, the possibility existed that another ether might give even better results. Reactions using THF were carried out using various reaction conditions. The THF systems gave much poorer results than the diethyl ether systems. Only in the case where the solvent was changed frequently (**E-69**), were products with reasonably high molecular weights produced.

3.3.4) Glutarate Reactions

Like the adipate, the glutarate was reacted with butane-1,4-diol in diethyl ether with and without molecular sieve for various times and under a variety of conditions (Table 9).

No	Solvent	Reaction time/days	Reaction Conditions	Mn	Mw
E-75	CH_2Cl_2	10	Blank	200	400
E-76	CH_2Cl_2	10	Molecular sieve	300	400
E-77	CH_2Cl_2	10	Solvent changed frequently	200	400
E-78	CH_2Cl_2	3+7	Solvent & enzyme changed	200	400
E-79	CH_2Cl_2	3	Blank	500	500
E-80	THF	10	Blank	300	600
E-81	THF	10	Molecular sieve	300	500
E-82	THF	10	Solvent changed frequently	300	700
E-83	THF	3+7	Solvent & enzyme changed	200	500
E-84	Toluene	10	Blank	2,900	4,400
E-85	Toluene	10	Molecular sieve	400	500
E-86	Toluene	10	Solvent changed frequently	3,000	4,600
E-87	Ether	3	Blank	10,700	843,000
E-88	Ether	3	Molecular sieve	200	200
E-89	Ether	10	Molecular sieve	-	-
E-90	Ether	3+7	Solvent & enzyme changed Molecular sieve	-	-
E-91	Ether	9	Blank	1,600	5,400
E-92	Ether	9	Molecular sieve	2,100	7,300
E-93	Ether	9	Solvent changed frequently	2,000	7,300
E-94	Ether	3+6	Solvent & enzyme changed	2,300	258,000

Table 9. The effect of using different reaction conditions and solvents on glutarate experiments **E-75 - E-94**

The glutarate results were not as conclusive as the adipate results. Again, experiments carried out in dichloromethane showed no indication that polymerisation had taken place. Also for the glutarate reactions in THF, no significant polymerisation was observed. The molecular weight results for reactions carried out in the presence of molecular sieve, were largely disappointing, with sample **E-92** giving the only reasonably high molecular weight. The product from this reaction was calculated to have Mn and Mw values of 2,100 and 7,300, respectively.

For the reactions carried out in ether, there appeared to be some inconsistencies. Whereas the 9-day reaction (**E-92**) gave a good result, **E-89**, a 10-day reaction carried out with added molecular sieve was unable to initiate a detector response for the GPC molecular weight analysis.

The real surprise was the observation of products with enormous molecular weight values. The Mw values for samples **E-87** and **E-94** were extremely high; 843,000 and 258,000, respectively. These were considered to be very encouraging results indeed, the products having a molecular weight easily in the range considered to be of commercial use. However, on closer investigation by NMR, it was thought that it was not in fact polymer that had been observed. It was hypothesised that a certain amount of polyester had become complexed with the enzyme. This resulted in the unusual solubility of the complex and also accounted for the extremely high molecular weights observed. This is discussed in more detail in chapter 4, section 4.4.1.

The GPC analysis for sample **E-87** reported a certain amount of insoluble material. Additionally, since the GPC columns used for the analysis were suited to low and medium range polymer detection, a proportion of the sample was reported to have been excluded from the columns. Thus, this resulted in a degree of distortion to the shape of the calculated distribution. Presumably, this could also help to explain why the apparent polydispersity was so high.

The reactions in toluene resulted in some unexpectedly high molecular weights. The 'blank' reaction, **E-84** and the reaction carried out with frequent solvent changes, **E-86**, showed comparable results. The reaction carried out with added molecular sieve, however, showed virtually no reaction had taken place.

3.4) Optimum Reaction Time

By this stage of the research work, it was realised that the best solvent was anhydrous diethyl ether and that the two esters which consistently produced the best results in a variety of different reaction conditions, were the two simplest esters; the glutarate and the adipate esters. It was also becoming evident that, contrary to all the progressive literature reports, the highest molecular weights that were being observed in this research project were in fact, when relatively short reaction times were employed. Obviously, if these findings are correct, they contradict current belief. A time study was carried out using the glutarate and adipate esters, both with and without molecular sieve. Experiments were carried out on the same scale as previous reactions, *viz* using 10 mmoles of either bis(2,2,2-trichloroethyl) glutarate or bis(2,2,2-trichloroethyl) adipate, 10 mmoles of 1,4-butanediol, 6g porcine pancreatic lipase and 50mL of anhydrous diethyl ether. A summary of the results is shown in Table 10.

Without Molecular Sieve				With Molecular Sieve			
Ester	Reaction Time/hours	GPC Mn	Mw	Ester	Reaction Time/hours	GPC Mn	Mw
Glutarate	8	3,200	5,500	Glutarate	8	4,000	8,000
Glutarate	24	4,100	6,100	Glutarate	24	5,700	9,500
Glutarate	72	3,500	6,100	Glutarate	72	4,600	8,000
Adipate	8	1,000	2,900	Adipate	8	1,800	6,600
Adipate	24	2,100	3,000	Adipate	24	4,500	6,500
Adipate	72	3,600	5,000	Adipate	72	6,500	9,000
Adipate	120	4,500	6,100	Adipate	120	5,600	8,100

Table 10. Summary of glutarate and adipate time-study experiments

The polymerisation reaction proceeded relatively rapidly, producing polyesters of several thousand Da in just a few hours. It was later established, during further work carried out after this research project, that the best polymerisation conditions were when short, i.e. 3 day reaction times were used in conjunction with molecular sieve[93]. Nevertheless, the majority of reactions carried out after these results were obtained, were still proceeded with using a reaction time of around 10 days. This was due to the fact that at this stage of the project, the benefits of using shorter reaction times were not fully appreciated.

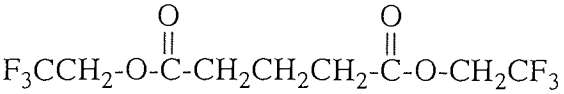
3.5) Alternative Leaving Groups

An alternative approach to the use of 2,2,2-trichloroethyl activated esters was to try completely different leaving groups which would also serve to activate the carboxyl function and make it favourable for transesterification reactions. A range of different activating substituents were selected which had the potential to be displaced readily by the competing diol nucleophile. The hypothesis was that the more easily that

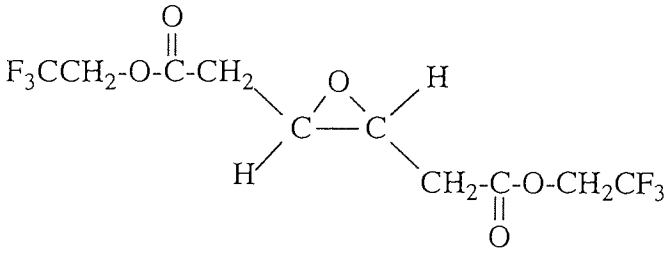
transesterification could be effected, the more efficiently polymerisation would be carried out, leading to larger molecular weight polymers.

The monomers chosen were bis (2,2,2-trifluoroethyl) glutarate and adipate, trimethyl silanol, phenol and cyclohexanone oxime esters and vinyl acetate. Three of the ester monomers, the vinyl, phenyl and oxime systems were chosen specifically to overcome the reversibility problem. On poly-transesterification, the vinyl ester produces an enol by-product, which rearranges to the more stable aldehyde. However, although this rearrangement is favourable with regards to the equilibrium position of the forward reaction, it was found, by Janssen *et al*, that the resulting aldehyde took part in unwanted side-reactions, which reduced the activity of the enzyme[94]. Neither the phenol or the hydroxylamine produced by the phenyl and oxime systems respectively, undergo enzyme catalysed transesterification. So, it was decided to investigate the potential of these various systems for polymerisation.

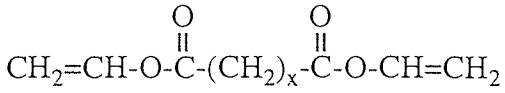
The compounds to be synthesised and their abbreviated names are illustrated below. The trimethyl silyl adipate was purchased from a commercial supplier.



Bis (2,2,2-trifluoroethyl) glutarate (**14**)

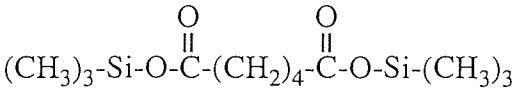


Bis (2,2,2-trifluoroethyl) (±) 3,4-epoxyadipate (**15**)

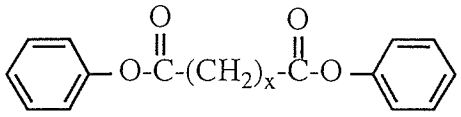


(x=3) - Divinyl glutarate (**16**)

(x=4) - Divinyl adipate (**17**)

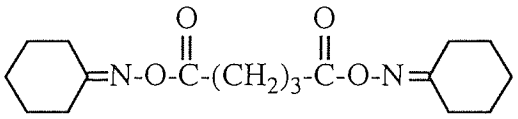


Bis (trimethylsilyl) adipate (**18**)



(x=3) - Diphenyl glutarate (**19**)

(x=4) - Diphenyl adipate (**20**)



Bis (cyclohexanone oxime) glutarate (**21**)

3.5.1) Synthesis of Bis (2,2,2-trifluoroethyl) glutarate and Bis (2,2,2-trifluoroethyl) (±)-3,4-epoxyadipate

Bis (2,2,2-trifluoroethyl) glutarate and bis (2,2,2-trifluoroethyl) (±)-3,4-epoxyadipate were the esters used to assess the effectiveness of the 2,2,2-trifluoroethyl leaving group. The esters were prepared bt the route used previously as for the synthesis of their 2,2,2-trichloroethyl equivalents described in chapter 2, using 2,2,2-trifluoroethanol in place of 2,2,2-trichloroethanol.

The use of the alternative trifluoroethyl leaving group had been reported relatively recently, by Morrow *et al*[83]. They had carried out a series of experiments using the monomer pair, bis (2,2,2-trifluoroethyl) glutarate and butane-1,4-diol. They found from their experiments that the presence of 2,2,2-trifluoroethanol caused termination of the polymerisation reaction. They established that the 2,2,2-trifluoroethanol accelerated the release of water which was bound to the 'anhydrous' enzyme. It was found that even though the enzyme had been thoroughly dried before use, there was still enough water bound to the enzyme to cause the polymerisation reaction to be terminated due to the competing hydrolysis reaction. An attempt was made to determine whether the alcohol was responsible for the release of water by reacting the diester monomer alone with the enzyme to see whether hydrolysis would occur. However, this did not occur and it became apparent, that hydrolyis only occurred in the presence of an alcohol. Also, the hydrolysis was not occuring to any significant extent at the start of the reaction when the concentration of butane-1,4-diol was at its greatest. This led to the conclusion that it was in fact, the trihaloethanol which was being produced during the course of the reaction, that was responsible for the release of enzyme-bound water.

Morrow reported that substitution of trifluoroesters for the trichloroesters in enzyme catalysed polytransesterifications gave higher molecular weight polymers. This is due to the fact that on polymerisation, the by-product of reaction is 2,2,2,-trifluoroethanol. Trifluoroethanol is quite volatile, with a boiling point of ~80°C compared with 151°C for trichloroethanol[95]. Consequently, when the trifluoroesters are used in conjunction with dimethoxybenzene, a high boiling solvent, a vacuum can be applied to the mixture and the relatively low boiling alcohol, 2,2,2-trifluoroethanol, removed. Thus, the removal of the by-product pushes the reaction equilibrium in favour of the forward reaction, i.e. polymerisation. Morrow *et al* reported that with the periodic application of a vacuum to the reaction mixture, they were able to achieve much higher molecular weights than with their previous system. The highest molecular weight product obtained using this system had a Mn value of 31,000, Mw of 39,000 and a reaction time of 18 days.

3.5.2) Syntheses of Divinyl Glutarate and Divinyl Adipate

The idea of using a vinyl group to activate the ester was inspired by Fang and Wong[34]. They demonstrated the advantage of using such a group. Since an esterification or transesterification reaction is reversible, this can reduce the yield of the product or polymer due to the thermodynamic equilibrium that exists between the reactants and products as was shown above. If the products can somehow be removed or altered, this serves to favour the forward reaction and hence promote polymer synthesis. By using a vinyl group, the by-product of the polymerisation reaction is an unstable enol. This enol rearranges to the stable aldehyde and is effectively removed from the reaction mixture, so promoting product formation. Fang and Wong used this approach to effect simple esterifications for mainly peptide and carbohydrate systems. Various workers, in particular Fang and Wong[34], have reported the use of vinyl esters extensively for enzyme catalysed transesterification reactions[96, 97]. In view of the promising work carried out by various workers, the vinyl system appeared to be a good proposition for polytransesterification reactions.

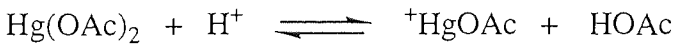
To synthesise the vinyl esters, the method of German *et al* was employed[98]. They used a long, commercially available vinyl ester, vinyl versatate-10, to synthesise lower esters by transvinylation in the presence of the catalysts mercuric acetate and sulphuric acid. The reagents were stirred together in an argon atmosphere. The product vinyl ester was removed by continuous distillation as it was being formed. This is possible since the vinyl esters formed generally have boiling points which are 40-50% below those of the acids from which they are derived. Also, the vinyl versatate is reported to have a relatively high boiling point so remains in the reaction flask during distillation.

The acids stated in the literature, were all relatively low boiling liquids with the highest boiling acid having a boiling point of 160°C, whereas adipic and glutaric acids are solids

at ambient temperature with the relatively high boiling points of 265°C / 100mm and 200°C / 20mm respectively[95].

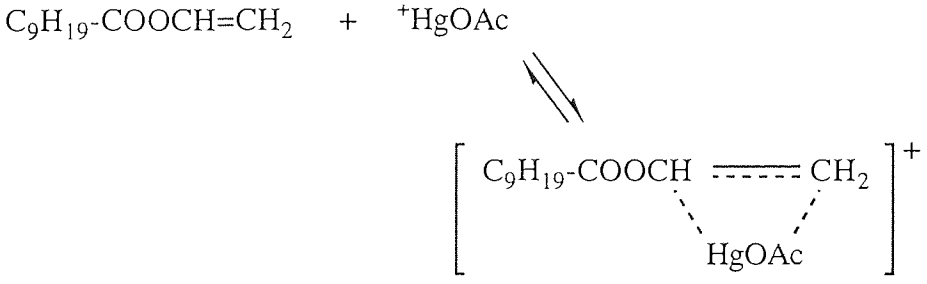
The reaction mixture was left to reflux for several hours and the acetic acid produced was neutralised with excess triacetate hydrate. The acetic acid boils at a temperature lower than that of the vinyl glutarate and adipate so had to be removed or else destroyed. Initially, sodium hydrogen carbonate was used, but this was too powerful a base and it was believed that partial cleavage of the product ester was occuring. When the acetic acid had been neutralised with sodium hydrogen carbonate, the vinyl glutarate or vinyl adipate was removed by vacuum distillation.

The reaction is thought to proceed as shown below. The mechanism is shown for glutaric acid. First the catalyst acts to form a cation with the acid:



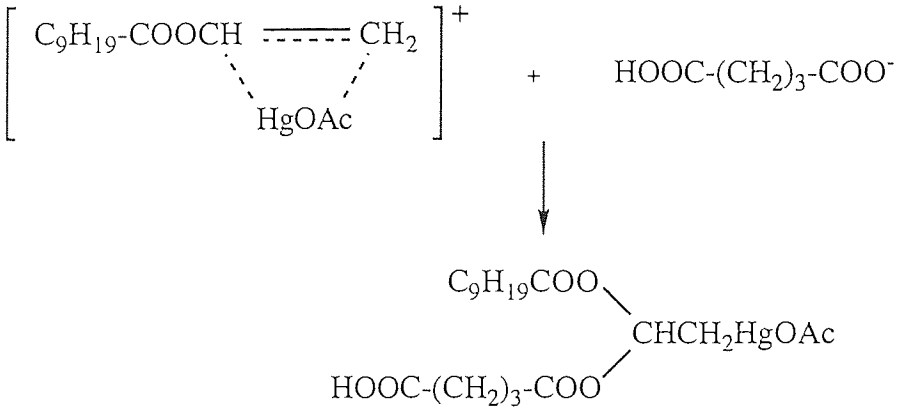
Scheme 7. Formation of mercury (ii) acetate cation

This cation then forms a complex with the vinyl versatate.



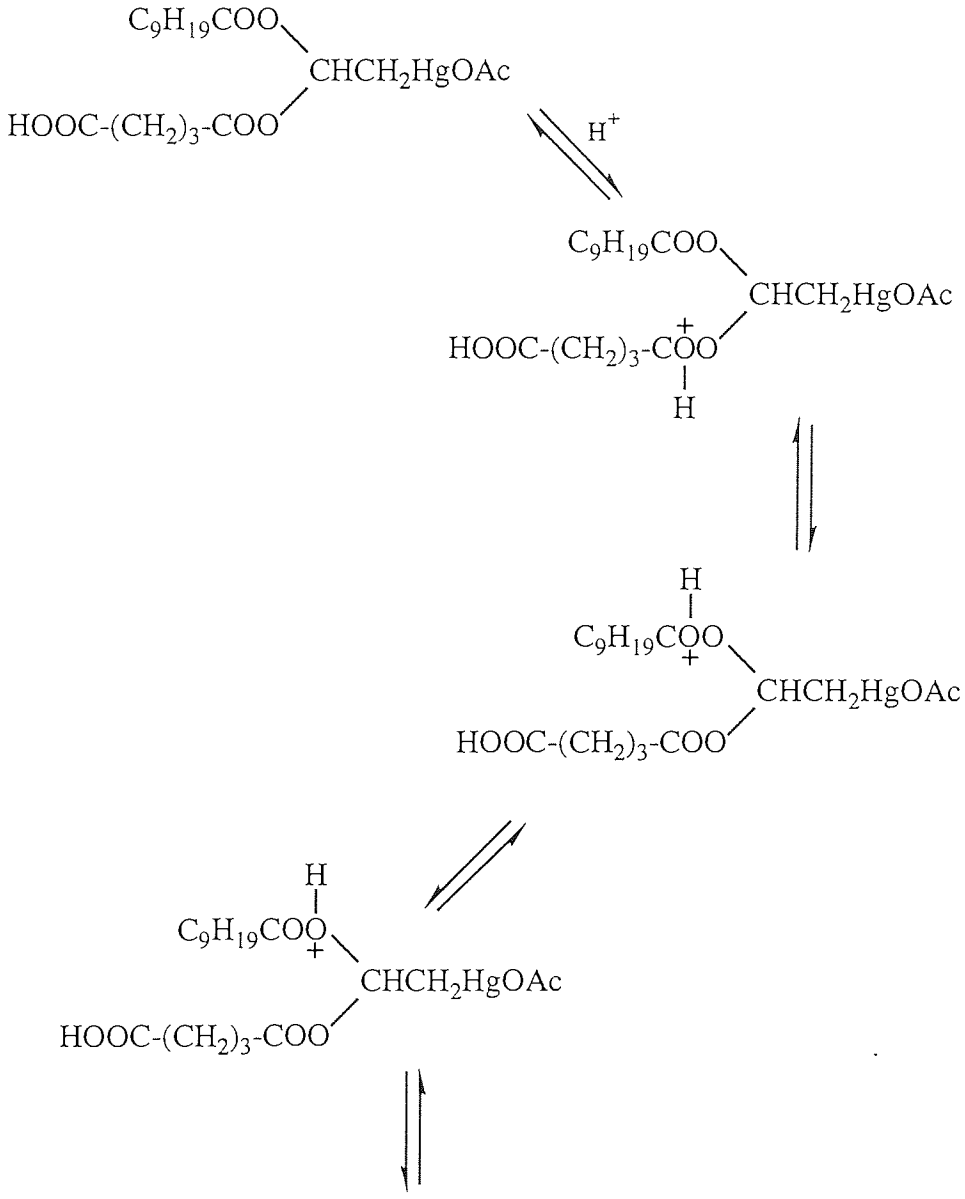
Scheme 8. Formation of a complex between mercury (ii) acetate and vinyl versatate

The complex ion then forms an intermediate with the glutaric acid anion.



Scheme 9. Formation of the reaction intermediate

Finally, the intermediate reacts with the sulphuric acid and rearranges resulting in the formation of the vinyl glutarate:



$$HOOC\text{-}(CH_2)_3\text{-}COOCH{=}CH_2 \quad + \quad {}^{+}HgOAc \quad + \quad C_9H_{19}COOH$$

[A similar procedure is repeated for the vinylation of the remaining acid moiety.]

Scheme 10. Mechanism for the formation of vinyl glutarate

For subsequent syntheses of vinyl esters, a much simpler method was employed using the procedure described by Swern and Jordan[99]. This involved a single stage synthesis of the vinyl ester from the parent acid and vinyl acetate. The product diester was isolated as described in the previous synthesis and the same catalysts, mercuric acetate and

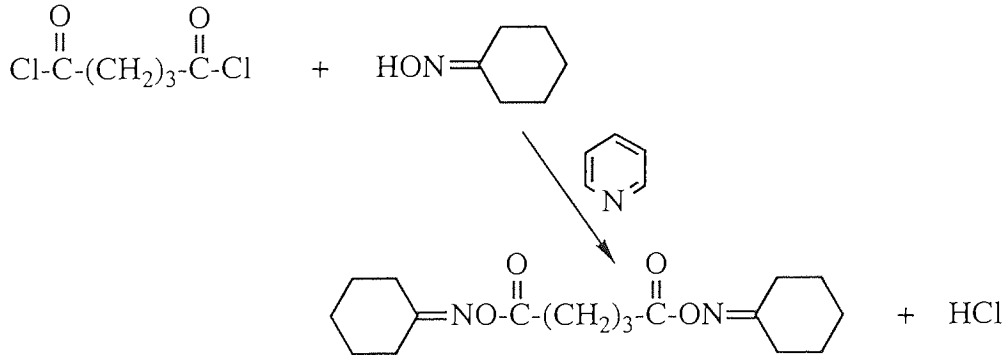
sulphuric acid, were also used. The reaction is thought to proceed via a similar mechanism, with the vinyl acetate adopting the role of the vinyl versatate.

