Studies leading to the large scale synthesis of polyesters using enzymes

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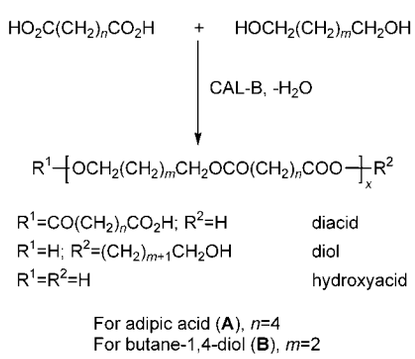
The ability of immobilised *Candida antarctica* lipase to catalyse the condensation of diols and diacids was studied. The range of acids investigated (in condensations with butane-1,4-diol) included adipic, maleic, fumaric, itaconic, (*E*)-hex-3-enedioic and acetylenedicarboxylic acids, while the diols studied, in condensations with adipic acid, encompassed ethane-, propane-, butane-, pentane- and hexane-diols as well as (*Z*)-but-2-ene-1,4-diol and but-2-yne-1,4-diol. The oligomerization of hexane-1,6-diol and adipic acid was most facile and was chosen for scale-up. A mechanism for the polycondensation process is proposed.

Introduction and background information

We have been interested for some time in the use of enzymes to catalyse the condensation of alkanediols and dicarboxylic acids (Scheme 1)[1] believing, amongst other things, that such a protocol would be more efficient (being operable at lower temperatures) than the conventional acid-catalysed procedure.[2] These pilot studies indicated that an hydrolase such as *Mucor miehei* lipase could effect the condensation of adipic acid and butane-1,4-diol, provided that the water being produced in the process was removed by molecular sieves.



Scheme 1 Enzyme-catalysed condensation of diol and diacid.

Further work in the Company's laboratories established first, that *Candida antarctica* lipase (CAL-B) was the preferred enzyme for the polymerization and, secondly, that dehydration of the system could be effected by performing the reaction without solvent *in vacuo*.[3]

Contemporaneously, enzyme-catalysed production of polyesters was being investigated in other laboratories. Morrow[4] and Jarvie[5] and co-workers used activated esters (*e.g.* trifluoroethyl esters) of carboxylic acids in couplings with butane-1,4-diol. Similarly Linko and co-workers used bis(2,2,2-trifluoroethyl) sebacate and bis(2-chloroethyl)succinate in enzyme-catalysed polymerizations, in a solvent such as diphenyl ether, with and without the application of vacuum to remove side products.[6] When sebacic acid itself was heated with butane-1,4-diol and *Mucor miehei* lipase in diphenyl ether at 37 °C for seven days with periodic applications of vacuum, a polymer M_w 42000 was obtained. While the use of expensive esters and/or costly solvents makes such processes non-viable commercially, the use of lipases in polymerization processes was becoming more firmly established.

The recent work of Russell *et al.* is also relevant and noteworthy. The American team first coupled divinyl adipate and butane-1,4-diol using CAL-B in various solvents[7] before moving to a solvent-free system.[8] Using the vinyl diester in the process precludes the need for conducting the polymerization *in vacuo* (the released vinyl alcohol tautomerizes to acetaldehyde and takes no further part in the reaction) but we surmise the methodology is not commercially viable due to the prohibitively high cost of the diesters. Similarly enzyme-mediated preparation of polyesters from ω-hydroxyacids[9] or macrocyclic lactones/anhydrides[10] suffers from the relatively high costs of the substrates.

In summary, we believe that commercially-viable production of simple[11] polyesters using enzyme technology will involve the condensation of a cheap diacid such as adipic acid (A) and an inexpensive diol such as butane-1,4-diol (B). Further progress towards commercialization of the process for the production of diacid $(AB)_nA$, diol $B(AB)_n$ and/or hydroxyacid $(AB)_n$ (see Scheme 1) demanded studies aimed at the following.

(a) An understanding of the mechanism of the polymerization in order to optimize the reaction conditions before scale up.

(b) Estimation of the ranges of substrates that may undergo CAL-B promoted polymerization under the optimum conditions, to allow access to different products.