3.5.3) Synthesis of Diphenyl Glutarate and Diphenyl Adipate

The phenyl esters were prepared by esterification of the respective acids with phenol. The resulting phenyl esters which were obtained in excellent yields, were recrystallised from cyclohexane.

3.5.4) Synthesis of Bis (Cyclohexanone Oxime) Glutarate

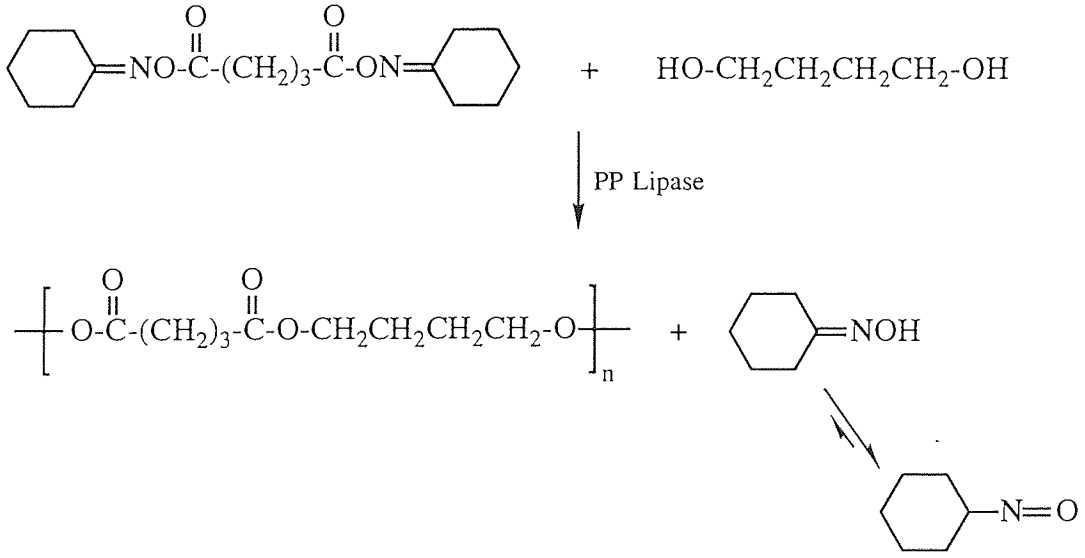
The oxime glutarate synthesis involved a simple condensation reaction using the bis chloride and cyclohexanone oxime in pyridine:



Scheme 11. Formation of bis (cyclohexanone oxime) glutarate (**21**)

Glutaryl dichloride was reacted with cyclohexanone oxime in the presence of pyridine to give the product, bis (cyclohexanone oxime) glutarate in quantitative yield. The resulting reaction mixture required careful vacuum distillation, using a high vacuum (0.2mm Hg),

to remove the oxime ester. Kumar and Ghogare used oxime esters as irreversible acyl transfer agents, in lipase catalysed reactions[100]. The concept of oxime ester use is similar to that of enol esters, in that any transesterification reactions carried out using these materials will result in the production of a by-product which rearrange to a more stable form on formation. This means that the undesirable reversible reaction is effectively ruled out due to the thermodynamic unfavourability of the reaction. Kumar and Ghogare found that reactions carried out using oxime esters had accelerated rates in comparison to analogous reactions carried out with enol esters.



Scheme 12. The polymerisation of bis(cyclohexanone oxime) glutarate with butane-1,4-diol. The hydoxylamine by-product tautomerises to the more stable nitroso-form.

3.5.5) Polymerisation of Bis(2,2,2-trifluoroethyl) Glutarate and Bis(2,2,2-trifluoroethyl) (±)-3,4-Epoxyadipate

Attempts were made to repeat the polymerisation carried out by Morrow[83] polytransesterification experiments using the bis (2,2,2-trifluoroethyl) glutarate (**14**).

The bis (2,2,2-trifluoroethyl) glutarate (**14**) was reacted with butan-1,4-diol in the high boiling solvent, dimethoxybenzene, using PPL as a catalyst and identical conditions to those described by Morrow. It had been reported that it was possible to remove the 2,2,2-trifluoroethanol as it was formed by periodically applying a high vacuum to the system. This proved impossible in our system.

To ascertain whether it was possible to remove the trifluoroethanol, an attempt was made to remove trifluoroethanol, dissolved in dimethoxybenzene. A high vacuum was applied to the stirred solution to try to remove the alcohol. It was not possible to remove the alcohol at ambient temperature. Consequently, the stirred solution was gradually warmed to about 60°C. Aliquots of the solution were removed periodically and then analysed by gas chromatography. Eventually, trifluoroethanol, which has a b.p. of 80°C at atmospheric pressure, could only be removed when the temperature of the mixture was raised to about 45°C, 0.05mm Hg.

The reaction was repeated several times but all attempts failed. Additionally, the high boiling 1,2-dimethoxybenzene was extremely difficult to remove on completion of the reaction. Since it proved impossible to remove the alcohol and the reaction itself gave no high molecular weight products, this method was abandoned. However, it was decided to use two 2,2,2-trifluoroethyl substituted esters for polymerisation reactions with alternative solvents.

Two series of experiments were carried out using bis(2,2,2-trifluoroethyl) glutarate and bis(2,2,2-trifluoroethyl) (±)-3,4-epoxyadipate in anhydrous diethyl ether using different reaction conditions. All of the bis(2,2,2-trifluoroethyl) (±)-3,4-epoxyadipate reactions showed no evidence of reaction. Reactions carried out using bis(2,2,2-trifluoroethyl) glutarate showed that some polymerisation had taken place. Consequently, further series of reactions were carried out using a range of different solvents.

The reactions carried out are summarised in the table below.

No.	Solvent	Reaction time/days	Conditions	Mn	Mw
E-95	CH_2Cl_2	10	Blank	500	700
E-96	CH_2Cl_2	10	Molecular sieve	800	1,200
E-97	CH_2Cl_2	10	Solvent changed frequently	500	700
E-98	CH_2Cl_2	3+7	Solvent and enzyme changed	500	800
E-99	THF	10	Blank	500	1,400
E-100	THF	10	Molecular sieve	800	1,500
E-101	THF	10	Solvent changed frequently	300	300
E-102	THF	3+7	Solvent and enzyme changed	500	800
E-103	Toluene	10	Blank	1,700	3,400
E-104	Toluene	10	Molecular sieve	1,000	2,500
E-105	Toluene	10	Solvent changed frequently	1,500	3,100
E-106	Toluene	3+7	Solvent and enzyme changed	2,100	4,000
E-107	Ether	9	Blank	1,000	1,700
E-108	Ether	9	Molecular sieve	2,000	4,800
E-109	Ether	9	Solvent changed frequently	1,700	4,400
E-110	Ether	3+6	Solvent and enzyme changed	1,200	3,200

Table 11. Reactions of bis(2,2,2-trifluoroethyl) glutarate with butane-1,4-diol (E-95 - E-110)

For the reactions carried out in dichloromethane and THF, no significant reaction occurred. However, there was a slight improvement for the reactions carried out with

added molecular sieve. Again, it can be said with the present knowledge that molecular sieve use is more beneficial when used in conjunction with shorter reaction times of, for example, 3 days[93].

The best results were observed for the reactions carried out in toluene and diethyl ether. Although, the results obtained with these latter two solvents gave reasonable molecular weight values, there was no improvement made on the results obtained using bis(2,2,2-trichloroethyl) glutarate. With a view to obtaining a measure of the relative leaving group ability of the trifluoro and trichloroethyl systems a time study of the PPL catalysed reaction of bis (2,2,2-trifluoroethyl) glutarate with butane-1,4-diol in anhydrous diethyl ether was carried out:

No.	Reaction time	Mn	Mw
E-111	1 hour	-	-
E-112	3 hours	500	800
E-113	7 hours	1,600	5,700
E-114	1 day	1,100	5,500
E-115	2 days	1,300	4,800
E-116	3 days	1,300	6,300
E-117	6 days	1,100	5,300

Table 12. Time study of the PPL catalysed reaction of bis (2,2,2-trifluoroethyl) glutarate (**14**) with butan-1,4-diol, in anhydrous diethyl ether

It would appear from Table 12 that in the initial stages the trifluoro system is more reactive than the trichloro system. It is interesting to note that the reaction was already approaching a molecular weight maximum after as little as 7 hours and appears to plateau at this point. This phenomenon of the reaction reaching a maximum molecular weight value after a relatively short reaction time, was noted in a previous time study experiment (see section 3.4). Again, with hindsight, it can be said that improved molecular weights

may have been observed for this short reaction time time-study if molecular sieve had been used.

3.5.6) Polymerisation of Glutarate and Adipate Esters Activated with Alternative Leaving Groups

Once the esters (divinyl glutarate, divinyl adipate, diphenyl glutarate, diphenyl adipate, oxime glutarate and TMS adipate) had been synthesised and purified they were dried thoroughly in a vacuum dessicator over phosphorus pentoxide for several weeks. The PPL catalysed reactions of these various esters with butan-1,4-diol, were all performed in anhydrous diethyl ether, at ambient temperature for 9 days, but after 3 days half of the reaction mixture was removed and analysed. The products did not appear to be very soluble in the usual GPC solvent, dichloromethane. A better solvent for the products was found to be 1,2-dichlorobenzene. Unfortunately, it was reported that there was negligible detector response when this was used as the solvent. When 1,2-dichloro-benzene was used as the GPC solvent, the samples were first dissolved in the boiling solvent (bp=180°C) and then analysed at 140°C. This was done, apparently, due to the high viscosity of the solvent. However, it was felt that this may have had a harmful effect on the samples and possibly caused their thermal degradation. Additionally, for the samples run in 1,2-dichlorobenzene an inappropriate column, more suited to lower molecular weight polymers, had been used for the sample analyses. Consequently, the samples from the first run in 1,2-dichlorobenzene showed no GPC response. Therefore, a second analysis was performed using dichloromethane. Since, however, most or all of the samples supplied had been used in the initial dichlorobenzene analyses, a second analysis was not possible in most instances. A lot of these samples contained a good deal of undissolved material which consequently, was not able to be analysed by GPC. Usually, in the work-up procedure of a polyester sample, it was observed there was a direct correlation in the amount of material insoluble in ether and the resulting molecular

weight. When the reaction mixture was filtered off and the solvent removed from the filtrate, a viscous oil remained. This viscous oil was composed of unreacted reactants and the products of polymerisation. On addition of ether to the oil, the unreacted starting materials were dissolved in the ether leaving a polyester precipitate. It was found that small molecular weight polyesters or oligomers were precipitated from the ether as a sticky white solid. When however, the molecular weight was quite large, in the region of several thousand, the precipitate appeared as a viscous oil. The more viscous and abundant the oil and also the more readily it was precipitated from the ether, the higher the observed molecular weight. A reaction summary table is shown below.

No	Ester	Reaction time/days	Mn	Mw	Comments
E118	Divinyl glutarate	3	-	-	Sample depleted
E119	Divinyl glutarate	9	-	-	Insoluble material
E120	Divinyl adipate	3	150	2,800	Insoluble material & inappropriate column
E121	Divinyl adipate	9	150	3,150	Insolb material & inapp column
E122	Diphenyl glutarate	3	350	7,450	Inappropriate column
E123	Diphenyl glutarate	9	400	6,200	Insolb material & inapp column
E124	Diphenyl glutarate	3	250	250	-
E125	Diphenyl glutarate	9	250	250	-
E126	Oxime glutarate	3	200	4,500	Inappropriate column
E127	Oxime glutarate	9	250	6,300	Insolb material & inapp column
E128	TMS adipate	3	-	-	No detector response
E129	TMS adipate	9	250	900	-

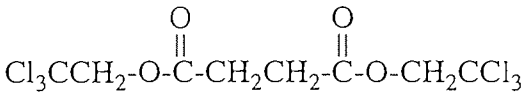
Table 13. The attempted PPL catalysed reactions of various activated esters with butane-1,4-diol in anhydrous diethyl ether

The results proved extremely dissapointing as high molecular weight products seemed imminent. During the product isolation stage, every indication was given that, except for the trimethyl silanyl adipate, all of the reaction mixtures contained high molecular weight material. Large amounts of viscous oil precipitates were obtained when ether was added to the filtrate. It is felt that the physical appearance of the products is highly indicative of the resulting polyester. On consideration, it was thought very likely that the undissolved material in the samples dissolved in dichloromethane, could contain high molecular weight material. Ideally, these experiments should have been repeated but all of the ester monomers had been used up and the project research time had come to a close.

3.6) Other Reactions

Throughout the research project and particularly in the early stages, a variety of different systems were investigated. Most showed minimal success but served to form a better understanding and clearer overall picture of enzyme catalysis. A brief report of the miscellany of approaches are given in this concluding section. The work involving the succinate ester and that using ultrasound treatment was carried out in the very early stages of the project.

3.6.1) Reactions of Bis (2,2,2-trichloroethyl) Succinate



Bis (2,2,2-trichloroethyl) succinate (**7**)

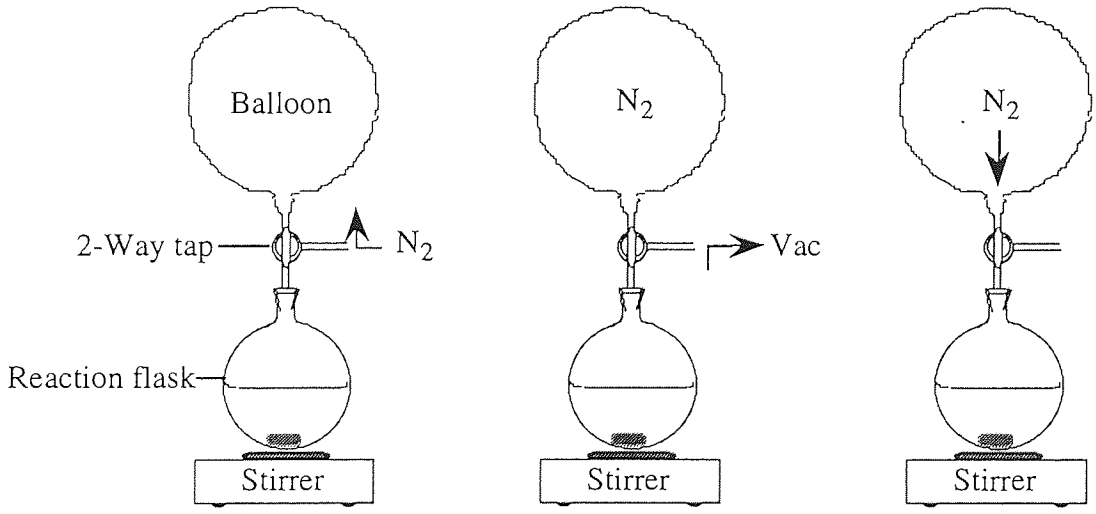
This ester was synthesised from succinic acid and 2,2,2-trichloroethanol using DCC and DMAP, as described for the Wallace and Morrow ester in section 2.2.

Two reactions were set up in anhydrous conditions; one using anhydrous THF with a continuous flow of nitrogen gas and the other under a static nitrogen atmosphere in anhydrous isopropyl ether.

3.6.1.1) Continuous Flow Method

The reaction mixture (**E-130**) was left to stir in THF at ambient temperature for over two weeks. The reaction mixture was stirred using a magnetic stirrer, in a 2-neck round bottom flask fitted with a nitrogen inlet and a Vigreux column to minimise solvent loss by evaporation. A gas trap filled with silicone oil was fitted at the top of the Vigreux column.

3.6.1.2) Static Nitrogen Atmosphere



Step 1 - First the balloon was filled with nitrogen

Step 2 - The reaction flask was evacuated

Step 3 - The flask was flushed with nitrogen from the balloon

Figure 11. The static nitrogen-atmosphere reaction vessel

In this system (**E-131**) the reaction vessel was evacuated and subsequently filled with nitrogen. This process was carried out several times to ensure the reaction vessel had been thoroughly purged with nitrogen and was repeated daily. Finally, the tap from the nitrogen balloon was left open to the reaction vessel so that a slight positive pressure of nitrogen always existed. This mixture in isoproyl ether was also left to stir at ambient temperature for the same length of time.

No polymer product was observed. However, from the results it was evident that the static system was not a good approach to adopt. Due to the repeated evacuating of the reaction vessel there was significant solvent loss. Additionally, it was felt that this sort of system was prone to leaks. With the continuous flow method there is a constant blanket of nitrogen covering the reactants. Hence, for subsequent reactions where a dry gas environment was deemed necessary, the former, continuous flow method was used.

3.6.1.3) Repeat of E-130 Using Argon

The nitrogen used in experiment **E-130** was not the especially dry grade (white spot), which should normally be used where anhydrous conditions are necessary. Therefore, the reaction was repeated using argon (**E-132**). This ensured that the environment was dry with the advantage of reduced cost. Additionally, the water condenser was replaced for a glass-packed column. This proved more efficient in reducing solvent loss. Again, this change was more advantageous in terms of resource, as no water was being used, especially in view of the lengthy reaction times involved.

A small amount of polymeric material was observed. It was found, by GPC anlysis, to have a Mn value of 800 and Mw of 950. This corresponds to a chain length of about 4.5 dimer repeat units. Although this result was not of great value, it was encouraging to find that an alternative monomer to the epoxy ester was being polymerised.

3.6.1.4) Elevated Temperatures

In order to maximise the efficiency of the enzyme, it was decided to try a few reactions using the optimum temperature of the porcine pancreatic lipase; this being around 40°C, the normal body temperature of a pig[101]. An alternative, yeast lipase, *Candida cylindracea*, was also employed at this same temperature for comparison purposes, although the optimum temperature for this enzyme is reported to be 55°C[67]. Both experiments were carried out in laboratory grade 'wet' THF as well as dry THF.

No reaction was observed with either the *Candida cylindracea* (**E-133**) or the porcine pancreatic lipase (**E-134**).

3.6.1.5) Ultrasound Treatment

Both the succinate and adipate esters were subjected to intermittent doses of ultrasound treatment (**E-135 - E-138**). The amount of time was usually 15 minutes in every hour for about 7 hours each day and was then left to stand, overnight. This process was continued for 5 days. The long times involved with the use of the ultrasound bath, caused the water to heat up. Consequently, this had to be changed fairly frequently so that the temperature of the bath did not exceed 40°C and thereby cause enzyme denaturisation.

3.6.2) Ring-Opening Polymerisation

As a result of the undesired cyclisation effect mentioned above, another idea came to light; the use of cyclic esters or lactones. If instead of using straight chain diesters, cyclic esters were used, they could act as a form of activated diester. Here, the

thermodynamic equilibrium of the forward reaction is made favourable due to the inclination of the lactone to ring-open and to undergo transesterification. Hall and Schneider reported that for their work on non-enzymatically catalysed ring-opening polymerisations, four-, seven- and eight-membered rings polymerised in almost all cases[102]. For the four-membered ring, polymerisation is relatively easy due to the ring-strain of the molecule. For the seven- and eight-membered rings the polymerisation driving-force is due to the crowding caused by the abundance of hydrogen atoms in the ring.

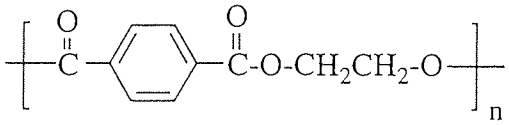
Ring-opening polymerisation undergone by Knani *et al*, yielded some moderately successful results[68]. The largest polyester produce this way had a Mw (by NMR) of 1,900 and degree of polymerisation of 16.

The two lactones attempted were β and γ-butyrolactone, using two different solvents; THF and dichloromethane. Poly(β-butyrolactone) is one of the constituents of the commerically available biodegradable co-polymer, BIOPOL, mentioned in section 1.1. In view of this, it was considered useful if a similar polymer could be polymerised in this manner. The major drawbacks of this monomer are, however, that it is a cancer suspect agent as well as a mutagen. Unfortunately, since the γ-butyrolactone, is a five-membered ring it does not polymerise under the usual reaction conditions. Only under considerable pressure and quite high temperatures (2000 MPa and 160°C) has it been reported to polymerise and even then, in low yield[103]. This is due to the fact that the ring is not under any ring strain nor is it crowded by hydrogen atoms as is the case for rings having more than seven or eight atoms. None of the reactions were successful.

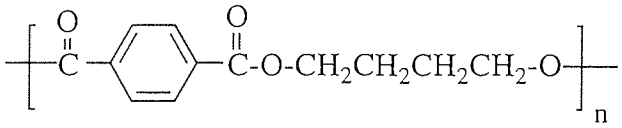
3.6.3) Enzymic Synthesis of PET and PBT

An attempt was made to effect enzymic polymerisation of two polymers quite unrelated to the project work. The two polymers, attempted were poly(ethylene terephthalate) and

poly(butylene terephthalate), both of which are already widely used and readily available commercially.



Poly(ethylene terephthalate) (**22**)



Poly(butylene terephthalate) (**23**)

Firstly, terephthalic acid was esterified with 2,2,2-trichloroethanol to activate it for subsequent transesterification with either 1,4-butanol or 1,2-butylene glycol. Then the polymerisation was carried out as usual in anhydrous diethyl ether, at ambient temperature and left to react for about 10 days.

The reactions were not successful and were only attempted once to see if there was even slight reaction or oligomer formation. If this had been the case, then it would have seemed worthwhile to pursue the reaction further. Conditions such as the reaction solvent could have been changed to determine whether it was feasible to optimise this synthetic technique for use as a realistic alternative to the existing polymerisation process.

3.7) Conclusions

Several important conclusions emerged from these various studies. The first was that anhydrous diethyl ether was the best solvent for the PPL catalysed transesteriication reactions of the glutrate and adipate esters with butane-1,4-diol. To date there has been minimal success with reactions that have been carried out using THF and especially dichloromethane. It is quite surprising that THF was in any way successful, as this goes against the present understanding that solvents miscible with water are not favourable for enzymatic slurry reactions such as these[37]. Dichloromethane, on the other hand, has a polarity similar to that of diethyl ether and is immiscible with water[92]. It would perhaps, at first sight, seem a good choice of solvent for polymerisation reactions. However, there are no reports favouring the use of dichloromethane as a reaction solvent and our results have served to confirm that it is not a good solvent for polytransesterification reactions. THF showed one good result with an adipate system, but not for the glutarate systems. The adipate reaction **E-69**, for which the solvent was changed frequently, had a Mn value of 2,100 and Mw value of 8,300.

Toluene showed some promise for the glutarate reactions, but the results were irreproducible. However, the solvent providing, consistently the best results for both the adipate and glutarate reactions was diethyl ether. Typically Mn values obtained were in the range 2,000-6,000 with Mw values in the range 5,000-9,000.

From the NMR analysis, it was not really possible to accurately determine the molecular weight. However, the NMR spectra did provide some useful information concerning the polymerisation reaction. Particularly, for all of the NMR spectra of experiments without molecular sieve, the predominant polyester end-group was always the hydroxy function. Where molecular sieve was used, the NMR spectra always showed an excess of the ester function. These NMR observations are discussed in more detail in chapter 4.

The second important conclusion was that the use of molecular sieve promoted the polytransesterification reactions. For the adipate experiments there appeared to be significant improvement in the molecular weights with the use of molecular sieve. Its inclusion in the glutarate experiments did not show such a conclusive beneficial effect as for the adipate experiments, but certainly did not show any negative effects either.

The third significant conclusion was that contrary to the vast majority of literature reports, shorter reaction times gave higher molecular weight products than with longer reaction times. The optimum reaction time for the adipate and glutarate polytransesterification reactions with butane-1,4-diol was around 3 days

The most startling results achieved, were for reactions the three glutarate experiments carried out in ether, **E-87**, **E-65** and **E-94**. All of these showed extremely high molecular weight values. The Mn values were 10,700, 900 and 2,300 respectively and the Mw values were 843,000, 56,000 and 258,000, respectively. The Mn values for **E-65** and **E-94** were not high, but all three samples had high Mw values. This showed that there was a certain amount of material in each sample at least, which was of high molecular weight. NMR analysis of these high molecular weight materials showed unusual spectra which led to an unexpected finding. It was believed that the large molecular weights were due possibly, to complexing of the enzyme itself with the polymester sample, rather than just very high molecular weight material. Evidence for this hypothesis is discussed in more detail in chapter 4.

The use of alternative leaving groups to the usual trihaloethyl type seemed very promising. Unfortunately however, the GPC results did not reflect the encouraging physical observations made of the resulting products. It was felt that the poor GPC results could well be due to the GPC technique employed and more specifically the inability of being able to find an appropriate solvent, rather than as a result of non-polymeric material being analysed. Therefore, more research into alternative leaving

groups was considered highly desirable and likely to be very promising. Unfortunately, insufficent project research time was available for continued work in this topic.

However, as mentioned in section 3.3.5.2), further work was carried out after this project work was completed, using the vinyl systems[93]. These systems have consistently produced relatively high molecular weight polyesters, with Mn values of ~10,000 and polydispersities of less than 2.0. It is thought that the molecular weights have been restricted to ~10,000, due only to poisoning of the enzyme catalysts.

The miscellany of experiments described in towards the end of this chaptert did not provide any advantages over the more usual methods described in preceding sections. There were, however, some informative ideas that came to light whilst carrying out these reactions, such as the lack of need for an inert atmosphere. This knowledge enabled reactions to be carried out far more conveniently using conventional 'quickfit' vessels.

4) NMR & GPC Analysis of the Results

4.1) Introduction

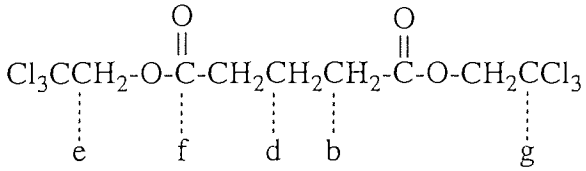
Both GPC and NMR were used for the analysis of the synthesised polymers. NMR was the technique employed predominantly by Wallace and Morrow for the determination of Mn values and to follow the progress of the reaction[84]. However, it was felt that whereas NMR was very useful in revealing essential information regarding the nature of the polymer, it was quite limited, as a tool for molecular weight measurements. In fact, Morrow *et al* themselves reported in a recent publication of an extreme case where the molecular weight determined by NMR, had been overestimated to be 37,000 but by GPC was shown to be only 3,300[83]. Roberts *et al* who used the same NMR analytical method to calculate the molecular weight of polyesters synthesised enzymatically from adipic acid and 1,4-butanediol, found that the method was only accurate for oligomers of up to four units in length[85]. The Wallace and Morrow calculation involves a comparison of the integrals of the polymerised methylene groups and the unpolymerised end-groups. This gives an indication of the degree of polymerisation (DP). The DP value is then multiplied by the molecular weight of the repeat unit to give a Mn value for the polymer. Depending on the particular case, the unpolymerised end-groups chosen for the calculation could be the hydroxy group, the ester group or even the unpolymerised or terminal, methylene group (a*-see section 4.2.1). However, in practice it was found by us that with any polymer having a relative molecular weight greater than a few thousand, the ratio of the polymer methylene groups to end groups was too large for any reasonable degree of accuracy to be achieved.

Since NMR end-group analysis did not appear to be too reliable in this investigation, Mn values were determined by both NMR and GPC and the Mw values by GPC. The results tabulated and discussed in this chapter are those where both NMR and GPC analyses were carried out on the product polymers.

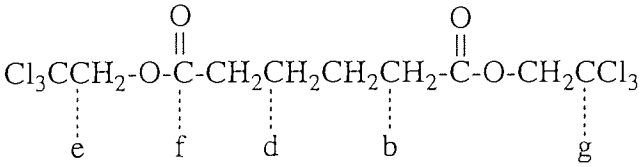
4.2) Calculation of Molecular Weights by NMR

4.2.1) Assignment of NMR Absorptions

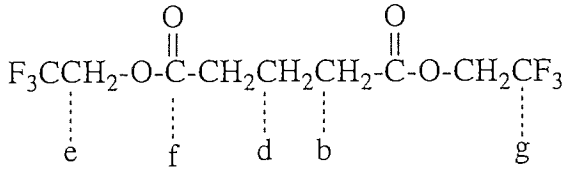
For polymers, NMR spectra can appear quite complex. In order to determine the molecular weight of a polymer from a spectrum, it is necessary to correctly assign all of the relevant peaks. The best approach to do this, is to first analyse the spectrum of the monomers; in this case the diester and diol. The information gained from the monomers can then be used to help assign the absorption peaks in the polymer spectrum. The polyesters for which these calculations were carried out were those showing, most consistently, the best results. These were derived from the trifluoro and trichloro glutarate esters and the trichloroadipate. For these esters, the chemical shifts were assigned for the different ^{1}H and ^{13}C environments. These are shown below.



Bis (2,2,2-trichloroethyl) glutarate (**8**)



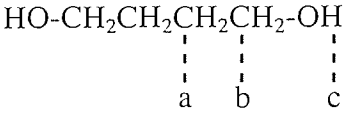
Bis (2,2,2-trichloroethyl) adipate (**9**)



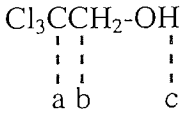
Bis (2,2,2-trifluoroethyl) glutarate (**14**)

C/H	Glutarate/δ		Adipate/δ		TRIF-Glutarate/δ	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
b	2.3	33	2.3	33	2.3	19
d	1.9	20	1.6	24	1.9	32
e	4.6	74	4.7	74	4.4	77
f	-	171	-	171	-	171
g	-	95	-	95		123

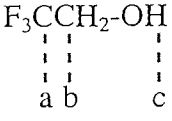
Table 14. Chemical shift values for the different ^{1}H and ^{13}C environments in Bis (2,2,2-trichloroethyl) adipate and glutarate and Bis (2,2,2-trifluoroethyl) glutarate



Butane-1,4-diol - [**Diol**]



2,2,2-Trichloroethanol - [**TRIC**]

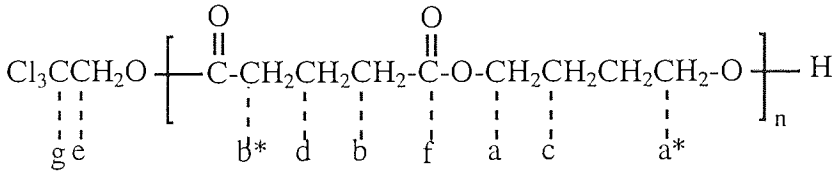


2,2,2-Trifluoroethanol - [**TRIF**]

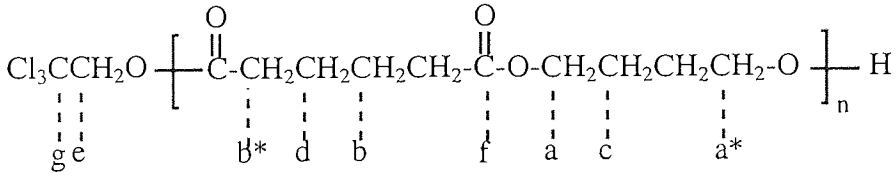
Proton	Diol	TRIC	TRIF
a	1.6		
b	3.6	3.5	4.4
c	5.2	4.2	4.2

Table 15. Chemical shift values for the different ^1H environments in Diol, TRIC and TRIF

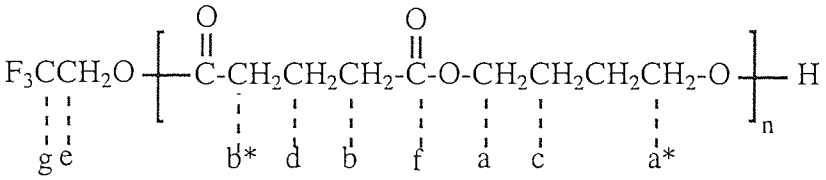
From these monomer spectra it was possible to assign all of the absorptions in the polymer spectra. The absorptions values are shown below. The protons denoted by an asterisk represent the terminal or non-polymerised methylene groups of the polymer. The chemical shifts of these protons correspond to the chemical shifts of the respective monomers. That is, b* has the same chemical shift value as the corresponding methylene from the monomer, bis(2,2,2-trichloroethyl) glutarate. Similarly, a* has the same value as for the corresponding methylene in butane-1,4-diol.



Polyester from synthesis of bis(2,2,2-trichloroethyl) glutarate with butane-1,4-diol - [**Glut**] (**24**)



Polyester from synthesis of bis(2,2,2-trichloroethyl) adipate with butane-1,4-diol - [**Adip**] (**25**)



Polyester from synthesis of bis(2,2,2-trifluoroethyl) glutarate with butane-1,4-diol - [**TRIF-Glut**] (**26**)

$^{1}H/^{13}C$	Glutarate/δ		Adipate/δ		TRIF-Glutarate/δ	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
a	4.0	64	4.0	64	4.0	64
a*	3.6	-	3.6	-	3.6	-
b	2.3	33	2.3	34	2.3	33
b*	2.5	-	2.5	-	2.5	-
c	1.6	25	1.6	25	1.6	25
d	1.9	20	1.6	24	1.9	20
e	4.6	-	4.7	-	4.4	-
f	-	173	-	173	-	173
g	-	-	-	-		-

Table 16. Chemical shift values for the different ^{1}H and ^{13}C environments in the polyesters, Glut, Adip and TRIF-Glut

Examples of some typical NMR spectra for the polymers are shown in figures 12-17, overleaf.

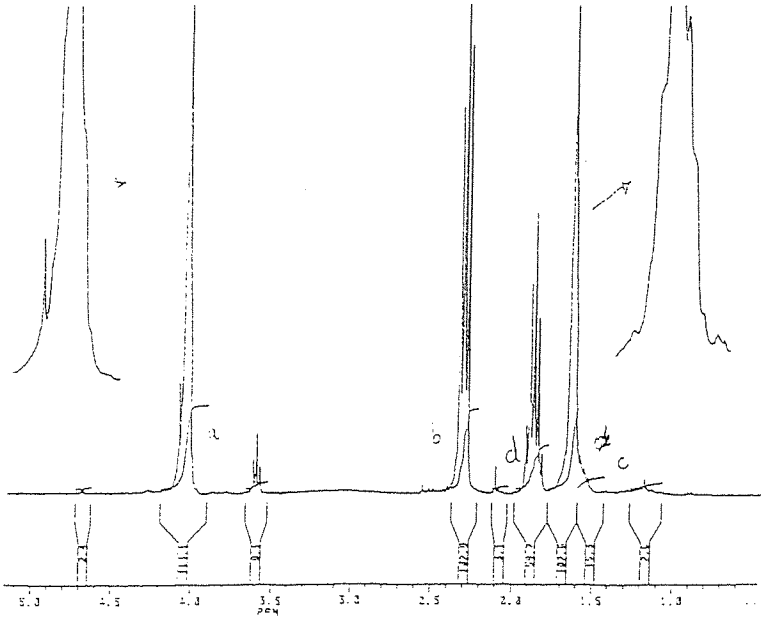


Figure 12. A typical ^{1}H NMR spectrum of a glutarate polyester (sample no. **E-94**)

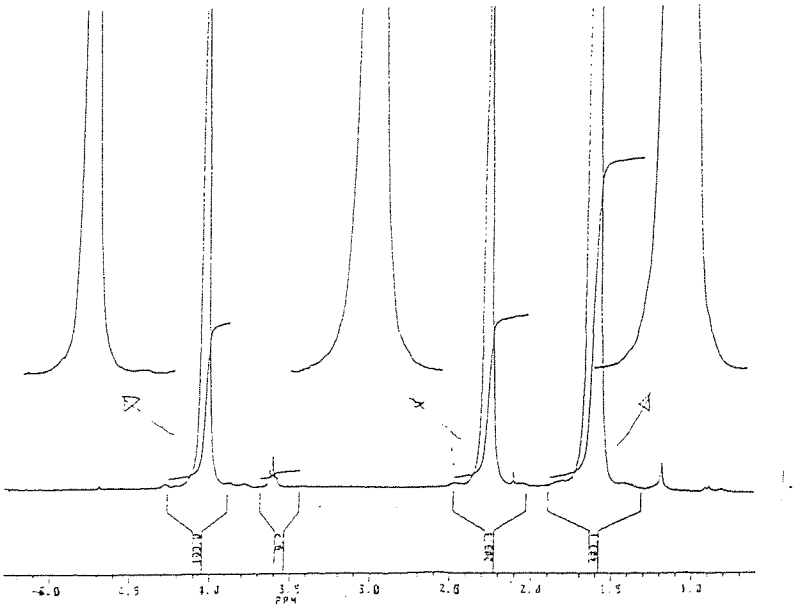


Figure 13. A typical ^{1}H NMR spectrum of an adipate polyester (sample no. **E-61**)

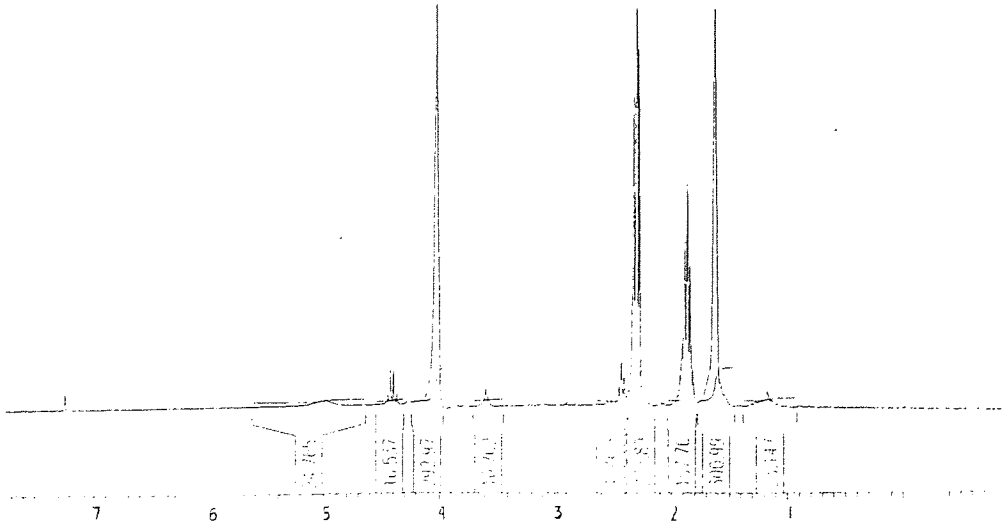


Figure 14. A typical ^{1}H NMR spectrum of a TRIF-glutarate polyester (sample no. **E103**)

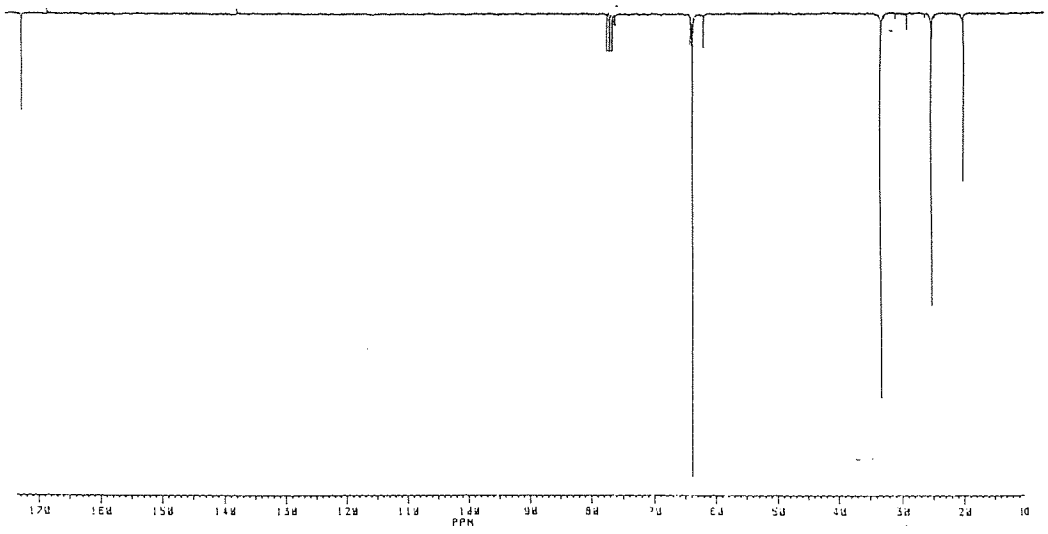


Figure 15. A typical ^{13}C NMR spectrum of a glutarate polyester (sample no. **E-94**)

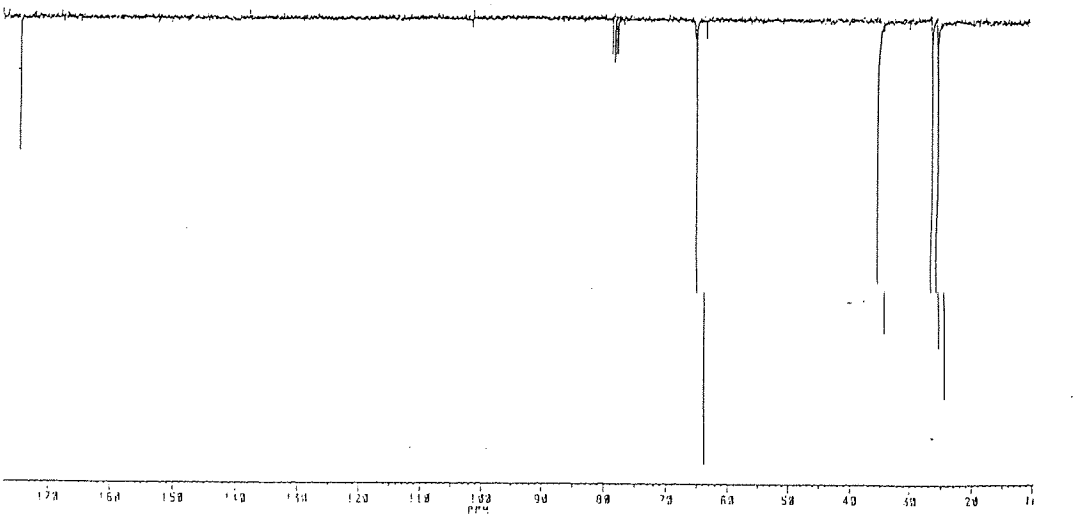


Figure 16. A typical ^{13}C NMR spectrum of an adipate polyester (sample no. **E-61**)

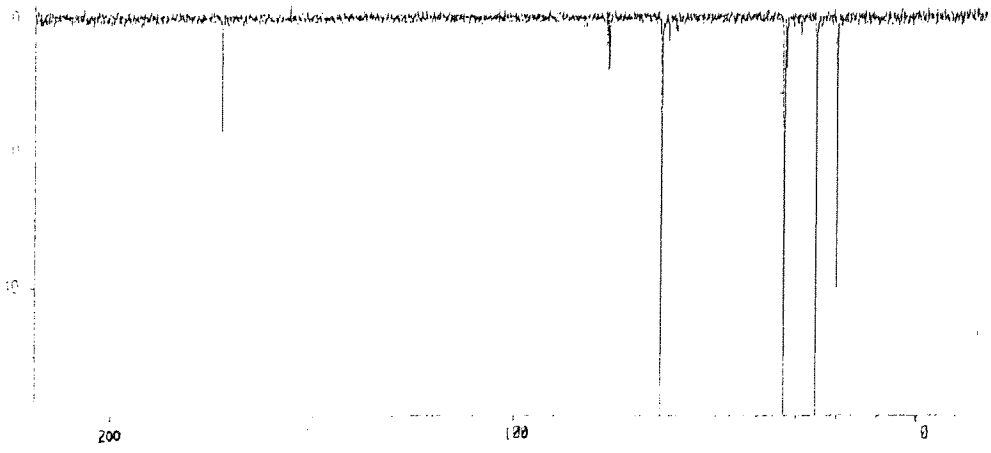


Figure 17. Typical ^{13}C NMR spectrum of a TRIF-glutarate polyester (sample no **E103**)

4.2.2) Molecular Weight Calculations by End-Group Analysis

Once having correctly assigned all of the absorptions the molecular weights were calculated. To calculate the molecular weight from the NMR spectra, the integral of **a**, δ 4.0, which corresponds to 4 protons was divided by the combined values of the integrals for **a***, δ 3.6, and **e**, δ 4.4-4.7 (The δ-values are slightly different for the **e** proton of the esters). These, combined, are proportional to 4 protons. Wallace and Morrow used the end-group methylene values for **b***, δ 2.5, and **a***, δ 3.6. However, it was found that it was not possible to use the **b*** integral value as it appears at δ 2.5 and overlaps with the **b** integral value which occurs at δ 2.3. Hence the integral value for **e** was used instead. This value appears much further downfield and is clearly defined. The other components present in the reaction mixture were butane-1,4-diol and 2,2,2-trihaloethanol.