(c) Assessment of the constitution of the polymers derived from the enzyme-catalysed process, in order to ascertain differences between the new material and polyester produced by the conventional methods.

The progress made in all of these areas is detailed in this paper.

Results and discussions

Mechanism of the polymerization

The standard protocol for condensation of adipic acid (A) and butane-1,4-diol (B) for laboratory scale (both simple flange flask, 0.5–5 litre, and automated lab reactor 0.5–1 litre), through pilot plant (20 litre to 0.5 tonne), up to manufacturing scale (2 tonne steel vessel) is as follows.

Stage 1 acid (A) and diol (B) (1 : 1 ratio) together with an aliquot of water (1.8% w/w) were stirred at 40 °C for

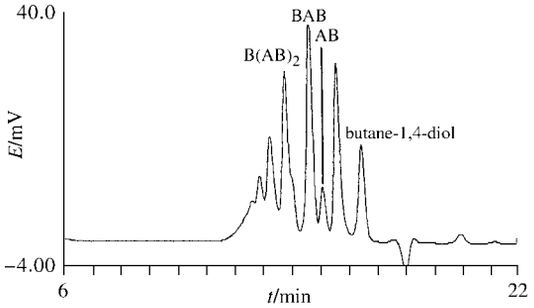


Fig. 1 GPC trace of oligomers produced after Stages 1 and 2 for butane-1,4-diol and adipic acid.

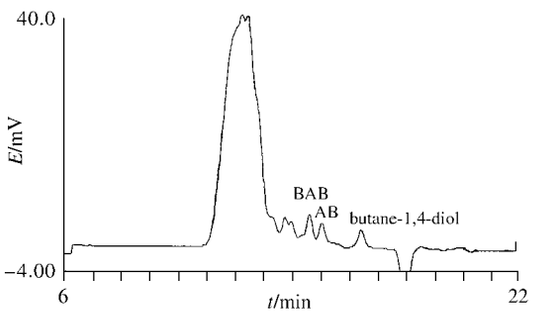


Fig. 2 GPC trace of polymers produced after Stage 3 for butane-1,4-diol and adipic acid.

1 h to ensure saturation of B with A (much of acid A remains insoluble);

Stage 2 immobilized CAL-B was added (0.35% w/w) and stirring at 40 °C continued for 4 h;

Stage 3 temperature raised to 60 °C and vacuum applied (100 ± 5 mbar) before stirring for 17 h to give a suspension of the immobilized enzyme in an homogeneous solution;

Stage 4 immobilized enzyme removed by filtration;

Stage 5 oligomer mixture stirred for 7 h at 60 °C under vacuum (10 ± 3 mbar);

Stage 6 polymer mixture stirred for further 24 h at 60 °C under vacuum (10 ± 3 mbar).

Analysis of the components of the reaction mixture was aided considerably by synthesizing the markers AB, BAB and $(AB)_2$ as well as obtaining $B(AB)_2$ by preparative-scale gel permeation chromatography (GPC).[12]

A sample taken 4 h after the start of Stage 3 (Fig. 1) shows clearly the predominance of hydroxy-terminated oligomers BAB and $B(AB)_2$ as well as butanediol and (insoluble) adipic acid. This lent support to our postulate[1] that incremental growth of the polymer occurs, with AB being the key building block. The low concentration of AB in the mixture follows from the fact that this moiety readily acylates the lipase and the lipase–AB complex is readily attacked by B, BAB or $B(AB)_2$, the most abundant nucleophiles. As expected $(AB)_2$ is a minor product (seen as a shoulder on the $B(AB)_2$ peak) since little AB is available to attack the lipase–AB complex.

GPC analysis (Fig. 2) of the reaction mixture after Stage 3 shows the presence of polymeric material of low dispersity ($M_w/M_n = 1.5$) and only small amounts of A (0.3%), B (2.1%) and AB (3.0%). A control reaction run in the absence of enzyme gave no reaction.

A lipase-catalysed reaction of AB with a five-fold excess of B (to mimic the conditions in the standard acid–diol process) was conducted. Six hours after the commencement of Stage 3 of the process GPC analysis of the mixture showed BAB and butane-1,4-diol (B) as the major components with a smaller amount of $B(AB)_2$ and traces of $B(AB)_3$ and AB (Fig. 3).

In a parallel experiment BAB was found to be largely

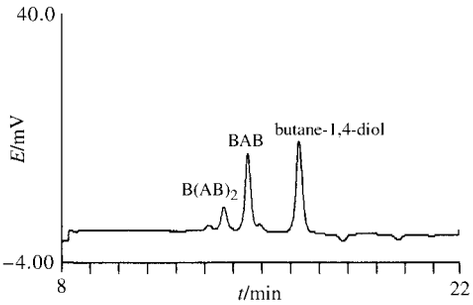


Fig. 3 GPC trace of oligomers produced after Stages 1 and 2 and 6 h into Stage 3 for AB and a 5-fold excess of butane-1,4-diol.

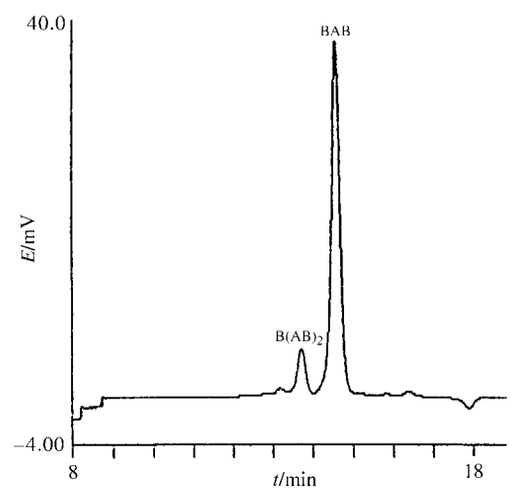


Fig. 4 GPC trace of oligomers produced after Stages 1–3 for BAB and a 5-fold excess of butane-1,4-diol.

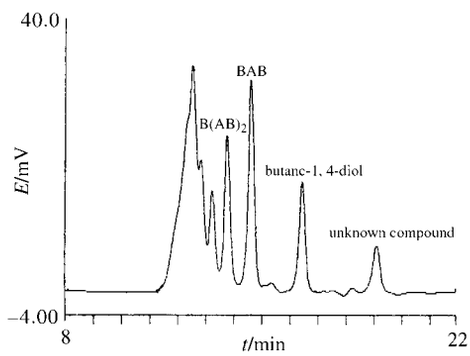


Fig. 5 GPC trace of oligomers produced for BAB and a 5-fold excess of butane-1,4-diol in toluene at 60 °C, atmospheric pressure.

unreactive when treated with lipase at 60 °C. GPC analysis after 17 h showed a small amount of $B(AB)_2$ but no butanediol (Fig. 4). Confirmation that the lipase was active at the end of the 17 h period was accomplished by the addition of a small amount of AB (*ca.* 7 mol% with respect to BAB). Stirring this mixture for 4 h at 60 °C led to the formation of higher molecular weight oligomeric species. This experiment shows that, in solvent-free conditions, BAB only slowly acylates CAL-B by a process of transesterification.

Parenthetically BAB *does* react extensively when heated in anhydrous toluene at 60 °C for 17 h, in the presence of CAL-B. A large array of oligomeric products and a substantial quantity of butane-1,4-diol (B) were formed (Fig. 5) indicating that transesterification *does* take place in this medium. (No reaction occurs in the absence of enzyme.) Such a radical change in substrate specificity of an enzyme when operating in different conditions is not unexpected, having been observed on a number of occasions previously.[13]