In early NMR work carried out by Wallace and Morrow, the degree of polymerisation was calculated by dividing half of the **b** integral value (a methylene group from the polyester half of the polymer) by the integral value of **b*** (a methylene group from the polyester half of the polymer which is terminated with 2,2,2-trichloroethanol). This was based on the premise that there was always an equal number of ester functions reacting as there were hydroxy functions. It was evident, however, that this was not the case. In fact, there always appeared to be an excess of hydroxy-terminated polymers present. This implies that the original method of molecular weight determination would give too high an estimate for the molecular weight. If there is an excess of hydroxy-terminated end-groups, then there would be only a small amount of **b*** protons. In their later work, they used as the 4-proton end-groups, the combined integral values of **b** and **e**. This time their results showed that there were more of the hydroxy end-groups than the trihaloethyl end-groups. It was noted by us that in many instances, this was true. However, there were some notable exceptions which showed the reverse selection. That is, there was an excess of the ester end-groups instead of hydroxy end-groups. What

these predominantly ester end-group polymers had in common, was that they all had undergone polymerisation in the presence of molecular sieve. The molecular sieve had somehow caused the ester end-groups to be more prevalent in the resulting polymer.

Two typical spectra of glutarate polyesters are shown below. One is of a polymerisation where molecular sieves were employed and one where no sieves were used.

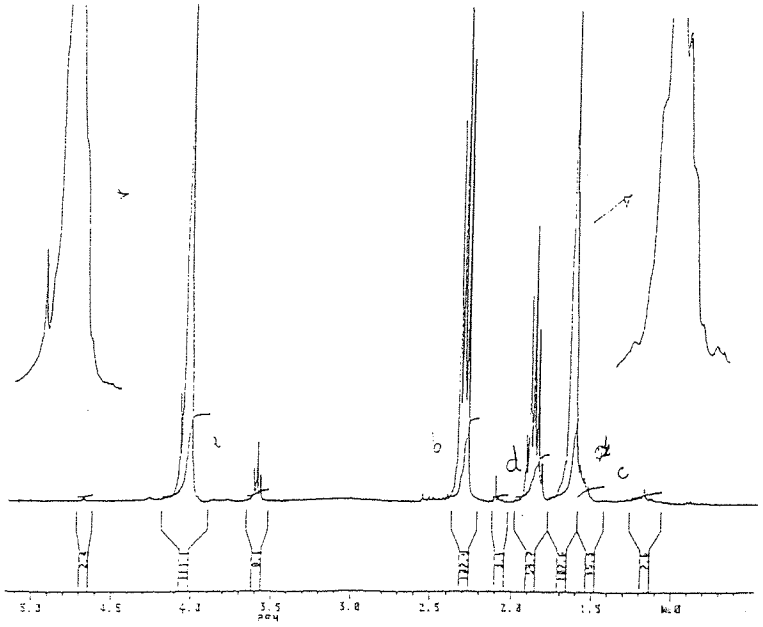


Figure 18. A typical NMR spectrum of a glutarate polyester (no molecular sieve)

[Experiment no. **E-94**]

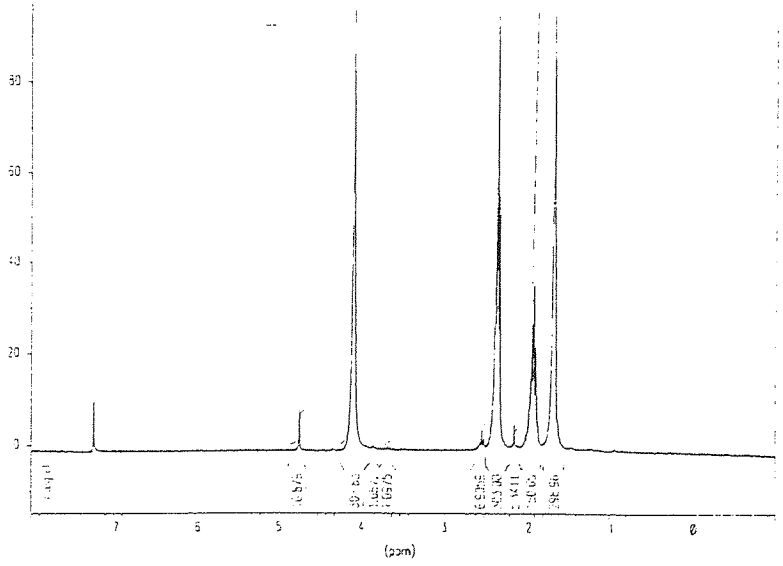


Figure 19. A typical NMR spectrum of a glutarate polyester (with molecular sieve)

[Experiment no. **E-139**]

As can be seen from either spectra, the peak correponding to **b*** overlaps with that of **b** itself. Also it can be seen quite clearly that there is a much greater excess of hydroxy-terminated polymers to ester-terminated polymers in the absence of molecular sieves. Conversely, when sieves are present, the ester-terminated polymers are prevalent. If the two values for the different end-groups, **a*** and **e** are added together, this means that whatever the actual ratio of hydroxy terminated ends to ester terminated ends, the combined integral value should give an exact 4 proton measure with which to compare the polymerised 4-methylene groups with.

An example of an actual calculation to estimate the molecular weight of a glutarate ester is as follows:

Area of a = 113

Area of a* = 9.1

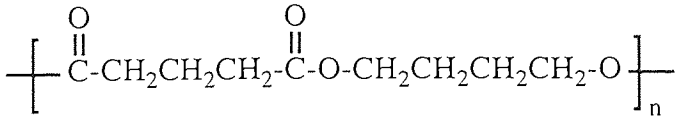
Area of e = 2.4

$$\text{Degree of Polymerisation (DP)} = \frac{\text{area of a}}{\text{area of a* + area of e}}$$

$$= \frac{113}{9.1 + 2.4}$$

Hence DP = 9.8

Molecular weight = DP x molecular weight of polymer repeat unit



(27)

$C_9H_{14}O_4 = 186$

$$= 9.8 \times 186$$

Hence, molecular weight $= \underline{1,800}$

These calculations were carried out for all of the samples having reasonable NMR spectra from which the relevant integrals values could be obtained. The calculated molecular weights from the NMR spectra were compared to the values from GPC analysis and have been tabulated below.

4.3) Comparison of Molecular Weights Calculated by NMR and GPC

4.3.1) Polymers Synthesised from Bis (2,2,2-trichloroethyl) Glutarate

The experiments shown in bold text were carried out with added molecular sieve. In these particular cases the ester end-group is not predominating. For experiment number **E-81** (a glutarate reaction carried out in THF, with molecular sieve present), the spectrum was very unclear and there appeared to be a good deal of overlapping peaks. These overlapping peaks were all due to the fact that there had been very little reaction and the product mixture contained a range of small oligomers and starting ester. In fact, experiments number **E-80 - E-83** (glutarate reactions in THF) inclusive, all showed no evidence of polymerisation, which in turn led to a very confusing NMR spectrum. The integral values for these experiments were all estimated as there was extensive overlapping and it was not possible to obtain 'clean' integral values For experiments carried out in dichloromethane (**E-75 - E-78** inclusive), the NMR spectra were even more difficult to interpret. Additionally, the molecular weights determined by GPC were very low, showing essentially unreacted starting ester and so it was not considered worthwhile to tabulate the molecular weight comparisons.

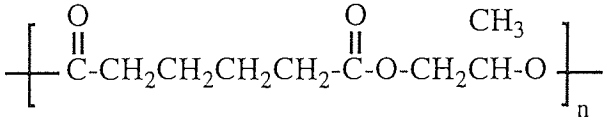
No.	Solvent	Reactn Time (days)	a/δ	b/δ	c/δ	d/δ	a*/δ	e/δ	Mn NMR	Mn GPC
E-91	Ether	9	113	108	108	59	9.1	2.4	1,800	1,500
E92	Ether		170	168	194	88	11.6	-	2,700	2,050
E-93	Ether	9	112	113	125	58	7.0	-	3,000	2,000
E140	Ether	9	153	160	164	81	9.7	-	2,900	1,300
E81	THF	9	38	43	26	20	20.4	4.6	300	250
E-80	THF	9	45	65	47	23	22.4	6.5	300	300
E-82	THF	9	34	32	32	32	6.6	6.2	500	250
E-83	THF	9	35	42	40	42	16.2	6.6	300	200

Table 17. ^{13}C NMR Chemical shifts & molecular weights of polymers synthesised from bis(2,2,2-trichloroethyl) glutarate

4.3.1.1) Results & Discussion

There appeared to be good correlation of molecular weights determined by both NMR and GPC for the very low molecular weight experiments, **E-81 - E-83**, inclusive. Although the actual molecular weights themselves were very low, it was reassuring that the values obtained using the two different analytical techniques, were in agreement. The molecular weights determined for oligomers and polymers using NMR, were all substantially higher than the molecular weights observed by GPC analysis. This led to the question of whether that was always the case. It also prompted the question of whether GPC or NMR provided a more realistic value of molecular weight. It was expected that a more accurate value was obtained using GPC since it is a technique intended for molecular weight determination. However, it must also be realised that GPC relies on comparison and is as good as the internal standards allow. In other words, if

the polystyrene standards against which the molecular weights were compared behaved in a completely different way to that of the polymer under investigation, then there would be increased likelihood of obtaining a false value. For this reason it was considered necessary to obtain an ester of known molecular weight which was similar in structure to the polyesters under investigation. Most of the work done by workers in this field used the universal internal GPC marker, polystyrene. In certain respects, since all of the work being carried out in this field used the same reference material, it should not greatly matter whether the molecular weight determination was very accurate, since all of the polymers produced using this approach were polyesters. Hence, the values will show the correct relative magnitude. Neverthless, it was thought useful to establish how reliable a standard polystyrene was. Thus, poly(1,2-propylene glycol adipate)



was obtained commercially and analysed by GPC against polystyrene standards to determine its molecular weight. It was reported, by light-scattering, to have a molecular weight of 4,000[104]. The GPC analysis reported it to have a molecular weight of ~4,500. This value was a little higher than the one quoted by light-scattering. So if these different techniques consistently showed the same trend, light scattering predicted the lowest molecular weight value, then GPC analysis, with NMR end-group analysis giving the highest value of all. However, these results were for particular samples and were carried out at particular times. It is not true to state that this was always true. A good approach perhaps would be to have used the commercial polyester sample as a reference each time a set of products was being analysed. This would guarantee that different batches of samples analysed on different occasions could be compared accurately by ensuring the molecular weights of the reference sample were consistent.

4.3.2) Polymers Synthesised From Bis(2,2,2-trichlorethyl) Adipate

Firstly before analysing the actual figures a few important points should be made. There was a marked contrast in the spectra of the adipate and glutarate polyesters. For the adipates, the NMR spectra were very clean, with the integration values being very straightforward and easy to read. For both the adipates and glutarates, however, it was observed for the GPC analysis, that many of the samples contained some insoluble material.

No.	Solvent	Molec Sieve	Reactn Time (days)	a/δ	b/δ	c+d/δ	a*/δ	e/δ	Mn NMR	Mn GPC
E-58	Ether	-	10	30	35	71	7.4	20.5	200	300
E-70	Ether	-	3	133	141	289	-	-		3,300
E-61	Ether	-	3+7	200	208	409	9.7	-	4,100	2,100
E-60	Ether	-	10	201	204	407	10.2	-	3,950	1,900
E-71	Ether	Yes	3							6,000
E-72	Ether	Yes	3+7							2,200
E-74	Ether	Yes	10							1,550
E-73	Ether	-	10	80	82	161	6.5	-	2,450	1,450
E-69	Ether	-	15	123	124	248	6.4	-	3,850	2,050
E141	Toluene	-	10	157	143	299	12.2	2.8	2,100	2,200
E142	Toluene	-	10	143	140	301	7.9	2.3	2,800	1,300
E143	Toluene	-	3+7	78	78	159	3.2	1.0	3,700	1,000

Table 18. ^{13}C NMR and chemical shifts of polymers synthesised from bis(2,2,2-trichlorethyl) adipate

4.3.2.1) Results & Discussion

Except for sample **E-71** (a 3-day adipate reaction carried out in ether) the GPC results themselves were somewhat lower than anticipated and differed quite substantially from the NMR calculated values. On average the GPC Mn values were approximately half the Mn values determined by NMR. This was especially disappointing in view of the exceptionally good quality of the NMR spectra, from which it was thought that accurate molecular weight values could be obtained. The end-group peaks on the NMR spectrum for sample **E-71** were too small, relative to the polymeric methylene groups, for an integration value to be calculated. Another very interesting and important fact to note is that the GPC run for samples **E-70** (a 3-day adipate reaction carried out in ether) and **E-71** was carried out on a different occasion to the other samples. Samples **E-70** and **E-71** were analysed over a month before any of the other series of ether samples, **E-58 - E-74**. The time of analysis for **E-70** and **E-71** was about 5 weeks after having been worked up. The other samples in this series were analysed after a period of about 11 weeks. Additionally, there was no mention of solubility problems with either sample **E-70** or **E-71** . With the other samples in this ether series, there was a significant amount of insoluble material reported to have been present.

4.3.3) Polymers Synthesised From Bis(2,2,2-trifluoroethyl) Glutarate

For the trifluoroglutarate reactions, the NMR spectra showed a good deal of overlapping peaks, which in turn, made the integration values very difficult to calculate.

No	Solvent	Molec Sieve	Reac Time /days	a/δ	b/δ	c/δ	d/δ	a*/δ	e/δ	Mn NMR	Mn GPC
E107	Ether	-	9	181	181	291	118	50.4	-	700	1,000
E108	Ether	Yes	9	174	168	102	234	32.2	-	1,000	2,000
E109	Ether	-	9	126	125	159	73	20.2	-	1,200	1,700
E114	Ether	-	1	254	296	303	154	10.0	6.0	2,950	1,050
E116	Ether	-	6	247	286	297	146	12.1	11.7	1,950	1,300
E117	Ether	-	10	214	260	299	140	23.4	6.0	1,500	1,100
E103	Toluene	-	9	293	274	300	158	10.8	16.5	2,000	1,600
E105	Toluene	-	9	280	285	300	160	12.0	16.0	1,800	1,450
E-95	CH_2Cl_2	-	9	241	244	189	331	171	40.9	200	500
E-96	CH_2Cl_2	Yes	9	165	166	128	274	89.2	20.5	300	800
E-98	CH_2Cl_2	-	3+6	230	223	154	228	74.4	17.1	500	500
E-99	THF	-	9	214	244	139	168	93.9	-	400	500
E100	THF	Yes	9	267	283	176	326	93.2	18.1	450	700
E102	THF	-	3+6	152	160	145	156	92.5	24.7	250	500

Table 19. ^{13}C NMR and molecular weights of polymers synthesised from bis(2,2,2-trifluoroethyl) glutarate

4.3.3.1) Results & Discussion

All of the GPC molecular weight values of the trifluoroglutarate samples shown above seemed, on the whole, to correlate with the values observed by NMR, even though the values were not particularly high.

4.4) Spectra of High Molecular Weight Polyesters

A typical NMR spectrum obtained for one of the extremely high molecular weight polymers produced, is shown below.

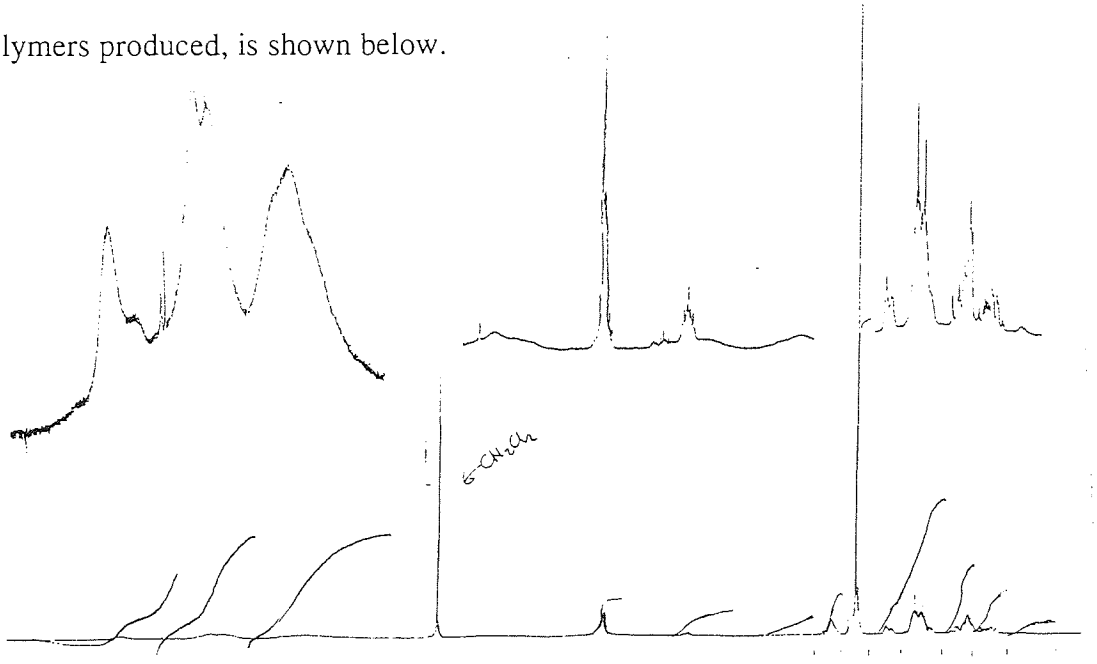


Figure 20. NMR spectrum for a glutarate polyester, sample **E-65** (Mw 55,800)

No.	Ester	Solvent	Reaction Time/days	GPC	
				Mn	Mw
E-87	Glutarate	Ether	3	10,700	843,000
E-65	Glutarate	Ether	3+7	900	55,800

Table 20. Summary of reaction conditions for experiments **E-87** and **E-65**

4.4.1) Results & Discussion

As was expected, in general, the higher the molecular weight reported, the cleaner was the spectrum. However, there were a few notable exceptions. The NMR spectra for samples, **E-87** and **E-65** proved very unusual. The extremely high molecular weight material was also quite extraordinary. It would be expected that with such a high

molecular weight, the NMR spectrum would be very simple. However, this was not the case and thus led to questions about the nature of the material. Firstly, the facts of the material were reconsidered:

(i) the molecular weight was extraordinarily high;

(ii) the material proved to be quite insoluble in most organic solvents, being only partially soluble in DMSO;

(iii) the NMR spectrum was fairly complex and showed the presence of a broad range of carbon and proton environments, including an unexpected region of aromatic moieties.

Whilst investigating the properties of this material, another unusual fact emerged. Whereas sample **E-65** was insoluble both in dichloromethane and in water, it was readily soluble in a mixture of the two solvents. This led to the idea that what had actually occurred was that the enzyme had become entrappped within a polymer matrix. The presence of the two solvents, therefore, enabled the two components to be solubilised; the enzyme in water and the polyester in dichloromethane. It was hypothesised that the polyester had grown sufficently large enough to ensnare an enzyme molecule or had perhaps even complexed with the enzyme. Whatever the exact form, it was considered highly probable that the enzyme and polymer were somehow linked. This hypothesis fitted very well in explaining the various phenomena concerning the sample, such as the high molecular weight detected by GPC.