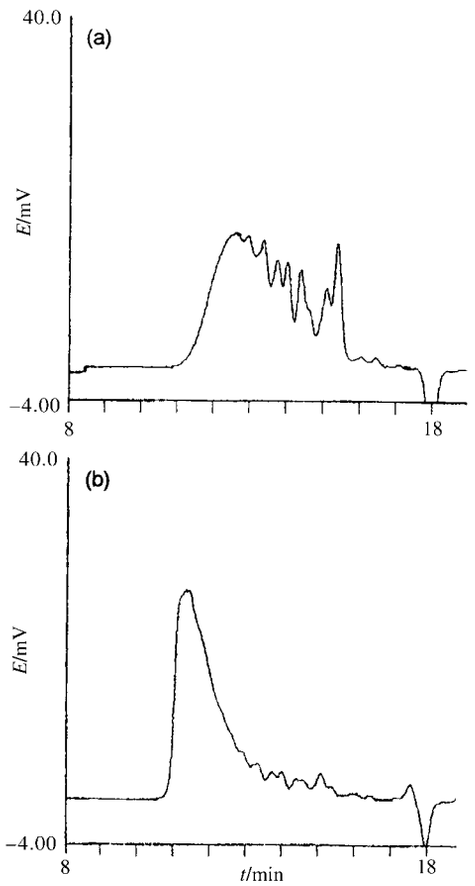


Fig. 6 GPC trace of polymer produced after (a) Stage 5 and (b) Stage 6 for butane-1,4-diol and adipic acid.

Thus the pathway of the oligomerization process up to the end of Stage 3 seems clear. Adipic acid (A) acylates the enzyme; the acyl–enzyme complex is attacked by butane-1,4-diol (B) releasing AB. AB then acylates the enzyme *via* the acid terminus and the enzyme–AB complex is attacked by B to give BAB which does not react with the enzyme but, in due course, attacks an enzyme–AB complex to give $B(AB)_2$. Continuation of the cycle gives $B(AB)_n$.

At Stage 4 of the process the immobilized enzyme is removed. It was found that the recovered enzyme retained only 35% of its original activity. Stage 5 entailed stirring the reaction mixture for 7 h, under vacuum (10 ± 3 mbar). Stage 6 involved reaction for a further 24 h period under similar conditions. The amount of polymer produced increases during these latter phases, while the low weight oligomers disappear (Fig. 6 a→b). At this point in the programme it was deemed necessary to prohibit the deactivation of the enzyme (to allow it to be recycled) and to explain the continuation of the polymerization in Stage 5.

The latter phenomenon was considered to be due to a small amount of the lipase being leached from the support during Stages 2 and 3. Application of a higher vacuum (10 ± 3 mbar) thus aids the removal of water shifting the equilibrium further in the direction of polyesterification. Three experiments were in accord with this hypothesis. First it was shown that non-immobilised CAL-B was, indeed, able to catalyse the oligomerization/polymerization observed in Stages 3 *and* 5 of the process. In the second experiment Chirazyme® (*covalently* immobilized CAL-B) was employed. After Stage 3, an analysis of the oligomeric material showed M_w 601; M_w/M_n 1.9. The enzyme was *not* removed but the higher vacuum applied. Further polymerization ensued (M_w 1041; M_w/M_n 2.0). Water (8% w/w) was added and after 0.5 h some breakdown of the polymeric material was observed to have occurred (M_w 885; M_w/M_n 2.3). Application of the higher vacuum for 24 h reformed the higher weight materials (M_w 1024; M_w/M_n 2.0). Obviously in the relatively non-polar oligomeric mixture (essentially in the absence of adipic acid and butanediol) ester bond cleavage *can* take place and molecular weight changes *can* occur either to greater values or to lesser values depending on the nucleophile (oligomer or water) that attacks the acyl–enzyme complex. In a complementary experiment Chirazyme® was used but filtered off after the initial oligomerization stage; application of high vacuum then failed to convert the oligomer to polymer. In short it seems that a small amount of free enzyme catalyses the oligomer to polymer transformations in Stage 5 of the process.

The deactivation of the enzyme by 65% after one cycle was initially a cause for concern; it was crucial from an economic standpoint that the enzyme should be recyclable, up to ten times. Gratifyingly it was found that addition of 0.02 mol% of triethylamine (TEA) to the polymerization of A and B, catalysed by immobilized CAL-B, had a very positive effect, the activity of the recovered lipase being raised to *ca.* 90% of the original value. By supplementing recovered enzyme with 10% of fresh immobilized CAL-B, the same batch of enzyme was recycled 13 times through the A–B–TEA protocol. The reduction in the deactivation of the enzyme also led to the formation of higher molecular weight polymer after Stage 3 of the process (M_w 1327; M_w/M_n 1.8).