To confirm the hypothesis it was decided to try to separate the two components and analyse them by NMR. A mixture of the two solvents, dichloromethane and water, was added in an attempt to dissolve each component in its preferred solvent, as mentioned above. Then the solvents were removed using a rotary evaporator and each residue analysed by NMR. If the hypothesis were true, then the expected results should show the presence of polyester from the dichloromethane fraction and enzyme from the water

fraction. This in fact, was the outcome, proving that the product was an enzyme/polyester complex. The NMR spectrum from the aqueous, enzyme-containing fraction showed the presence of lipase alone. The NMR spectrum of the dichloromethane fraction showed the presence of polyester as well as some lipase. Presumably, the lipase was also partially soluble in the organic phase. If the product had been a simple, uncomplexed mixture then it could have been partially dissolved by both organic and aqueous solvent. In other words, addition of water would have dissolved the enzyme component of the mixture and similarly, addition of an organic solvent such as dichloromethane would have caused the dissolution of the polyester portion. However, the product was totally insoluble in either solvent separately, implying that the two components were complexed, thus preventing the dissolution of the other component in its respective solvent. Only when both organic and aqueous solvent components were added was there complete and immediate dissolution of the product.

4.5) Conclusions

1) For samples number **E-87**, **E-65** and **E-94**, which had Mw values of 843,000, 56,000 and 258,000 respectively, it is likely that the phenomenon of polyester/enzyme complexing was occurring. This was disappointing in that the large molecular weight polymers which were formerly thought to have been synthesised were not in fact being made. However, there was some consolation in the discovery of the curious behaviour demonstrated by the complexing enzyme and polyester. Unfortunately, the amount of product available was minimal and so the analysis carried out was limited. Ideally, a more thorough investigation into the nature of the complex needed to be done in order to determine the usefulness of this effect. It may be that the polyester completely enclosed the enzyme and had become physically entangled with it. Or else it may, perhaps have been partly bonded with it, being loosely held by ionic interactions.

2) On analysing the NMR spectra, it was evident that as a technique for calculating the size of the polyesters, it was only accurate where the relative molecular weight was less than several thousand. However, the lower the molecular weight, the more complicated the spectrum. This is due to the fairly close molecular weight range of oligomeric products causing extensive absorption overlapping and making the interpretation of the spectra very difficult.

3) For the majority of the polyester spectra analysed in this work, the predominating end-group was the hydroxy group. This indicated that the polymer products were generally being terminated with diol monomers. When however, 4Å molecular sieve was used, often the complete reverse was true and the polyesters showed a dominance of ester terminated end-groups. One possible explanation which has been advanced to explain this phenomenom is that the diol monomers are more able than the diester monomers to access the active site around the enzyme. Thus, the presence of excess diols causes the polyester to be terminated with mainly hydroxy functions. It is possible that the molecular sieve is able to adsorb the diol and draw it away from crowding the enzyme's active site which would allow the diester monomer more opportunity to react with the polyester at the active site and account for the relatively greater ester end-capping, when molecular sieve is present.

4) For the polyester products analysed by both NMR and GPC, it was found that on average, NMR molecular weight predictions were higher than those found by GPC. This could be due to a number of reasons:

(i) The error margin in the NMR spectra, due to signal noise was too great to obtain accurate ratios of polymer to non-polymer methylene groups;

(ii) The highest molecular weight material, being more difficult to solubilise, was not being detected by GPC analysis. This problem was less significant for NMR analysis, as more time was allowed for the dissolution of samples;

(iii) Since for GPC analysis, polystyrene is used as the calibration standard this could very easily give a false indication of the actual molecular weight. The structural nature of the polyesters being analysed differs considerably from that of polystyrene.

5) The practice of running an internal standard of known molecular weight with every batch of samples analysed by GPC is a good way of ensuring that consistent results are obtained from one run to another. Additionally, the use of a reference sample such as poly(1,2-propylene glycol adipate), is likely to give a more realistic molecular weight value than polystyrene due to a greater structural similarity.

6) The GPC and NMR molecular weight results for the bis(2,2,2-trifluoroethyl) glutarate showed much better correlation than those of either bis(2,2,2-trichloroethyl) glutarate or bis(2,2,2-trichloroethyl) adipate. It was thought that this may be due to the lengthy standing times between the polyester sample work-up and analysis. It may be that the samples were being degraded to some extent, on standing. A difference in the shelf-life between the trifluoro-polyesters and the trichloro-polyesters would explain the difference in molecular weight correlations. Whether this hypothesis is correct or not, it was felt that the samples should have been analysed as soon as possible, preferably within 1 day of the termination of the reaction . This would serve to rule out the additional factor of polyester degradation.

5) Conclusions & Future Work

5.1) Conclusions

The most interesting and significant conclusions gathered from this research work are given below.

1) Anhydrous diethyl ether gave consistently good results. Interestingly, even though diethyl ether was the best solvent for the polytransesterification reaction, the polymers themselves were quite insoluble in this solvent. It seems that the reaction was able to proceed quite favourably, with the reactants present in the form of a slurry. This raises intriguing issues concerning the interfacial mechanism of action of the lipases.

Toluene also gave some encouraging results. For example, reactions **E-84** and **E-86**, produced polymers with Mn values of 2,900 and 3,000, respectively and Mw values of 4,400 and 4,600, respectively.

Use of the cyclic ether, THF gave polymers with molecular weights (Mw) values of 2,300 and 8,200. However, the majority of the polymerisations carried out in THF gave oligomers. At first this was thought to be due to the hydrophilic nature of THF. Since it is water miscible, it was thought that the THF was able to interact closely with the enzyme and thus denature it. Tests have shown that the catalytic activity of PPL is reduced by the action of THF[93].

Dichloromethane proved to be a poor solvent for the polymerisation reactions, producing only oligomers, even though it was a good solvent for the actual polymers.

2) Solvent dryness emerged as an important factor. In the early work 'wet' solvents had been employed as supplied by the manufacturers, without having undergone any stringent drying process. This appeared appropriate as conventional wisdom suggested

that a certain amount of water was necessary for the enzyme catalyst to function properly. From the results obtained, however it became evident that even with thorough drying of all the reaction components, there was still sufficient water present for the enzyme to catalyse the polymerisation reactions. Too much water has a detrimental effect on the polymerisation reaction, due to hydrolysis. Much improved molecular weights were observed when molecular sieve was added to the reactions.

3) From time studies, two factors emerged that are contrary to current thinking. Firstly, the optimum reaction times were much shorter than previously reported and secondly, if the reactions were left for several days, the molecular weight actually **decreased**, which may be due to in situ hydrolysis of the polyesters. Interestingly, the decline was also observed with reactions where molecular sieve was used. It might have been expected that hydrolysis would be suppressed if the sieve had absorbed all excess water. Also the NMR spectra of the products showed that there was a much smaller ratio of hydroxy to ester terminated end-groups with molecular sieve (see conclusions, section **4.5**). If in this case hydrolysis makes a significant contribution to the declining molecular weight, it must be assumed that the efficiency of the sieve is inadequate.

An alternative hypothesis that may account for the lowering of the ratio of hydroxy to ester end-groups and would also rationalise the lower molecular weights is the adsorbtion of butane-1,4-diol by the sieve. If the molecular sieve was saturated with butanediol molecules, then it would not be fully effective in removing excess water.

The highest molecular weight products were obtained using PPL suspended in anhydrous diethyl ether, in the presence of molecular sieve. The 72 hour reaction of bis(2,2,2-trichloroethyl) adipate with butane-1,4-diol gave the highest molecular weight polymer observed to date (Mn 6,500 and Mw 9,400). The more highly reactive glutarate gave similar molecular weights (Mn 5,700 and Mw 9,500) but in the much shorter time of 24 hours. The extremely high molecular weights obtained for glutarate/butanediol

experiments **E-87**, **E-65** and **E-94**, (which had Mw values of 843,000, 56,000 and 258,000, respectively), were probably due to the porcine pancreatic lipase (molecular weight ~50,000) becoming complexed with the polyester.

4) In the original work carried out by Wallace and Morrow[79], the molecular weights were determined by NMR end-group analysis. Our observations led us to believe that molecular weight calculations carried out by NMR end-group analysis are only accurate for polyesters with molecular weights not exceeding several thousand. This assessment was subsequently confirmed by Morrow *et al*[83] who subsequently reported that molecular weights calculated using NMR spectra can be significantly overestimated.

However, NMR analysis was useful for our work in that it provided information on the polymerisation reaction. For example, from the NMR spectra of the polyesters, it was clear that in the absence of molecular sieve, there was a larger proportion of hydroxy-terminated end-groups to ester terminated ones. This was probably one of the main reasons why the polyesters tended to be restricted to a molecular weight (Mn) never exceeding 10,000.

The polymerisation of bis(2,2,2-trifluoroethyl) glutarate in diethyl ether resulted in a polyester with a Mn value of 1,600 and Mw value of 6,300. Morrow *et al*[83] reported that polyesters of high molecular weights could be obtained using 2,2,2-trifluoro activated esters and a high boiling solvent (see section 3.5.1). An essential feature of the work was that the etrifluoroethanol was periodically removed by placing the system under vacuum. However, we found that the 2,2,2-trifluoroethanol could not be removed efficiently, even under a high vacuum at 40-45°C.

5) In addition to the bis(2,2,2-trihaloethyl) adipate and glutarate systems, a number of other precursors were investigated, *viz* :

(i) bis(2,2,2-trichloroethyl) succinate, *trans*-3-hexenedioate and (±)-3,4-epoxyadipate, (ii) diphenyl glutarate and adipate, (iii) bis(2,2,2-trifluoroethyl)*trans*-3-hexendioate, (iv) divinyl glutarate, (v) *N,N'*-glutaryl dicyclohexanone oxime.

It must be emphasised that the polytransesterification reactions were generally carried out for 10 days before the products were analysed and in some cases, in not entirely anhydrous conditions. It is feasible that in the earlier stages of these reactions, higher molecular weight products may have been produced but that they were subsequently degraded.

5.2) Future Work

Some of the most interesting discoveries were made towards the end of the research project. For example, some very promising work was carried out on the alternative activated leaving groups. It was felt that the phenol and vinyl groups showed particular promise and should be investigated more closely. In fact, currently, work is being continued by our research group in the area of alternative good leaving groups and so far has been focussed mainly on work involving vinyl esters but is also now concerned with investigating the potential of phenol esters.

As mentioned in point 1 of the conclusions, it was felt that the molecular weight of the polyesters was restricted due to the growing polymer chain being surrounded by an excess of butanediol monomers. Using molecular sieve could help to reduce the diol monomer density around the polymer chain and hence aid in providing an equal diester/diol ratio for the propagating polymer chain. However, this hypothesis of polyester 'swampling' still needs to be confirmed. It is felt that at this stage, what is needed primarily, is to elucidate a clear and accurate picture of the polymerisation process. One way to achieve this is to have a time study, with a series of reactions carried out for different lengths of time. The products of each of these reactions could

then be isolated and then chromatographically separated. It is felt that a successful separation could offer tremendous insight into the polymerisation reaction and help to disclose the reasons behind the molecular weight limitations.

It is felt that it would be interesting to try to make more of the complexed material which was thought to have been synthesised in samples **E-87**, **E-65** and **E-94**. If such an enzyme/polymer complex could be made in a controlled way, potentially it could be very useful. For example, the polyester surrounding it could be made to release the enzyme under certain aqueous/organic solvent conditions. Hence, it might be useful as a transportation means for the enzyme.

5.3) Concluding Remarks

The long term aim of this project was to investigate the potential of using enzymes as catalysts in the synthesis of polyesters, with a view to making truly biodegradable polymers. Some thought also, however, must be given to the implications of such an undertaking. The concept of making biodegradable polymers does at first thought, seem an attractive idea when posed with the problems caused by non-degradable waste. However, perhaps the narrow treatment of a symptom is just that; narrow. If the sole purpose of making biodegradable polyesters is to reduce the amount of unsightly litter, then this may tend to encourage the volume of litter created in the first place; out of sight, out of mind. Most 'litter' tends to be packaging material and the relative inexpense of polymers encourages its excessive use. Obviously a certain amount of packaging is necessary, but perhaps in view of todays consumer demands, long term logic would suggest that recycling is a far more desirable approach. The uses of polymers in todays world are enormous and they offer tremendous advantages. Coupled with the ability to biodegrade, their potential could be unlimited. It is only hoped that they will be used fittingly.

6) Materials & Methods

6.1) Reagents

All reagents were used as supplied unless otherwise stated.

Diethyl ether and THF were dried first over KOH and then over sodium wire for at least several days. Thereafter, they were distilled, just prior to use, discarding the first 10% fraction.

Dichloromethane was dried over calcium hydride powder for at least several days and then distilled just prior to use, discarding the first 10% fraction.

Toluene was distilled just prior to use, discarding the first 10% fraction.

Butane-1,4-diol was distilled, discarding the first 10% fraction and stored in a dessicator over phosphorus pentoxide, at ambient temperature. All of the acid and ester monomers synthesised were also stored in a desiccator over phosphorus pentoxide, at ambient temperature. The enzyme samples were stored in a dessicator over phosphorus pentoxide, at 5°C.

4Å Molecular sieves were dried by heating in a furnace at 600°C for several hours and were then transferred to a dessicator containing phosphorus pentoxide.

Compound	R.M.M.	Supplier
Acrylic acid	72	Aldrich
Adipic acid	146	Fisons
Butane-1,4-diol	90	Aldrich
2-Butene-1,4-diol	88	Aldrich
t-Butylcatechol	166	Aldrich

Butylene glycol	64	Aldrich
β-Butyrolactone	86	Lancaster
γ-Butyrolactone	86	Aldrich
Caprylic acid	144	Sigma
Celite 545	-	BDH
m-Chloroperoxybenzoic acid	172.5	Aldrich
Cyclohexanone oxime	113	Aldrich
Dichloromethane	85	Aldrich
N,N'-dicyclohexylcarbodiimide [DCC]	206	Aldrich
Diethyl ether	74	Aldrich
1,2-Dimethoxybenzene	138	Aldrich
4-(Dimethylamino) pyridine [DMAP]	122	Aldrich
Ethanol	46	Aldrich
Ethylene diol	**62**	Aldrich
Gelatin	-	Sigma
Glass beads (mesh ~100)	-	
Glutaric acid	132	Aldrich
Glutaric anhydride	114	Aldrich
Glutaryl dichloride	169	Aldrich
Glycerol	**92**	Aldrich
Glycolic acid	76	Aldrich
Heptane	100	Aldrich
Hexane-1,6-diol	118	Aldrich
Hydrochloric acid	36.5	BDH
trans-b-Hydromuconic acid	144	Aldrich
2-Hydroxybenzoic acid	**138**	Aldrich
2-Hydroxycaproic acid	**132**	Aldrich
12-Hydroxydodecanoic acid	216	Aldrich
Hydroxyisobutyric acid	**104**	Aldrich

2-Hydroxypyridine	**95**	**Sigma**
Isopropyl ether	102	Aldrich
Lipase (*Candida cylindracea*, Type VIII-S)	~65,000	Sigma
[700-1500 units/mg using olive oil]		
Lipase (Porcine pancreatic), Type II,	~50,000	Sigma
[35-70 units per mg protein using triacetin]		
Lipase (Lipozyme)	-	Novo
DL-Malic acid	134	Fisons
Malonic acid	104	BDH
Mercuric acetate	319	Aldrich
Molecular sieves (4Å)	-	
Octanol	130	Aldrich
Poly(caprolactone) [PCL] diol 1250	1250	Aldrich
Poly(caprolactone) triol 300	300	Aldrich
Poly(ethylene glycol) [PEG] 200	200	Aldrich
PEG 1000	1000	Aldrich
Phenol	94	Fisons
Phosphate buffer solution (pH 7.0)	-	
Potassium acetate	98	Aldrich
Potassium chloride	74	Aldrich
Potassium nitrate	101	Aldrich
Pyridine	79	Aldrich
Silica Gel	Art 11695	Merck
Sodium *bis*-2-(ethylhexyl) sulphosuccinate	444	Sigma
Sodium chloride	58.5	
Sodium hydrogen carbonate	84	Aldrich
Sodium phenoxide	116	Aldrich
Succinic acid	118	Hopkins & Williams
Sulphuric acid	98	Aldrich

Terephthalic acid	166	Hopkins & Williams
Tetrahydrofuran [THF]	72	Aldrich
Toluene	92	Aldrich
2,2,2-Trichloroethanol	150	Aldrich
Triethylamine	101	Aldrich
2,2,2-Trifluoroethanol	100	Vickers
Trimethylsilyl adipate	290	Aldrich
1-(Trimethylsilyl)-imidazole	**140**	Aldrich
Vinyl acetate	86	Aldrich
Vinyl adipate	**198**	Aldrich
Vinyl versatate-10	198	Kemira

6.2) Methods of Analysis

Infrared spectra were recorded on a Perkin Elmer 1710 Fourier Transform Infrared Spectrometer. Solid samples were prepared as KBr discs and liquids as thin films between NaCl plates.

NMR spectra were recorded on a Bruker AC 300 spectrometer. The ^{13}C spectra were recorded as either APT (Attached Proton Test) or DEPT (Distortionless Enhanced Polarisation Transfer) spectra, using the following conditions:

Pulse width	4.0μs, 40°
Relaxation delay	3 s
Sampling interval	32k
Total data points (FID)	32k

GPC was performed by RAPRA Technology Ltd., Shawbury, Shrewsbury, Shropshire, SY4 4NR, U.K. using a Knauer 64 HPLC pump. The column dimensions were 300 x 7.5mm and were packed with a heavily cross-linked polystyrene/divinylbenzene PL gel (10μ diameter mixed bed D). A Knauer differential refractometer was used as a detector. The GPC system was calibrated with polystyrene standards. The molecular weight averages were computated using Viscotek software.

The ultrasound bath used was a Camlab Transsonic T310 (220-240V, 0.14A, 50-60Hz, HF Freq. 35kHz).

6.3) Non-Polymer Syntheses

6.3.1) Enzyme Operations

6.3.1.1) Synthesis of a Microemulsion-Based Gel Using Lipase

Sodium bis-2-(ethylhexyl) sulphosuccinate [AOT] (0.89g,) was dissolved in heptane (5.3mL) and the solution maintained at 55°C using a thermostatted water-bath. *Candida cylindracea* lipase (2.5mg) was dissolved in distilled water (3 drops) and this solution then added to the AOT solution which was subsequently returned to the water-bath. Distilled water (2.4mL) was warmed to 55°C and stirred quite fast using a magnetic stirrer until a vortex was formed. Gelatin (1.4g) was added quickly to the stirred water in one go. This was found to be the best approach at achieving complete dissolution of the gelatin. The lipase/AOT solution was added to the gelatin solution and stirred until a homogeneous mixture was formed. The resulting mixture was poured into a petri dish and left overnight, at ambient temperature, for the gel to form.

6.2.1.2) Synthesis of a Microemulsion-Based Gel Using Lipase and a pH 7 Buffer

The method for this synthesis was identical to that of the enzyme-containing gel above, except that a pH 7.0 phosphate buffer was used instead of water.

(6.3.1.3) Synthesis of Ethyl Octanoate Using an Enzyme-Containing Microemulsion-Based Gel

Octanoic acid (1.44g, 10mmoles) and ethanol (0.46g, 10mmoles) were added to heptane (30mL). An enzyme containing gel from the previous experiment was added to the

reaction mixture which was sealed and left to shake in a thermostatted water-bath at 25°C, overnight. Subsequently, the gel was filtered off. At this stage two attempts were undergone on separate occasions, to extract the product ester. For the first attempt, the heptane was removed using a rotary evaporator and the remaining viscous oil loaded onto a silica column in an attempt to isolate the ester by flash chromatography. The column was eluted with 3:1 hexane/diethyl ether and 2% triethylamine. The various fractions were analysed by GC using a silicone grease column at 200°C and FTIR using KBr discs. For GC analysis, the retention times of the fractions had values that corresponded to that for unreacted octanoic acid. For FTIR, the carbonyl stretch (C=O: ~1713cm^{-1}), also had the same value as for octanoic acid. The desired product, ethyl octanoate has a C=O adsorption at 1739cm^{-1}. This was established by the acid-catalysed synthesis of the ester.

The second approach was to attempt isolation the product ester by vacuum distillation. The liqiud removed was analysed by GC and FTIR and found to be heptane. The distillate was also analysed using both GC and FTIR and found to be unreacted octanoic acid. However, both reaction attempts were unsuccessful.

(6.3.1.4) Synthesis of Ethyl and Octyl Octanoate Using Enzyme-Containing Microemulsion-Based Gels

For both of these reactions, a 10 molar equivalent of alcohol to acid was used in order to direct the reaction equilibrium towards the products. Octanoic acid (1.58mL, 10mmoles) and ethanol (5.8mL, 100mmoles) were added to heptane (30mL). An enzyme-containing gel was added to the reaction mixture which was left at 37°C for 2 days. A similar procedure was used for the attempted synthesis of the octyl ester except that ethanol was replaced with octanol (13.0mL, 100mmoles). The large quantity of octanol used caused the complete dissolution of gel. For the ethanol-containing reaction there was partial

dissolution of the gel. Samples were taken from both reactions and analysed by FTIR. They were found to contain unreacted starting materials and so were subsequently discarded.

(6.3.1.5) Synthesis of Ethyl Octanoate Using the Buffered Gel

Octanoic acid (1.58mL, 10mmoles) and ethanol (0.58mL, 10mmoles) were added to heptane (30mL). The buffered gel from section 6.2.1.2, was added to the reaction flask, which was stoppered and left shaking at 25°C for 7 days. After this time the gel was filtered off and washed with heptane. The solvent from the filtrate was subsequently removed using a rotary evaporator to give a pale yellow oil in low yield. This was analysed by GC and found to have a retention time consistent with that found for the same ester made using a conventional acid catalyst. GC-silicone resin column at 230°C: retention time = 1.22 minutes.