The addition of amines to enzymatic, particularly lipase-catalysed, reactions is not novel,[14] although it has not been reported previously, to our knowledge, for a solvent-free system. The explanation for the protective effect of the tertiary amine is not clear-cut; we postulate that the small amount of base may exert its influence by moderating the decrease in the pH of the medium caused by dissolution of some of the adipic acid in the diol–water mix. The work of Halling[15] and others[16] is insightful in this regard and the report by Turner and co-workers,[17] on the beneficial effect of triethylamine in a different transformation, concludes that the effect of the base is due, at least in part, to scavenging of acid in the medium.

Subsequently both the butylene and hexylene adipates were scaled up progressively in the pilot plant; the hexylene adipate was then selected as the most promising candidate for development and passed to manufacture on the tonne scale; after minor modifications to the protocol (see Experimental section) the reaction was operated in routine fashion.

Ranges of diols and diacids undergoing polymerization on lipase-catalysis

(a) Variation in the diol component. The ability of lower and higher homologues of butane-1,4-diol to couple with adipic acid was assessed using the standard protocol up to the end of Stage 3. In addition the extent of inactivation of the immobilized catalyst was determined (Table 1). Clearly ethylene glycol and propane-1,3-diol are not the most promising substrates. As discussed above butane-1,4-diol affords oligomers and the enzyme is partially deactivated (in the absence of triethylamine). It was interesting to note that pentane-1,5-diol and hexane-1,6-diol not only formed oligomers but had little deactivating effect on the enzyme, assuming that *ca.* 10% of the protein is leached from the carrier in each case. Because of the decreased deactivation of the enzyme in these two cases the addition of triethylamine to the reaction mixture is not necessary. It is likely that the decreased solubility of adipic acid in the media is beneficial to the latter processes.

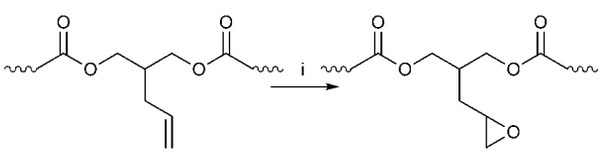
It seems that increasing the polarity of the medium (the diol), deactivates the enzyme and little coupling takes place. Thus, it was of interest to see if a propane-1,3-diol with a non-polar side chain attached at C-3 would undergo polymerization. 2-Allylpropane-1,3-diol was chosen for this study for two reasons. First, diols of this type would not undergo polymerization under conventional conditions without interfering

Table 1 Polymerization of various simple diols $HOCH_2(CH_2)_n$-CH_2OH with adipic acid

Substrate	Extent of oligomerization		Residual enzyme activity (%)
	M_w	M_w/M_n	
Ethylene glycol $n = 0$	194	1.3	6
Propane-1,3-diol $n = 1$	526	1.8	8
Butane-1,4-diol $n = 2$	606	1.9	35
Pentane-1,5-diol $n = 3$	846	1.9	≥90
Hexane-1,6-diol $n = 4$	784	2.4	88

side-reactions taking place.[18] Thus the advantage of using the mild conditions permitted in the enzyme-based strategy would be emphasized. Secondly, if poly(2-allylpropane-1,3-diyl adipate) could be produced, the pendant double bond in the polymer could be utilized to cross-link the chains.

2-Allylpropane-1,3-diol was prepared in 80% yield from diethyl 2-allylmalonate using lithium aluminium hydride. This 1,3-diol formed high molecular weight oligomeric species (M_w 1810; M_w/M_n) when coupled with adipic acid using immobilized CAL-B as catalyst, using as the protocol the standard procedure up to the end of Stage 3. ^{1}H NMR spectroscopy gave a C*H*$_2$OH to C*H*$_2$OCOR ratio of *ca.* 1:4 and ^{13}C NMR spectroscopy showed no discernible signal in the region 177 ppm (where *C*O$_2$H resonances should appear) indicating B(AB)$_4$ was typical of the oligomers present in the mixture. For comparison 2-allylpropane-1,3-diol and adipic acid were polymerized under conventional conditions, at 220 °C under nitrogen using Ti(O^{i}Pr)$_4$ as catalyst. GPC revealed polymer was formed (M_w 5204; M_w/M_n 2.5) but the material was isolated as a yellow gel, suggesting that side reactions or some decomposition had occurred. Epoxidation of enzymically-prepared poly-(2-allylpropane-1,3-diyl adipate) was achieved using *m*-chloroperoxybenzoic acid (MCPBA) (Scheme 2).



Scheme 2 Epoxidation of polyester with pendant allyl group. *Reagents and conditions*: i) MCPBA, CH_2Cl_2, room temp., 48 h.

^{1}H NMR spectroscopy clearly showed the absence of the olefinic moiety while new signals in the region 2.4–2.9 ppm indicated the presence of the terminal epoxide group. Potentially, this epoxypolymer could be cross-linked using a diamine.

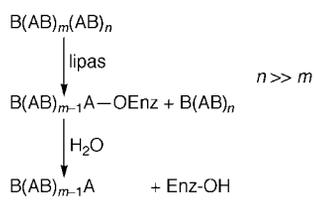
Condensation of commercially available (*Z*)-but-2-ene-1,4-diol with adipic acid under the standard solvent-free conditions gave little oligomer (M_w 358, M_w/M_n 1.7), while but-2-yne-1,4-diol was unreactive.

(b) Variation in the diacid component. Brigodiot and co-workers studied lipase-catalysed reactions of dimethyl maleate and dimethyl fumarate with hexane-1,6-diol in toluene at 60 °C and reported polymer formation over a 15-day period.[19] Thus we elected to study the reactions of maleic acid and fumaric acid with butane-1,4-diol under solvent-free conditions; itaconic acid, (*E*)-hex-3-enedioic acid and acetylenedicarboxylic acid (butynedioic acid) were added to the list. Only acetylenedicarboxylic acid showed any tendency to form higher molecular weight oligomers (M_w 574; M_w/M_n 4.9) but in a separate experiment virtually the same result was obtained in the absence of enzyme (*i.e.* quite unlike the adipic acid case). The other above-mentioned diacids gave $M_w < 370$ indicating little ester formation.

In the light of these studies adipic acid/butane-1,4-diol with triethylamine added in the standard procedure and adipic acid/hexane-1,6-diol without triethylamine were prime candidates for exploitation.

Constitution of polymers derived from the enzyme-catalysed processes

The proposed mechanism for formation of polymers under enzymic catalysis suggests that the number of acid end-groups in the polymeric species will be minimal. This is, indeed the case as displayed with exquisite clarity by MALDI-TOF spectroscopy. The MALDI-TOF spectra produced from enzymatically-prepared poly(hexane-1,6-diyl adipate) (procedure taken to Stage 6) and the conventionally prepared analogue are shown in Fig. 7a and 7b respectively. (The signals represent $[M + Na]^+$ ions.) Comparison of Fig. 7a and 7b immediately conveys two points. First, the enzymatic product has a slightly broader spread of acid/hydroxy-ended species than the conventional material. More importantly, the latter contains a large number of acid-terminated species of molecular weight >1000 Da, seen as the $[M + Na]^+$ signals of lower intensity. By analogy to the work of Russell,[20] we estimate that about one quarter of the middle-weight oligomers are *mono* acid-ended. Note that titrimetric methods showed that the polyester from the enzymatic and the conventional processes had similar numbers of acid end-groups, strongly suggesting the statistical distribution of acid-ended species is skewed towards low-molecular weight species in the enzymatic case. This is exactly what is expected if, in the late-stage transesterification processes leading to high-molecular weight material, only small oligomeric acid-ended species are released into the milieu by attack of residual water on the acyl-enzyme complex (Scheme 3). Note that the production of macrolactones, which would produce peaks in the mass spectrum at (M − 18) shadowing those of the open-chain polyesters, is certainly not evident in the polymeric region, but such species cannot be excluded from being present in the oligomeric region. The formation of such species has been recorded previously[21] and the mass spectrum, though more complicated for lower values of molecular weight, does show signals in the appropriate position.