(6.3.1.6) Synthesis of Ethyl Octanoate Using Reverse Micelles

Firstly the reverse micelles were formed. *Candida cylindracea* lipase (3mg) was dissolved in 5 drops of pH 7.0 phosphate buffer. Sodium *bis*-2-(ethylhexyl) sulphosuccinate [AOT] (0.01g, 0.02mmoles) was added and the whole mixture put in an ultrasound bath for ~5 minutes to ensure an even dispersion. AOT (0.88g, 2mmoles) was dissolved in heptane (5.3mL) and then the lipase mixture was added. Octanoic acid (1.58mL, 10mmoles) and ethanol (0.58mL, 10mmoles) were added to heptane (30mL). Then the enzyme-containing reverse micelles were added to the heptane mixture. Finally, the reaction flask was stoppered and left shaking in a thermostatted water bath at 37°C for 7 days. After this time, the solvent was removed on a rotary evaporator and the reaction mixture was analysed by GC without prior product purification. The mixture showed the

presence of ethyl octanoate. GC-silicone resin column at 230°C: retention time = 1.23 minutes.

(6.3.1.7) Synthesis of Octyl Octanoate Using Reverse Micelles

The synthesis and purification techniques were carried out as for the synthesis of the ethyl octanoate above, except that octanol (1.3mL, 10mmoles) was used instead of ethanol. The product mixture was also analysed by GC and showed the presence of octyl octanoate. The product was confirmed by comparing the retention time with that of a sample prepared by conventional acid catalysis. GC-silicone resin column at 270°C: retention time = 4.15 minutes.

6.3.1.8) Adsorption of Lipase Onto Glass

A pH 7.2 phosphate buffer (~20mL) of concentration 0.2M, was added to PP lipase (10g). Glass beads (50g) with mesh ~100 were added to the enzyme solution. The mixture was stirred gently, poured into an evaporating dish and left to dry at ambient temperature for ~1 week. During this time, the mixture was stirred occasionally to aid drying. The resulting free-flowing enzyme mixture was transferred to a vacuum dessicator over phosphorus pentoxide at 5°C.

(6.3.1.9) Stabilisation of Lipase Using Potassium Salts

Three different potassium salts were incorporated with lipase at different concentrations.

Twelve lipase samples of 9.80g each were used. Potassium acetate, potassium chloride

and potassium nitrate were incorporated with the enzyme samples at 25%, 50%, 75% and 100% w/w. For each enzyme/salt sample approximately 25mL of pH 7.2 phosphate buffer was used to dissolve the mixtures. The sample numbers (in brackets) and the mass (in grams) of potassium salt added to each 9.80g enzyme portion are shown in the table below.

CH_3COOK	KCl	KNO_3	% w/w
(**1**) 9.80	(**5**) 9.80	(**9**) 9.80	100
(**2**) 7.35	(**6**) 7.35	(**10**) 7.35	75
(**3**) 4.90	(**7**) 4.90	(**11**) 4.90	50
(**4**) 2.45	(**8**) 2.45	(**12**) 2.45	25

Table 21. Experiments using enzyme/potassium salts mixtures

The samples were subsequently freeze dried for a period of several weeks. During the freeze-drying process, the samples were periodically crushed up to aid drying.

6.3.2) Ester Syntheses

6.3.2.1) Synthesis of Bis (2,2,2-trichloroethyl) *trans* 3-hexenedioate (2)

Trans-β-hydromuconic acid (30.0g, 208mmoles), 2,2,2-trichloroethanol (63.0g, 420mmoles) and anhydrous dichloromethane (300mL) were placed in a 1 litre, three-necked round-bottom flask. The flask was equipped with an argon inlet, magnetic stirrer-bar and two condensers in series, i.e. one on top of the other. The lower condenser was a water condenser and the uppermost, a glass-packed distilling column. *N,N'*-dicyclohexylcarbodiimide [DCC] (86.5g, 420mmoles) was first melted by using a warm air drier, prior to being weighed and then dissolved in anhydrous dichloromethane (220mL). The DCC solution and 4-dimethylamino-pyridine [DMAP] (0.6g, 5mmoles)

were added, in rapid succession, to the flask containing the stirred slurry. A vigorous exotherm ensued which caused the dichloromethane to reflux. The reaction was left to react for ~12 hours or overnight, after which time a voluminous white precipitate of *N,N'*-dicyclohexylurea was formed. The precipitate was filtered off and the filtrate washed successively with 3 x 400mL of 5% aqueous HCl, 3 x 400mL of saturated aqueous sodium bicarbonate solution and 3 x 400mL of saturated aqueous sodium chloride solution. After drying over magnesium sulphate overnight, the solvent was removed using a rotary evaporator to give a light yellow/brown precipitate. The product was recrystallised twice from cyclohexane to give an off-white precipitate (58.5g, 69%, m.p. 60-61°C), Lit[79]. 95% (before recrystallisation), m.p.60-61°C. The product was analysed by NMR.

FTIR (cm^{-1};KBr): 2856 (w; ν(C-H)); 1747 (s; ν(C=O))

^1H NMR (δ $CDCl_3$): 5.78 (m, 2H; –C**H**=); 4.73 (s, 4H; -C**H**$_2$CCl_3); 3.25 (m, 4H; -C**H**$_2$-CO_2-)

6.3.2.2) Synthesis of Bis (2,2,2-trichloroethyl) (±)-3,4-Epoxyadipate (1)

Bis(2,2,2-trichloroethyl) *trans* 3-hexenedioate (11.5g, 28mmoles) was dissolved in anhydrous dichloromethane (70mL) in a 1 litre, 3-neck round bottom flask equipped with a magnetic stirrer and an argon inlet. *m*-Chloroperoxybenzoic acid (85%) (12.1g, 70mmoles) was dissolved in anhydrous dichloromethane (125mL) and this solution was added to the reaction flask. The reaction mixture was left to stir at ambient temperature under an argon atmosphere for 3 days after which time a voluminous precipitate appeared. The excess *m*-chloroperoxybenzoic acid was neutralised by adding excess saturated aqueous sodium sulphite solution. To affirm that all of the acid had been destroyed, the mixture was tested using starch/iodide paper. The blue paper turning white was an indication that all of the peroxide had been destroyed. Consequently, the

insoluble *m*-chlorobenzoic acid was filtered off and the filtrate washed successively with saturated sodium sulphite solution (2 x 200mL), saturated sodium bicarbonate solution (2 x 200mL) and saturated sodium chloride solution (2 x 200mL). After drying over magnesium sulphate, the dichloromethane was removed using a rotary evaporator to yield a colourless oil (9.5g, 80%) which crystallised on standing. The product was recrystallised twice from cyclohexane. m.p. 53-54°C. [Lit.[79] 95%, m.p. 55-56°C.]

FTIR (cm^{-1};KBr): 2935 (s; ν(C-H)); 1761 (s; ν(C=O)); 1402 (s; ν(C-O))

^1H NMR(δ $CDCl_3$): 4.74 (s, 4H; -C**H**$_2$CCl_3) 3.20 (m, 2H; **H**-C-O); 2.74 (m, 4H; -C**H**$_2$CO_2-)

6.3.2.3) Synthesis of Bis (2,2,2-trichloroethyl) Glutarate (8)

The procedure for the glutarate ester synthesis was similar to that of the Wallace and Morrow synthesis described above in section 6.3.2.1, but was carried out on a larger scale. The quantities of reagents used were as follows:

Glutaric acid	39.6g, 300mmoles
2,2,2-Trichloroethanol	89.4g, 600mmoles
DCC	123.6g, 600mmoles
DMAP	0.9g, 7mmoles
CH_2Cl_2	500+250mL

The resulting off-white precipitate was recrystallised twice from cyclohexane to yield a white precipitate (77.4g, 61%), m.p.41-42°C, [Lit.[84] 91%, m.p. 41-42°C.].

FTIR (cm^{-1};KBr) 2927 (s; ν(C-H)); 1746 (s; ν(C=O))

^1H NMR(δ $CDCl_3$): 4.70 (s, 4H; -C**H**$_2$CCl_3) 2.53 (t, 4H; -C**H**$_2$CO_2-); 2.00 (pent, 2H; -C**H**$_2$-)

6.3.2.4) Synthesis of Bis (2,2,2-trichloroethyl) Succinate (7)

The procedure for the succinate ester synthesis was similar to that of the Wallace and Morrow synthesis[79] described above in section 6.3.2.1, but was carried out on a larger scale. The quantities of reagents used were as follows:

Succinic acid	23.6g, 200mmoles
2,2,2-Trichloroethanol	59.6g, 400mmoles
DCC	82.4g, 400mmoles
DMAP	0.55g, 4mmoles
CH_2Cl_2	300+150mL

The resulting off-white precipitate was recrystallised twice from hexane to yield 42g (55%); m.p. 48-49 °C

FTIR : ν(C-H) 2939cm^{-1}, ν(C=O) 1757cm^{-1}, ν(C-O) 1143cm^{-1}

^{1}H NMR(δ $CDCl_3$): 4.70 (s, 4H; -C**H**$_2$CCl$_3$) 2.87 (s, 4H; (C**H**$_2$)$_2$)

6.3.2.5) Synthesis of Bis (2,2,2-trichloroethyl) Adipate (9)

The procedure for the adipate ester synthesis was similar to that of the Wallace and Morrow synthesis described above in section 6.3.2.1, but was carried out on a larger scale. The quantities of reagents used were as follows:

Adipic acid	58.4g, 400mmoles
2,2,2-Trichloroethanol	119.2g, 800mmoles
DCC	164.8g, 800mmoles

DMAP	1.4g, 11mmoles
CH_2Cl_2	700+300mL

The resulting off-white precipitate was recrystallised twice from cyclohexane to give a 50% yield. m.p.=47-48°C, [Lit.[84] 96%, m.p. 44.5-46°C.];
^{1}H NMR(δ $CDCl_3$): 4.66 (s, 4H; -CH_2CCl_3) 2.43 (t, 4H; -CH_2CO_2-); 1.89 (pent, 4H; (-CH_2CH_2-)

6.3.2.6) Synthesis of Bis (2,2,2-trichloroethyl) Terephthalate

Terephthalic acid (30.00g, 181mmoles), 2,2,2-trichloroethanol (53.86g, 361mmoles), DCC (74.46g, 361mmoles), DMAP (0.52g, 4.29mmoles) and anhydrous dichloromethane (400+200mL) were used for the synthesis of the title ester. The procedure carried out was as described for the synthesis and work-up of *bis*(2,2,2-trifluoroethyl) glutarate. The light yellow product was recrystallised twice from toluene to give a white solid in quantitative yield. m.p. 104-106°C [Lit[106]. m.p. 106°C.]

FTIR (cm^{-1};KBr): 3293 (s; aromatic ν(C-H)); 2932, 2883 (s, m; aliphatic ν(C-H)); 1736 (s; ν(C=O)); 1698, 1645 (m, s; ν(C=C)); 1545 (m; ν(C-O))

6.3.2.7) Synthesis of Bis (2,2,2-trifluoroethyl) Glutarate (14)

The procedure for the glutarate ester synthesis was slightly different to that of the Wallace and Morrow synthesis described in section 6.2.2.1. Glutaric acid (48.0g, 360mmoles), 2,2,2-trifluoroethanol (72.7g, 727mmoles) and anhydrous CH_2Cl_2 (600mL) were placed in a 1 litre flask as described previously. This time, however, no argon was used. The reaction mixture was stirred and placed in an ice bath at ~0°C. DMAP (1.1g, 9mmoles)

was added and the mixture allowed to stir for a further 15 minutes. Then DCC (150g, 727mmoles) dissolved in CH_2Cl_2 (300mL), was added with continued stirring for an additional 10 minutes. The ice-bath was removed and the reaction mixture allowed to stir at ambient temperature for 4 days. The product was isolated as described in section 6.3.2.1 and recrystallised from cyclohexane to yield a white precipitate (128g, 54%). [Lit.[83] 90%, 0.05mm Hg; 55-58°C.]

FTIR (cm^{-1};KBr): 2937 (s; ν(C-H)); 2860 (m, ν(C-H)); 1761 (m; ν(C=O))

^1H NMR (δ $CDCl_3$): 4.33 (q, 4H; -OC**H**$_2$CF$_3$); 2.35 (t, 4H; -C**H**$_2$CO$_2$-); 1.86 (m, 2H; -C**H**$_2$-); 1.57, 1.16 (m; impurity)

6.3.2.8) Synthesis of Bis (2,2,2-trifluoroethyl) *trans* 3-Hexenedioate

The procedure was similar to that carried out for the the synthesis of bis(2,2,2-trifluoroethyl) glutarate above except that *trans*-β-hydromuconic acid (30.0g, 207mmoles) was used instead of glutaric acid. Also the quantities of the other components were as shown below.

2,2,2-trifluoroethanol	63.00g, 414mmoles
anhydrous CH_2Cl_2	400mL + 220mL
DMAP	0.6g, 5mmoles
DCC	86.7g, 414mmoles

The product was recrystallised twice from cyclohexane to give a white precipitate (33g, 52%). Analysis by FTIR was carried out to determine whether the reaction had been successful: ν(C-H) 2932cm^{-1}, ν(C=O) 1748cm^{-1}, ν(C=C) 1660cm^{-1}, ν(C-O) 1158cm^{-1}

^1H NMR (δ $CDCl_3$): 5.63 (m, 2H; –C**H**=); 440 (s, 4H; -C**H**$_2$CF$_3$); 3.43 (q, 4H; -C**H**$_2$-CO$_2$-)

6.3.2.9) Syntheses of Bis (2,2,2-trifluoroethyl) Malic Acid and Bis (2,2,2-trifluoroethyl) Malonic Acid

Both malic and malonic acids were dried in a vaccuum oven for ~4 hours before undergoing reaction. DL-Malic acid (13.40g, 100mmoles), 2,2,2-trifluoroethanol (20.0g, 200mmoles), CC lipase (5g) and 4Å molecular sieves (~20g) were added to anhydrous THF (80mL). The reaction mixture was stirred in a stoppered flask at ambient temperature for ~1 day. The enzyme was filtered off and washed with THF. The solvent from the filtrate and combined washings was removed using a rotary evaporator. The resulting oil was analysed by FTIR and found to be unreacted starting acid.

A similar procedure was used for the esterification of malonic acid (10.40g, 100mmoles). The FTIR results of this product also showed the presence of unreacted starting acid.

6.3.2.10) Synthesis of Divinylglutarate (16)

Glutaric acid (13.2g, 100mmoles) and vinyl acetate (86.0g, 1mole) were mixed together in a flask supplied with an argon line. The reaction mixture was warmed to ~60°C to dissolve the glutaric acid. Mercuric acetate (0.40g, 1mmole) was added and the reaction mixture stirred at 50-60°C for appproximately 30 minutes. Then one drop of 98% sulphuric acid was added and the reaction mixture heated under reflux for ~3hours. The mixture was allowed to cool and subsequently sodium acetate trihydrate was added to neutralise any sulphuric acid. The reaction mixture was distilled under a vacuum of 0.05 mmHg. The excess vinyl acetate distilled off at ~30°C and the product, divinylglutarate distilled off at ~50°C. The product was analysed by FTIR.

FTIR (cm^{-1};KBr): 2943 (m; ν(C-H)); 1757 (s; ν(C=O)); 1648 (s; ν(C=C))

^1H NMR(δ $CDCl_3$): 5.95 (m, 2H;=C**H**); 5.20 (d, 4H; =C**H**$_2$); 2.48 (t, 4H; (-C**H**$_2$$CO_2$-)

2.10 (pent, 2H; -C**H**$_2$-)

6.3.2.11) Synthesis of *N,N'*-Glutaryl Dicyclohexanone Oxime (21)

Cyclohexanone oxime (22.6g, 200mmoles) was dissolved in anhydrous dichloromethane (~125mL). Pyridine (15.8g, 200mmoles) was added and the mixture stirred at ambient temperature for a few minutes, under an argon atmosphere. Glutaryl dichloride (16.90g, 100mmoles) was added slowly. On addition of the glutaryl dichloride an exotherm occured. The glutaryl dichloride was added at a rate which did not allow the exotherm to exceed ~50°C. The orange coloured precipitate was filtered, washed with dichloromethane and recrystallised twice from cyclohexane in quantitative yield.

FTIR (cm^{-1};KBr) 2937 (s; ν(C-H); 2860 (m, ν(C-H); 1761 ν(C=O); 1640 ν(C=N); 1449 ν(C-O)

^1H NMR(δ $CDCl_3$): 8.15 (m; solvent impurity); 7.28 (m; solvent impurity); 6.87 (m; solvent impurity); 2.10 (t, 8H; 2,6-positions cyclohex-ring); 1.92 (t,4H;-CH_2CO2-); 1.62 (p, 2H; -CH_2-); 1.26 (m, 12H; 3,4,5-positions cyclohex-ring)

6.3.2.12) Synthesis of Diphenyl glutarate (19)

Glutaric acid (13.2g, 100mmoles), phenol (18.8g, 200mmoles), DCC (41.2g, 200mmoles), DMAP (0.29g) and anhydrous dichloromethane (200+150mL) was used to synthesise the title ester. The procedure carried out was as described for the synthesis and work-up of bis(2,2,2-trifluoroethyl) glutarate except that phenol was used in place of the alcohol, 1,4-butanediol. The white product (obtained in quantitative yield) was recrystallised twice from cyclohexane and analysed by NMR, m.p. 45°C [Lit.[107] m.p. 45-46°C.]

^1H NMR(δ $CDCl_3$): 7.41 (t, 4H; *m*--Ph protons); 7.23(t, 2H; *p*-Ph protons); 7.12 (d4H; *o*--Ph protons); 2.73 (t, 4H; -CH_2CO_2-) 2.21 (pent, 2H; -CH_2-)

6.3.2.13) Synthesis of Diphenyladipate (20)

The procedure was exactly as for the synthesis of diphenylglutarate except that adipic acid (14.60g, 100mmoles) was used instead of glutaric acid. The white product (obtained in quantitative yield) was recrystallised twice from cyclohexane and analysed by NMR. m.p. 106-107°C [Lit.[108] m.p. 106°C.]

^{1}H NMR(δ $CDCl_3$): 7.25 (m, 4H; *o*-Ph protons); 7.19 (m, 2H; *p*-Ph protons); 7.07 (m, 4H; *m*-Ph protons); 2.65 (m, 4H; -CH_2CO_2-); 2.60 (m; impurity); 1.89 (m, 4H; -CH_2-CH_2-); 1.85(m;impurity)

6.4) Polyester Synthesis

6.4.1) Explorative Techniques

6.4.1.1) Polymerisation of Bis (2,2,2-trichloroethyl) Succinate with 1,4-Butanediol, Using a Nitrogen-Filled Balloon (E-131)

Bis(2,2,2-trichloroethyl) succinate (5.0g, 13mmoles), 1,4-butanediol (1.18g, 13mmoles) and porcine pancreatic lipase (4.0g) were added to anhydrous isopropyl ether (20mL) in a 100mL round bottom flask containing a magnetic stirrer bar. A three way tap onto which a balloon was fitted was used to stopper the flask. The ballon was first filled with nitrogen. The flask was evacuated using a vaccum pump and subsequently flushed with nitrogen from the balloon. This process of evacuating the flask and then flushing with nitrogen was repeated several times to ensure that all of the air had been driven from the flask and hence was filled with a dry nitrogen atmosphere. The reaction mixture was left to stir at ambient temperature for over 2 weeks. The lipase was filtered off and washed

with anhydrous THF. The filtrate was poured onto anhydrous diethyl ether to precipitate out the product polyester. A very small quantity of needle-like crystals were formed overnight. These were not analysed as there was an insufficient amount for either NMR or GPC.

6.4.1.2) Polymerisation of Bis (2,2,2-trichloroethyl) Succinate with Butane-1,4-diol Using a Nitrogen Line (E-130)

The same quantity of reagents were used as for the previous reaction except that THF was used instead of isopropyl ether. The reaction differed also from the previous one in that the a continuous flow or blanket of nitrogen was used. The reaction flask was fitted with a reflux condenser to minimise solvent loss by evaporation, as well as a nitrogen inlet tube and a gas bubbler or trap. The reaction was left for the same time period and worked up under the same conditions as mentioned above. A white precipitate formed immediately on addition to diethyl ether.

6.4.1.3) Polyester Synthesis Using an Argon Atmosphere (E-132)

Bis(2,2,2-trichloroethyl) succinate (5.0g, 13mmoles), butane-1,4-diol (1.18g, 13mmoles) and porcine pancreatic lipase (0.5g) were added to anhydrous THF (20mL) under an argon atmosphere. The reaction mixture was left to stir at ambient temperature for 2 weeks. The enyme was filtered off and washed with THF. The filtrate and washings were combined and the solvent removed using a rotary evaporator. The resulting viscous oil was poured onto cold diethyl ether to precipitate off the product polyester. A light brown precipitate was formed which was filtered off and analysed by GPC in THF : Mn 800, Mw 950

6.4.1.4) Synthesis of Polyesters From Butyrolactones

β-Butyrolactone (2.15g, 25mmoles) and porcine pancreatic (PP) lipase (5g) was added to anhydrous dichloromethane (20mL). The reaction was left to stir at ambient temperature, under a nitrogen atmosphere for 2 weeks. The reaction was worked up as described in section 6.4.1.7. No apparent reaction was observed.

This procedure was repeated for γ-butyrolactone. The procedure was again repeated for other γ-butyrolactone reactions using anhydrous THF as solvent, anhydrous dichloromethane and anhydrous toluene. Additionally, three other γ-butyrolactone reactions were carried out using *Candida cylindracea* (CC) lipase (1g) instead of PP lipase. The reactions were carried out using the solvents, anhydrous THF, anhydrous dichloromethane and anhydrous toluene. No apparent reaction was observed for any of the reactions.