Scheme 3 Late stage modification of polymer by enzyme-catalysed hydrolysis leading to release of low molecular weight acid-ended oligomers.

A second series of experiments was designed to give a more accurate estimate of the molecular weights of the high molecular weight enzymatic products since conventional end-group analysis is unreliable for polyesters with the low hydroxy values involved. The enzyme-derived products were compared, using standard light-scattering technique,[22] with a high molecular weight material, Dynacol™ 7361, produced in the conventional manner. All the enzymatic materials subjected to the test behaved consistently (Fig. 8) when the Debye function was plotted against concentration, but Dynacol™, the material prepared conventionally, was clearly anomalous to the general gradient trend. When GPC M_n data was correlated against the derived M_w figures (Fig. 9) clear evidence for disparity was

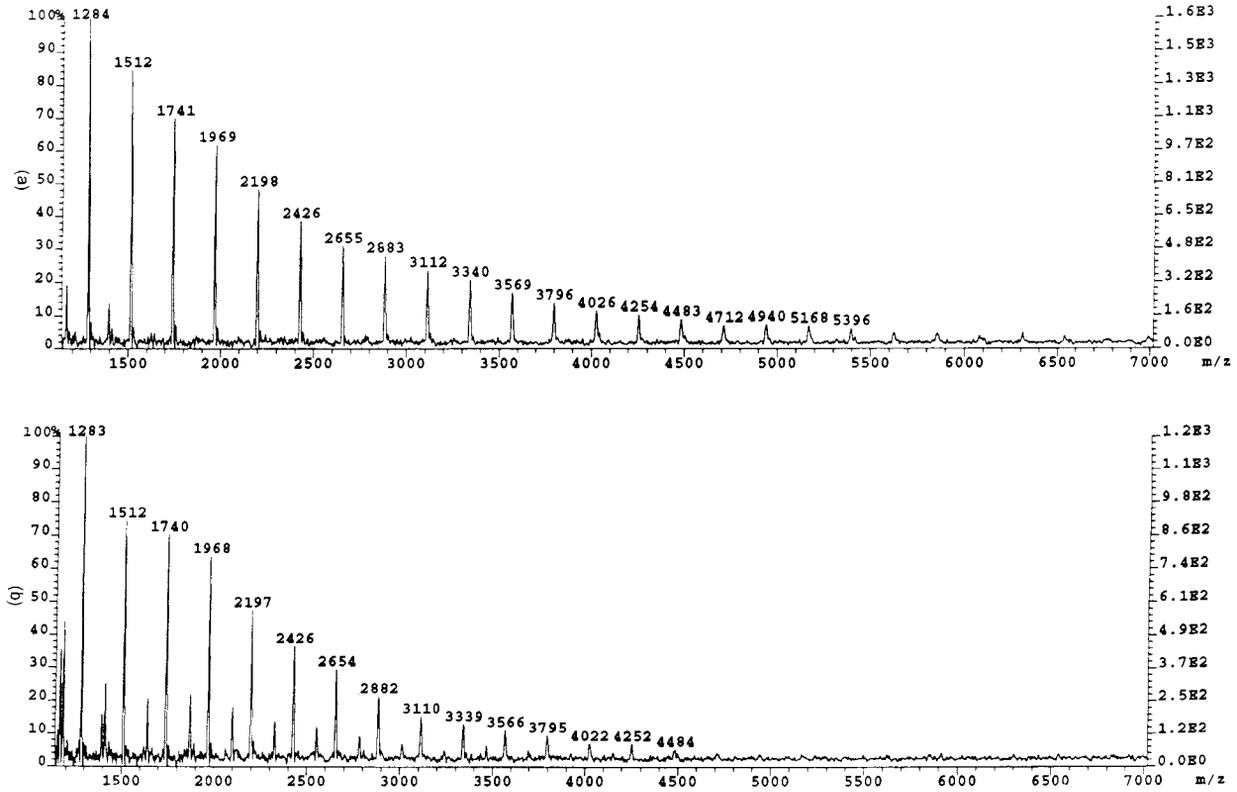


Fig. 7 MALDI-TOF mass spectrum of (a) enzymatically produced and (b) conventionally produced poly(hexane-1,6-diyl adipate).