6.4.1.5) Reactions at Elevated Temperatures (E-133, E-134)

Two reactions were set up in a thermostatted water bath at 43°C. The reaction flasks were stirred using a magnetic stirrer under a continuous nitrogen flow atmosphere at ambient temperature. The reaction flasks were fitted with water cooled reflux condensers to minimise the solvent lost by evaporation and a silicone oil filled gas trap. The reactions used the following reagents:

43:- Bis(2,2,2-trichloroethyl) succinate (5g, 13mmoles), butane-1,4-diol (2.25g, 25mmoles), THF (25mL), PP lipase (5g)

44:- Bis(2,2,2-trichloroethyl) succinate (5g, 13mmoles), butane-1,4-diol (2.25g, 25mmoles), THF (25mL), CC lipase (5g)

Both reactions were left for approximately 3 weeks. No apparent reaction was observed for either of the systems.

6.4.1.6) Polymerisation of Bis (2,2,2-trichloroethyl) (±)-3,4-Epoxyadipate (E-8)

Bis(2,2,2-trichloroethyl) (±)-3,4-epoxyadipate (5.54g, 13mmoles), butane-1,4-diol (0.59g, 6.5mmoles) and PP lipase (3.75g) were added to anhydrous diethyl ether (27mL) in a 100mL round bottom flask. The flask was fitted with a magnetic stirrer, nitrogen inlet line with oil trap and a glass-packed column condenser to minimise solvent loss by evaporation. The reaction mixture was left to stir at ambient temperature for 1 week. The enzyme was filtered off and washed with anhydrous dichloromethane. The filtrate and washings were combined and the solvent removed using a rotary evaporator, to yield ~3mL of a yellow oil. Anhydrous diethyl ether (~5mL) was added to the oil and stirred for ~1hour. The oil was miscible with the ether indicating that the polyester had not been formed. The ether was subsequently removed using a rotary evaporator and a fresh quantity of enzyme added to the oil. Dichloromethane (25mL), which had not been dried and was used as supplied by the manufacturer, was added to the enzyme and oil and the reaction left to stir, as set up previously, for an additional 5 days. The enzyme was filtered off and the washing process repeated. Diethyl ether (10mL) was added to the oil and stirred for ~1 hour. This time the oil was immiscible with the ether. The ether was decanted off and more ether added and the ether washing process repeated. The ether layers were combined and the solvent removed to yield a yellow/brown oil. This ether-layer oil was analysed by FTIR. The polyester oil was also analysed by NMR and GPC.

GPC (Viscosity results): Mn 6,100, Mw 8,700

GPC (Conventional results): Mn 2,250, Mw 4,050

^{1}H NMR(δ $CDCl_3$): 4.10 (s, 4H; O-($C\mathbf{H}_2$)$_2$); 3.89 (m, impurity); 3.60 (t, 2H; terminal HO-($C\mathbf{H}_2$)$_2$); 3.42 (q; solvent impurity); 3.16 (t, 2H; **H**-C-O); 2.60 (m, 4H; C(O)-($C\mathbf{H}_2$)$_2$; 1.68 (s, 4H; O-(CH_2-$C\mathbf{H}_2$)$_2$)

6.4.1.7) Polyester Synthesis Using Ultrasound (E-9 - E-20)

The reactions were set up in sealed flasks which, in turn, were set up in an ultrasound bath. The reactions were subjected to intermittent doses of 15 minutes of ultrasound radiation. On average the reactions underwent ultrasound treatment for about 15 minutes every hour. The water from the water bath tended to warm up so was changed fairly frequently, not being allowed to exceed 40°C. The reactions were set up with various reagents as follows:

E-9:- Bis(2,2,2-trichloroethyl) adipate (4.6g, 11mmoles), 1,4-butanediol (0.99g, 11mmoles), PP lipase (6g), anhydrous THF (25mL)

E-10:- Bis(2,2,2-trichloroethyl) succinate (4.2g, 11mmoles), 1,4-butanediol (0.99g, 11mmoles), PP lipase (6g), anhydrous THF (25mL)

E-11:- Bis(2,2,2-trichloroethyl) adipate (4.6g, 11mmoles), 1,6-hexanediol (1.3g, 11mmoles), PP lipase (6g), anhydrous THF (25mL)

E-12:- Bis(2,2,2-trichloroethyl) succinate (4.2g, 11mmoles), 1,6-hexanediol (1.3g, 11mmoles), PP lipase (6g), anhydrous THF (25mL)

E-13:- Bis(2,2,2-trichloroethyl) adipate (4.6g, 11mmoles), polyethylene glycol 200 (2.2g, 11mmoles), PP lipase (6g), anhydrous CH_2Cl_2 (20mL)

E-14:- Bis(2,2,2-trichloroethyl) succinate (4.2g, 11mmoles), polyethylene glycol 200 (2.2g, 11mmoles), PP lipase (6g), anhydrous CH_2Cl_2 (20mL)

E-15:- Bis(2,2,2-trichloroethyl) adipate (4.6g, 11mmoles), polyethylene glycol 1000 (11.0g, 11mmoles), PP lipase (6g), anhydrous CH_2Cl_2 (20mL)

E-16:- Bis(2,2,2-trichloroethyl) adipate (4.6g, 11mmoles), polycaprolactone diol 1250 (13.8g, 11mmoles), PP lipase (6g), anhydrous CH_2Cl_2 (20mL)

E-17:- Bis(2,2,2-trichloroethyl) adipate (4.6g, 11mmoles), polycaprolactone triol 300 (3.3g, 11mmoles), PP lipase (6g), anhydrous CH_2Cl_2 (20mL)

E-18:- Bis(2,2,2-trichloroethyl) succinate (4.2g, 11mmoles), polyethylene glycol 1000 (11.0g, 11mmoles), PP lipase (6g), anhydrous CH_2Cl_2 (20mL)

E-19:- Bis(2,2,2-trichloroethyl) succinate (4.2g, 11mmoles), polycaprolactone diol 1250 (13.8g, 11mmoles), PP lipase (6g), anhydrous CH_2Cl_2 (20mL)

E-20:- Bis(2,2,2-trichloroethyl) succinate (4.2g, 11mmoles), polycaprolactone triol 300 (3.3g, 11mmoles), PP lipase (6g), anhydrous CH_2Cl_2 (20mL)

Work-up Procedure

After 5 days the enzyme was filtered off and washed several times with CH_2Cl_2. The solvent from the filtrate and combined washings was removed using a rotary evaporator to yield a yellow oil. The oil was poured onto diethyl ether to precipitate out the product polyester. The mixture was stirred for ~1 hour. The ether was decanted off and more ether added and the ether washing process repeated. The precipitate was filtered off and

analysed by FTIR and GPC. The remaining ether solution was put on the rotary evaporator to remove the ether and the oil analysed by NMR.

Sample	Mn	Mw
E-9	150	150
E-10	150	150
E-11	150	150
E-12	150	150
E-13	150	150
E-14	150	150
E-15	800	1,500
E-16	1,650	7,000
E-17	850	3,150
E-18	500	1,100
E-19	1,150	6,550
E-20	550	2,100

Table 22. GPC results of expts **E-9 - E-20** (analysed in THF)

6.4.1.8) Polymerisation Using a Two-Chambered Vessel (E-40 - E-43)

A series of experiments were carried out using a two chambered vessel (see Figure 10) as used by Roberts *et al*[85].

(**E-42**):- The first chamber contained *trans*-β-hydromuconic acid (19.73g, 137mmoles), butane-1,4-diol (12.33g, 137mmoles) and *Candida cylindracea* lipase (6g). The second

chamber contained 4Å molecular sieves (~25g) and a magnetic stirrer. The reaction vessel was then filled (over the connecting mesh) with anhydrous diisopropyl ether (150mL). The reaction mixture was left to stir at ambient temperature for ~4 days. After this time, first the molecular sieves were filtered off and washed with THF and then the enzyme. The solvent from the filtrate and combined washings was removed using a rotary evaporator to yield a white solid. The solid product was analysed by FTIR and found to be unreacted starting material.

The reaction was repeated using adipic acid (20.00g, 137mmoles) instead of *trans*-β-hydromuconic acid (**E-40**).

Additionally both adipic acid (**E-41**) and *trans*-β-hydromuconic acid (**E-43**) underwent further reactions using lipozyme enzyme (6g) instead of CC lipase.

In all cases the products were analysed by FTIR and found to be unreacted starting material.

6.4.1.9) Polyesterification of *Trans*-β-hydromuconic acid at an Elevated Temperature (E-37)

Trans-β-hydromuconic acid (15.00g, 104mmoles), butane-1,4-diol (4.69g, 52mmoles), 4Å molecular sieves (~20g), anhydrous THF (150mL) and lipozyme enzyme (2.28g) were stirred in a stoppered flask at ~40°C for 4 weeks. It was observed that THF was not a good solvent for the acid, which was only partially dissolved. The reaction mixture was worked up as described in section 6.4.1.7.

6.4.1.10) Polyester Synthesis Using Excess Quantities of Enzyme (E-44, E-45, E-46)

(**E-44**):- Bis(2,2,2-trichloroethyl) adipate (4.6g, 11mmoles), PP lipase (3.2g) and butane-1,4-diol (0.99g, 11mmoles) were added to anhydrous diethyl ether (25mL). The mixture was stirred in a sealed flask at ambient temperature for 3 weeks.

(**E-45**):- A similar experiment was set up except using 5 times the amount of lipase *viz* 16.0g and 75mL of ether.

(**E-46**):- Again 5 times the amount of enzyme was used but added in batches over a period of two weeks. The reaction was set up with 3.2g of enzyme and 25mL of solvent. After 3 days an additional 3.2g of enzyme and 25mL of solvent were added. After a total time period of 6 days the same quantity of both enzyme and solvent were added. The remaining two 3.2g portions of enzyme were added after a total time period of 9 and 12 days. In summary a 3.2g portion of enzyme was added every 3 days until a total of 16.0g of enzyme had been added.

The reactions were worked up after 3 weeks, as described in the work-up procedure in section 6.4.1.7.

NMR :- very poor spectra were obtained

6.4.1.11) Polyester Synthesis Using Enzyme Adsorbed on Glass

Bis(2,2,2-trichloroethyl) succinate (4.2g, 11mmoles), butane-1,4-diol (0.99g, 11mmoles) and PP lipase either as a dry powder or adsorbed onto glass (see section 6.3.1.8) were added to diethyl ether (25mL). The reaction mixtures were left to stir in

sealed flasks at ambient temperature for 2 weeks. The reactions were set up with the following variations:

1) Used 'wet' ether as supplied by the manufacturer and glass/enzyme
2) Used dry ether and glass/enzyme
3) Used 'wet' ether as supplied by the manufacturer and dry powder enzyme
4) Used dry ether and dry powder enzyme

The reactions were worked up after 2 weeks, as described in the work-up procedure in section 6.3.1.7 and analysed by GPC. No apparent reaction.

6.4.1.12) Polyester Synthesis Reusing a Previous Polyester (E-47)

The product polyesters from experiments **E-44** and **E-45** were pooled together and reacted further with more diol and enzyme. The previous 'polyester' was calculated to have the structure $HO(CH_2)_4$-$OC(O)(CH_2)_4(O)CO$-$(CH_2)_4OH$ and molecular weight 290. The ester (0.16g, 0.6mmoles), butane-1,4-diol (0.05g, 0.6mmoles) and PP lipase (0.15g) were added to anhydrous dichloromethane (5mL). The mixture was stirred at ambient temperature for ~2 weeks in a sealed flask. After this time, the reactions were worked up as described in the work-up procedure in section 6.3.1.7 and analysed by GPC.

6.4.2) Advanced Modifications

6.4.2.1) Polyester Synthesis Using 1,2-Dimethoxybenzene

Bis(2,2,2-trichloroethyl) (±)-3,4-epoxyadipate (9.27g, 30mmoles), butane-1,4-diol

(1.35g, 15mmoles) and PP lipase (8.8g) was added to 1,2-dimethoxybenzene (75mL). The reaction mixture was stirred in a stoppered flask at ambient temperature for 9 days. During this time, the reaction mixture was subjected to a high vacuum (~0 mmHg), for an average of two, 10 minute sessions a day. This was done in order to remove any unwanted 2,2,2-trifluoroethanol produced as a by-product of the polymerisation reaction. On several occasions, whilst under vacuum, the reaction flask was heated to ~40°C to aid alcohol removal. After 9 days had elapsed the enzyme was filtered off and washed with dichloromethane. The 1,2-dimethoxybenzene was removed by vacuum distillation. The resulting product was analysed by FTIR and found to be unreacted starting material.

6.4.2.2) Comparison of Techniques Using Bis(2,2,2-trichloroethyl) Adipate and Bis(2,2,2-trichloroethyl) Glutarate

Two series of experiments were carried out using slightly different reaction conditions. The first set of experiments were undergone using bis(2,2,2-trichloroethyl) adipate. The second set of experiments were identical to the first set, except that bis(2,2,2-trichloroethyl) glutarate was the ester used.

Bis(2,2,2-trichloroethyl) adipate (4.09g, 10mmoles), butane-1,4-diol (0.90g, 10mmoles) and PP lipase (6g) were added to anhydrous dichloromethane (50mL). The reaction mixture was stirred in a stoppered flask at ambient temperature for 10 days. Prior to any stirring, the reaction mixture was put in an ultrasound bath for about 5 minutes in order to ensure a thorough and uniform dispersal of the components.

(**E-58**):- For this experiment both the enzyme and the solvent were changed after 3 days. Two thirds of the reaction mixture was removed from the flask whilst the remainder was allowed to continue reacting until 10 days had elapsed . For the two thirds removed the enzyme was filtered off and washed with dichloromethane. The solvent was removed

and the resulting oil was divided into two. The first half was added to diethyl ether and stirred for about 1 hour in order to precipitate out the product polymer. The ether was decanted off and more ether added to repeat the washing process. The ether was removed and the product allowed to dry at ambient temperature (**E-66**). The remaining half-portion was put on to react further using both fresh enzyme (0.3g) and fresh solvent (5mL). It was then left to react for a further 7 days, making a total of 10 days (**E-59**). The products **E-58** and **E-59** were worked up as for **E-66** and all three were analysed by both NMR and GPC.

The procedure was the same as above, except that anhydrous diethyl ether was used instead of dichloromethane. Products **E-70**, **E-60** and **E-61** were analogous to **E-66**, **E-58** and **E-59** respectively.

The procedure was the same as above, except that anhydrous diethyl ether was used instead of dichloromethane and 4Å molecular sieves (~20g) were used in addition. Fresh molecular sieves were also used after 3 days for **E-72**. Products **E-71**, **E-74** and **E-72** were analogous to **E-66**, **E-58** and **E-59** respectively.

The reactions for bis(2,2,2-trichloroethyl) glutarate (3.95g, 10mmoles) were carried out exactly as for the bis(2,2,2-trichloroethyl) adipate reactions.

Reaction numbers **E-79**, **E-62** and **E-63** were analogous to **E-70**, **E-60** and **E-61** respectively.

Reaction numbers **E-87**, **E-64** and **E-65** were analogous to **E-71**, **E-74** and **E-72** respectively.

All of the products were analysed by both NMR and GPC.

^{1}H NMR(δ $CDCl_3$): Sample **E-58**:- very unclear spectrum was obtained

Sample [**E-70**]: 4.03 (s, 4H; O-(C**H**$_2$-CH$_2$)$_2$); 3.59 (t,2H; terminal HO-(C**H**$_2$)$_2$); 2.26 (s, 4H; C(O)(C**H**$_2$)$_2$); 1.61 (m, 8H; (C**H**$_2$-C**H**$_2$)$_2$)

Sample [**E-61**]: 4.03 (s, 4H; O-(C**H**$_2$-CH$_2$)$_2$); 3.59 (t,2H; terminal HO-(C**H**$_2$)$_2$); 2.26 (s, 4H; C(O)(C**H**$_2$)$_2$); 1.61 (m, 8H; (C**H**$_2$-C**H**$_2$)$_2$)

Sample [**E-60**]: 4.07 (s; impurity); 4.03 (s, 4H; O-(C**H**$_2$-CH$_2$)$_2$); 3.59 (t,2H; terminal HO-(C**H**$_2$)$_2$); 2.26 (s, 4H; C(O)(C**H**$_2$)$_2$); 1.61 (m, 8H; (C**H**$_2$-C**H**$_2$)$_2$); 1.14 (m; vacuum grease impurity)

Sample [**E-79**]: very unclear spectrum was obtained

Sample	Mn	Mw - Comments
E-66	No detector response	
E-58	300	1,050
E-70	3,300	6,100
E-61	2,100	6,100
E-60	1,850	6,550
E-71	6,000	9,400
E-72	2,200	8,900
E-74	1,550	6,700
E-79	450	450
E-87	10,700	843,000 - contained some insoluble material
E-65	900	55,800

Table 23. GPC results of experiments **E-58 - E-87**, analysed in THF

6.4.2.3) Polymerisations of Bis(2,2,2-trichloroethyl) Adipate.(E-73, E-67, E-68, E-69)

Four experiments were undergone using bis(2,2,2-trichloroethyl) adipate under slightly different reaction conditions. Bis(2,2,2-trichloroethyl) adipate (4.09g, 10mmoles), butane-1,4-diol and PP lipase (6g) were used for each experiment. For experiments **E-73** and **E-69** the solvent used was anhydrous diethyl ether. For experiments **E-67** and **E-68** anhydrous THF was used. The reactions were stirred in sealed flasks at ambient temperature for 15 days. For experiment 111, after 3 days the enzyme was filtered off and washed with anhydrous dichloromethane. The filtrate and washings were combined and the solvent removed using a rotary evaporator. Then both fresh THF and lipase was added and the reaction resumed until a total time of 15 days had elapsed. For experiment 112, the solvent was removed using a rotary evaporator, every few days and replaced with new ether.

All of the experiments were worked up in the usual way as described in 6.3.1.7 and subsequently analysed by both NMR and GPC.

^{1}H NMR(δ $CDCl_3$): 4.08 (s; impurity); 4.03 (s, 4H; O-(CH_2-CH_2)$_2$); 3.62 (t,2H;
Sample [**E-73**] terminal HO-(CH_2)$_2$); 2.26 (s, 4H; C(O)(CH_2)$_2$); 1.61 (m, 8H; (CH_2-CH_2)$_2$)

Sample [**E-68**]:- Appeared to be spectrum of the enzyme

Sample [**E-69**]: 4.08 (s; impurity); 4.03 (s, 4H; O-(CH_2-CH_2)$_2$); 3.62 (t,2H; terminal HO-(CH_2)$_2$); 2.26 (s, 4H; C(O)(CH_2)$_2$); 1.61 (m, 8H; (CH_2-CH_2)$_2$)

Sample	Mn	Mw - Comments
E-73	1,450	4,750
E-68	300	2,350 -predominantly insoluble
E-69	2,050	8,250

Table 24. GPC results of expts **E-68, E-69, E-73** (analysed in THF)

6.4.2.4) Polymerisations of Bis(2,2,2-trichloroethyl) Glutarate, Bis(2,2,2-trifluoroethyl) Glutarate and Bis(2,2,2-trifluoroethyl) (±)-3,4-Epoxyadipate

All of the experiments were carried out on a 10 mmolar scale and had a total reaction time of 9 days. The quantities of reagents used for each experiment were as follows.

Note:- For experiments carried out with molecular sieves, ~10g of 4Å pore size sieves were used.

Bis(2,2,2-trichloroethyl) glutarate polymerisations:

Bis(2,2,2-trichloroethyl) glutarate (3.95g, 10mmoles)

Butane-1,4-diol (0.90g, 10mmoles)

PP lipase (6g)

Anhydrous solvent (50mL)

Bis(2,2,2-trifluoroethyl) glutarate polymerisations:

Bis(2,2,2-trifluoroethyl) glutarate (2.96g, 10mmoles)

Butane-1,4-diol (0.90g, 10mmoles)

PP lipase (6g)

Anhydrous solvent (50mL)

Bis(2,2,2-trifluoroethyl) (±)-3,4-epoxyadipate polymerisations:

Bis(2,2,2-trifluoroethyl) (±)-3,4-epoxyadipate (3.24g, 10mmoles)

Butane-1,4-diol (0.90g, 10mmoles)

PP lipase (6g)

Anhydrous solvent (50mL)

6.4.2.4.1) 'Blank' Experiments E-75, E-80, E-84, E-91, E-95, E-99, E-103, E-107, E-140, E-141

The appropriate reagents were added together in sealed flask and stirred at ambient temperature for 9 days. A range of different anhydrous solvents were used for the experiments.

E-91, E-107, E-140	-carried out in anhydrous diethyl ether
E-75, E-95	-carried out in anhydrous dichloromethane
E-80, E-99	-carried out in anhydrous THF
E-84, E-103, E-141	-carried out in anhydrous toluene

Thereafter, the reactions were worked up in the usual way as described in 6.3.1.7. The products were subsequently analysed by both NMR and GPC.

^{1}H NMR(δ $CDCl_3$): 4.68 (m, 2H; terminal $Cl_3C\mathbf{H}_2$); 4.07 (s; impurity); 4.04 (s, 4H; O-($C\mathbf{H}_2$-CH_2)$_2$); 3.60 (t, 2H; terminal HO-($C\mathbf{H}_2$)$_2$); 2.30 (t, 4H; C(O)($C\mathbf{H}_2$)$_2$); 1.87 (pent, 2H; CH_2-$C\mathbf{H}_2$); 1.64 (m, 4H; O-(CH_2-$C\mathbf{H}_2$)$_2$); 1.17 (m; vacuum grease impurity)

Sample [**E-91**]

Sample [**E-107**] 3.96 (s, 4H; O-(C**H**$_2$-CH$_2$)$_2$); 3.50 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 3.33 (q; solvent impurity); 2.23 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.79 (m, 2H; CH$_2$-C**H**$_2$); 1.62 (m, 4H; O-(CH$_2$-C**H**$_2$)$_2$); 1.14 (m; vacuum grease impurity)

Sample [**E-75**]: 4.93 (m, 2H; terminal Cl$_3$C**H**$_2$); 4.07 (s; impurity); 4.04 (s, 4H; O-(C**H**$_2$-CH$_2$)$_2$); 3.60 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 2.47 (m, impurity); 2.30 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.87 (pent, 2H; CH$_2$-C**H**$_2$); 1.64 (m, 4H; O-(CH$_2$-C**H**$_2$)$_2$); 1.17 (m; vacuum grease impurity)

Sample [**E-95**]: 4.37 (o, 2H; terminal F$_3$C-C**H**$_2$); 3.98 (s, 4H; O-(C**H**$_2$-CH$_2$)$_2$); 3.83 (m; impurity); 3.54 (t, 2H; terminal O-(C**H**$_2$)$_2$); 2.38 (m; impurity); 2.26 (m, 4H; C(O)(C**H**$_2$)$_2$); 1.88 (m, 2H; CH$_2$-C**H**$_2$); 1.60 (m, 4H; O-(CH$_2$-C**H**$_2$)$_2$); 1.27 (m; vacuum grease impurity)

Sample [**E-80**]: 5.39 (water impurity); 4.93 (m, 2H; terminal Cl$_3$C**H**$_2$); 4.07 (s; impurity); 4.04 (s, 4H; O-(C**H**$_2$-CH$_2$)$_2$); 3.60 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 2.47 (m, impurity); 2.30 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.87 (pent, 2H; CH$_2$-C**H**$_2$); 1.57 (m, impurity); 1.64 (m, 4H; O-(CH$_2$-C**H**$_2$)$_2$); 1.17 (m; vacuum grease impurity)

Sample [**E-99**] very poor spectrum was obtained

Sample[**E140**] 4.06 (s, 4H; O-(C**H**$_2$-CH$_2$)$_2$); 3.64 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 3.33 (q; solvent impurity); 2.34 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.90 (m, 2H; CH$_2$-C**H**$_2$); 1.66 (m, 4H; O-(CH$_2$-C**H**$_2$)$_2$)

Sample[**E141**] 4.67 (s, 2H; terminal F$_3$C-C**H**$_2$); 4.07 (s; impurity); 4.02 (s, 4H; O-(C**H**$_2$-CH$_2$)$_2$); 3.59 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 2.42 (m, impurity);

2.26 (s, 4H; C(O)(C**H**$_2$)$_2$); 1.61 (m, 8H; (C**H**$_2$-C**H**$_2$)$_2$); 1.14 (m; impurity)

Sample[**E103**] 4.40 (o, 2H; terminal F_3C-C**H**$_2$); 4.02 (s, 4H; O-(C**H**$_2$-CH_2)$_2$); 3.54 (t, 2H; terminal O-(C**H**$_2$)$_2$); 2.42 (m; impurity); 2.30 (m, 4H; C(O)(C**H**$_2$)$_2$); 1.90 (m, 2H; CH_2-C**H**$_2$); 1.60 (m, 4H; O-(CH_2-C**H**$_2$)$_2$); 1.27 (m; vacuum grease impurity)

Sample	Mn	Mw - Comments
E-91	1,550	5,350
E-107	1,000	1,700
E-75	200	450
E-95	500	700
E-80	300	600
E-99	500	1,400
E-140	1,350	8,350
E-84	2,850	4,500
E-141	750	3,100
E-103	1,650	3,400

Table 25. GPC results of experiments **E-84 - E-140** analysed in THF

6.4.2.4.2) Experiments Using 4Å Molecular Sieves (E-76, E-81, E-85, E-92, E-96, E-100, E-104, E-108)

All of these reactions, in addition to the reagents themselves, were undergone in the presence of approximately ~10g of 4Å molecular sieves. The solvents used for each experiment were as follows:

E-92, E-108 -carried out in anhydrous diethyl ether

E-76, E-96 -carried out in anhydrous dichloromethane

E-81, E-100 -carried out in anhydrous THF

E-85, E-104 -carried out in anhydrous toluene

Thereafter, the reactions were worked up in the usual way as described in 6.3.1.7. The products were subsequently analysed by both NMR and GPC.

^{1}H NMR(δ $CDCl_3$): 4.07 (s; impurity); 4.04 (s, 4H; O-(C**H**$_2$-CH_2)$_2$); 3.60 (t, 2H; Sample [**E-92**] terminal HO-(C**H**$_2$)$_2$); 2.30 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.87 (pent, 2H; CH_2-C**H**$_2$); 1.64 (m, 4H; O-(CH_2-C**H**$_2$)$_2$); 1.17 (m; vacuum grease impurity)

Sample [**E-108**]: 3.99 (s, 4H; O-(C**H**$_2$-CH_2)$_2$); 3.56 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 2.26 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.85 (m, 2H; CH_2-C**H**$_2$); 1.63 (m, 4H; O-(CH_2-C**H**$_2$)$_2$); 1.20 (m; vacuum grease impurity)

Sample [**E-76**]: 4.93 (m, 2H; terminal Cl_3C**H**$_2$); 4.07 (s; impurity); 4.04 (s, 4H; O-(C**H**$_2$-CH_2)$_2$); 3.60 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 2.47 (m, impurity); 2.30 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.87 (pent, 2H; CH_2-C**H**$_2$); 1.57 (m, impurity); 1.64 (m, 4H; O-(CH_2-C**H**$_2$)$_2$); 1.17 (m; vacuum grease impurity)

Sample [**E-96**] 4.40 (q, 2H; terminal F_3C-C**H**$_2$); 4.02 (m, 4H; O-(C**H**$_2$-CH_2)$_2$); 3.83 (q; solvent impurity); 3.59 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 2.42 (m; impurity); 2.31 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.91 (m, 2H; CH_2-C**H**$_2$); 1.66 (m, 4H; O-(CH_2-C**H**$_2$)$_2$); 1.23 (m; vacuum grease impurity)

Sample [**E-81**]:- very poor spectrum was obtained

Sample [**E-100**] 5.21 (s; impurity); 4.38 (q, 2H; terminal F_3C-C**H**$_2$); 4.25 (t; solventimpurity); 4.00 (s, 4H; O-(C**H**$_2$-CH_2)$_2$); 3.83 (q; solvent impurity); 3.54 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 2.42 (m; impurity); 2.26 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.91 (m, 2H; CH_2-C**H**$_2$); 1.60 (m, 4H; O-(CH_2-C**H**$_2$)$_2$); 1.14 (m; vacuum grease impurity)

Sample [**E-85**]:- very poor spectrum was obtained

Sample [**E-104**]: very poor spectrum was obtained

Sample	Mn	Mw
E-92	2,000	7,300
E-108	2,000	4,800
E-76	250	350
E-96	800	1,150
E-81	250	500
E-100	700	1,500
E-85	400	450
E-104	1,000	2,500

Table 26. GPC results of experiments **E-81 - E-108**, analysed in THF

<u>6.4.2.4.3) Experiments Undergoing Frequent Solvent Change (E-77, E-82, E-86, E-93, E-97, E-101, E-105, E-109, E-142)</u>

For these experiments the solvent was removed using a rotary evaporator and replaced with fresh anhydrous solvent. On average the solvent was changed every 2 days.

The solvents used for each experiment were as follows:

E-93, E-109 -carried out in anhydrous diethyl ether

E-77, E-97 -carried out in anhydrous dichloromethane

E-82, E-101 -carried out in anhydrous THF

E-86, E-105, E-142 -carried out in anhydrous toluene

Thereafter, the reactions were worked up in the usual way as described in 6.3.1.7. The products were subsequently analysed by both NMR and GPC.

^{1}H NMR(δ $CDCl_3$): 4.07 (s; impurity); 4.04 (s, 4H; O-(C**H**$_2$-CH_2)$_2$); 3.61 (t, 2H; Sample [**E-93**] terminal HO-(C**H**$_2$)$_2$); 2.30 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.87 (pent, 2H; CH_2-C**H**$_2$); 1.64 (m, 4H; O-(CH_2-C**H**$_2$)$_2$); 1.17 (m; vacuum grease impurity)

Sample [**E-109**]: 4.03 (s, 4H; O-(C**H**$_2$-CH_2)$_2$); 3.60 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 2.30 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.86 (m, 2H; CH_2-C**H**$_2$); 1.67 (m, 4H; O-(CH_2C**H**$_2$)$_2$); 1.23 (m; vacuum grease impurity)

Sample [**E-77**]: 4.93 (m, 2H; terminal Cl_3C**H**$_2$); 4.07 (s; impurity); 4.04 (s, 4H; O-(C**H**$_2$-CH_2)$_2$); 3.60 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 2.47 (m, impurity); 2.30 (t, 4H; C(O)(C**H**$_2$)$_2$); 1.87 (pent, 2H; CH_2-C**H**$_2$); 1.57 (m, impurity); 1.64 (m, 4H; O-(CH_2-C**H**$_2$)$_2$); 1.17 (m; vacuum grease impurity)

Sample [**E-97**]:- very poor spectrum was obtained

Sample [**E-82**]:- very poor spectrum was obtained

Sample [**E-101**]:- very poor spectrum was obtained

Sample [**E-86**]: 7.24 (m; impurity); 4.75 (m, 2H; terminal Cl_3C**H**$_2$); 4.00 (s, 4H; O-(C**H**$_2$-CH_2)$_2$); 3.55 (t, 2H; terminal HO-(C**H**$_2$)$_2$); 3.39 (q;

solvent impurity); 2.45 (m; impurity); 2.31 (m, 4H; $C(O)(CH_2)_2$); 1.92 (m; impurity); 1.81 (m, 2H; CH_2-CH_2); 1.62 (m, 4H; O-$(CH_2$-$CH_2)_2$); 1.14 (m; vacuum grease impurity)

Sample [**E-142**] 4.67 (s, 2H; terminal F_3C-CH_2); 4.07 (s; impurity); 4.02 (s, 4H; $O(CH_2$-$CH_2)_2$); 3.59 (t, 2H; terminal HO-$(CH_2)_2$); 2.42 (m, impurity); 2.26 (s, 4H; $C(O)(CH_2)_2$); 1.61 (m, 8H; $(CH_2$-$CH_2)_2$); 1.14 (m; vacuum grease impurity)

Sample [**E-105**] 4.36 (q, 2H; terminal F_3C-CH_2); 3.98 (s, 4H; O-$(CH_2$-$CH_2)_2$); 3.83 (q; solvent impurity); 3.54 (t, 2H; terminal HO-$(CH_2)_2$); 2.42 (m; impurity); 2.26 (t, 4H; $C(O)(CH_2)_2$); 1.91 (m, 2H; CH_2-CH_2); 1.60 (m, 4H; O-$(CH_2$-$CH_2)_2$); 1.14 (m; vacuum grease impurity)

Sample	Mn	Mw
E-93	1,900	7,300
E-109	1,700	4,350
E-77	200	450
E-97	450	700
E-82	250	700
E-101	300	300
E-86	4,600	3,000
E-142	1,350	4,250
E-105	1,450	3,100

Table 27. GPC results of expts **E-82 - E-109**, analysed in THF

6.4.2.4.4) Experiments Undergoing Both Solvent and Enzyme Change (E-78, E-83, E-94, E-98, E-102, E-106, E-110, E-143)

For all of these reactions, after 3 days the enzyme was filtered off and washed with anhydrous dichloromethane. The filtrate and combined washings were added together and the solvent removed using a rotary evaporator. Subsequently, both fresh enzyme and solvent was added and the reaction allowed to continue for an additional 6 days. Hence, the total reaction time corresponded to 9 days.

The solvents used for each experiment were as follows:

E-94, E-110	-carried out in anhydrous diethyl ether
E-78, E-98	-carried out in anhydrous dichloromethane
E-83, E-102	-carried out in anhydrous THF
E-106, E-143	-carried out in anhydrous toluene

Thereafter, the reactions were worked up in the usual way as described in 6.3.1.7. The products were subsequently analysed by both NMR and GPC.

^{1}H NMR (δ $CDCl_3$): Sample [**120**]: very poor spectrum was obtained

Sample [**E-78**]: 4.93 (m, 2H; terminal $Cl_3C\mathbf{H}_2$); 4.07 (s; impurity); 4.04 (s, 4H; O-($C\mathbf{H}_2$-CH_2)$_2$); 3.60 (t, 2H; terminal HO-($C\mathbf{H}_2$)$_2$); 2.47 (m, impurity); 2.30 (t, 4H; C(O)($C\mathbf{H}_2$)$_2$); 1.87 (pent, 2H; CH_2-$C\mathbf{H}_2$); 1.57 (m, impurity); 1.64 (m, 4H; O-(CH_2-$C\mathbf{H}_2$)$_2$); 1.17 (m; vacuum grease impurity)

Sample [**E-98**]: 4.33 (q, 2H; terminal F_3C-$C\mathbf{H}_2$); 3.93 (m, 4H; O-($C\mathbf{H}_2$-CH_2)$_2$); 3.74 (m; impurity); 3.49 (t, 2H; terminal HO-($C\mathbf{H}_2$)$_2$); 2.34 (m;

impurity); 2.21 (t, 4H; $C(O)(CH_2)_2$); 1.83 (m, 2H; CH_2-CH_2); 1.60 (m, 4H; O-(CH_2-CH_2)$_2$); 1.10 (m; vacuum grease impurity)

Sample [**E-83**]:- very poor spectrum was obtained

Sample [**E-102**]:- very poor spectrum was obtained

Sample [**E-143**] 4.67 (s, 2H; terminal F_3C-CH_2); 4.07 (s; impurity); 4.02 (s, 4H; $O(CH_2$-$CH_2)_2$); 3.59 (t, 2H; terminal HO-(CH_2)$_2$); 2.42 (m, impurity); 2.26 (s, 4H; $C(O)(CH_2)_2$); 1.61 (m, 8H; (CH_2-CH_2)$_2$); 1.14 (m; vacuum grease impurity)

Sample [**E-106**]:- very poor spectrum was obtained

Sample	Mn	Mw
E-94	2,200	258,000
E-110	1,150	3,200
E-78	200	400
E-98	500	850
E-83	200	500
E-102	500	750
E-143	1,000	3,700
E-106	2,150	4,000

Table 28. GPC results of expts **E-83 - E-143**, analysed in THF

6.4.2.5) Polymerisation of Bis(2,2,2-trichloroethyl) Terephthalate [Synthesis of Poly(butylene terephthalate)]

Bis(2,2,2-trichloroethyl) terephthalate (4.29g, 100mmoles), butane-1,4-diol (0.90g,

100moles) and PP lipase (6g) were added to anhydrous diethyl ether (50mL). The mixture was stirred in a stoppered flask at ambient temperature for 9 days. The enzyme was filtered off and washed with dichloromethane. After the reaction was worked up in the usual way, an abundant white precipitate was obtained. This was analysed by FTIR and NMR and found to be unreacted starting material.

FTIR (cm^{-1};KBr) 3289 (m; aromatic ν(C-H)); 2933, 2854 (s, m; ν(C-H)); 1738 (s; ν(C=O); 1698, 1648 (m, s; ν(C=C))

6.4.2.6) Polymerisation of Glutarate Esters Having Alternative Leaving Groups (E-118, E-127)

Three glutarate esters having leaving groups other than the usual 2,2,2-trihalo groups were polymerised.

E-118, E-119:- Bis(vinyl) glutarate (1.84g, 10mmoles), butane-1,4-diol (0.90g, 10mmoles), PP lipase (6g) and anhydrous diethyl ether (50mL) were stirred in a stoppered flask at ambient temperature for 3 days. Half of the reaction mixture was removed (**E-118**) and worked up in the usual way and analysed by GPC. The remaining portion (**E-119**) was allowed to continue further reaction until total time of 9 days had elapsed. After this time the reaction was worked up and analysed by GPC.

E-122, E-123:- An identical procedure was adopted as for experiment **E-118, E-119**, except that bis(phenyl) glutarate (2.84g, 10mmoles) was used instead of bis(vinyl) glutarate.

E-126, E-127:- An identical procedure was adopted as for experiment **E-118, E-119**, except that *N*,*N*'-glutaryl cyclohexanone oxime (3.22g, 10mmoles) was used instead of bis(vinyl) glutarate.

Sample	Mn	Mw
E-118	-	-
E-119	-	-
E-122	350	7,800
E-123	400	6,250
E-126	200	4,500
E-127	250	6,300

Table 29. GPC results of expts **E-118 - E-127**, analysed in CH_2Cl_2

6.4.2.7) Polymerisation of Adipate Esters Having Alternative Leaving Groups

Three adipate esters having leaving groups other than the usual 2,2,2-trihalo groups were polymerised.

E-120, E-121:- Bis(vinyl) adipate (1.98g, 10mmoles), butane-1,4-diol (0.90g, 10mmoles), PP lipase (6g) and anhydrous diethyl ether (50mL) were stirred in a stoppered flask at ambient temperature for 3 days. Half of the reaction mixture was removed (**E-120**) and worked up in the usual way and analysed by GPC. The remaining portion (**E-121**) was allowed to continue further reaction until toatl time of 9 days had elapsed. After this time the reaction was worked up and analysed by GPC.

E-124, E-125:- An identical procedure was adopted as for experiment **E-120, E-121**, except that bis(phenyl) adipate (2.98g, 10mmoles) was used instead of bis(vinyl) adipate.

E-128, E-129:- An identical procedure was adopted as for experiment **E-120, E-121**, except that bis(trimethylsilyl) adipate (2.90g, 10mmoles) was used instead of bis(vinyl) adipate.

Sample	Mn	Mw
E-120	150	2,750
E-121	150	3,150
E-124	250	250
E-125	250	250
E-128	-	-
E-129	250	900

Table 30. GPC results of expts **E-120 - E-129**, analysed in CH_2Cl_2

6.4.2.8) Polymerisation Reactions Using Potassium Salt-Stabilised Enzymes (E-24 - E-35)

Twelve reactions were set up each using bis(2,2,2-trichloroethyl) glutarate (3.95g, 10mmoles), butane-1,4-diol (0.90g, 10mmoles), PP lipase adsorbed with potassium salts (6g) and anhydrous diethyl ether (50mL). The enzymes used for the experiments were from experiment 134, section 6.2.1.9. The experiment numbers and enzyme/salt sample numbers used, are shown in the Table 31.

Experiment	Enzyme/Salt Sample
E-24	1
E-25	2
E-26	3
E-27	4
E-28	5
E-29	6
E-30	7

E-31	8
E-32	9
E-33	10
E-34	11
E-35	12

Table 31. Experiments **E-24 - E-35**, using enzyme/salt mixtures

All of the reactions were worked up in the usual way and analysed by GPC. The reactions proved unsuccessful and GPC data revealed the presence of unreacted starting ester in each case. Where there are no GPC results shown, this indicates lack of detector response.

Sample	**Mn**	**Mw**
E-24	200	250
E-25	200	250
E-28	250	300
E-29	250	250
E-30	200	250
E-31	250	350
E-35	250	300

Table 32. GPC results of experiments **E-24 - E-35**, analysed in CH_2Cl_2

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Appendix

In case of a future need to refer back to the original laboratory book, the following table lists the experiment numbers and their corresponding numbers as they appear in this thesis.

Thesis No	Expt No.	Thesis No	Expt No.	Thesis No	Expt No.
E-1	72A	E-24	180	E-47	68
E-2	72B	E-25	181	E-48	67A
E-3	72C	E-26	182	E-49	67B
E-4	72D	E-27	183	E-50	69D
E-5	76	E-28	184	E-51	69E
E-6	87	E-29	185	E-52	69F
E-7	66	E-30	186	E-53	69G
E-8	49	E-31	187	E-54	69H
E-9	51	E-32	188	E-55	74A
E-10	53	E-33	189	E-56	74B
E-11	52	E-34	190	E-57	74C
E-12	54	E-35	191	E-58	94R
E-13	55	E-36	84	E-59	94B
E-14	56	E-37	85	E-60	96R
E-15	57	E-38	90	E-61	95B
E-16	58	E-39	91	E-62	98R
E-17	59	E-40	79	E-63	98B
E-18	60	E-41	80	E-64	99R
E-19	61	E-42	81	E-65	99B
E-20	62	E-43	82	E-66	94
E-21	16	E-44	64A	E-67	110
E-22	12	E-45	64B	E-68	111
E-23	17	E-46	64C	E-69	112

Thesis No	Expt No.	Thesis No	Expt No.	Thesis No	Expt No.
E-70	95	E-95	129	E-120	165a
E-71	96	E-96	130	E-121	165b
E-72	96B	E-97	131	E-122	166a
E-73	109	E-98	132	E-123	166b
E-74	96R	E-99	139	E-124	167a
E-75	125	E-100	140	E-125	167b
E-76	126	E-101	141	E-126	168a
E-77	127	E-102	142	E-127	168b
E-78	128	E-103	170	E-128	169a
E-79	98	E-104	171	E-129	169b
E-80	135	E-105	172	E-130	28b
E-81	136	E-106	173	E-131	28a
E-82	137	E-107	121	E-132	29
E-83	138	E-108	122	E-133	43
E-84	152	E-109	123	E-134	44
E-85	153	E-110	124	E-135	51
E-86	154	E-111	157a	E-136	52
E-87	99	E-112	157b	E-137	53
E-88	100	E-113	157c	E-138	54
E-89	100R	E-114	157d	E-139	Ad32
E-90	100B	E-115	157e	E-140	144
E-91	113	E-116	157g	E-141	160
E-92	114	E-117	157h	E-142	162
E-93	115	E-118	164a	E-143	163
E-94	116	E-119	164b		