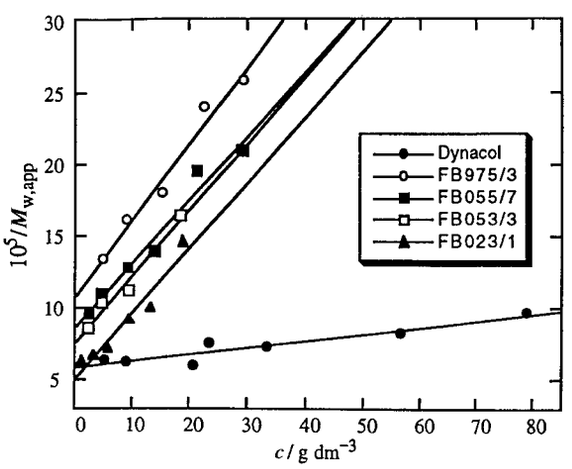


Fig. 8 Graph of Debye function *vs.* concentration from light scattering measurements for conventionally produced and a range of enzymatically produced poly(hexane-1,6-diyl adipate)s.

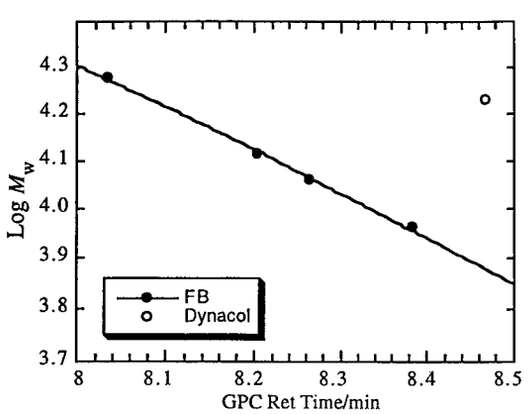


Fig. 9 Graph of absolute M_w derived from light scattering experiments against GPC retention time data for conventionally produced and a range of enzymatically produced poly(hexane-1,6-diyl adipate)s.

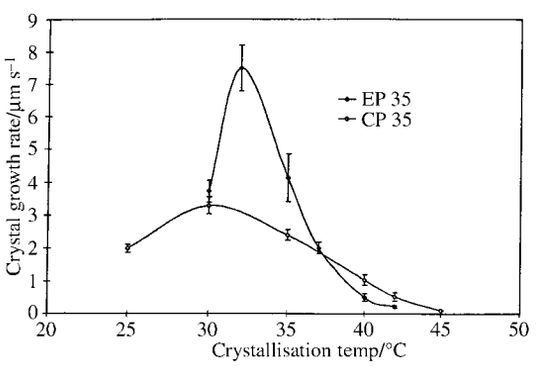


Fig. 10 Effect of synthesis route on crystallization behaviour of polyesters (3500 g mol^{-1}).

provided. We reason that whereas the enzymatic derivatives behave as discrete spheres in the solution, the conventional analogue is associated, to give an apparent molecular weight of 17000 Da (other physical data confirm that *ca.* 7000 Da is the actual molecular weight of the polymer).

Further, investigations on the rate of crystallization of an enzymatic polyhexylene adipate and its conventionally prepared analogue suggest a dramatic enhancement of efficiency for crystallization of the enzymatic derivative which indicates a fundamental difference in the ordering of the polymeric matrix. Fig. 10 shows the crystallization behaviour of two polyhexylene adipates of corresponding molecular weight (both 3500 Da): the conventionally produced derivative (CP35) and its enzymatically produced analogue (EP35). Though at 30 °C both have similar crystalline growth rate, above this temperature the enzymatic polyester demonstrates superior crystal growth, a 3.8-fold enhancement being measured over the conventional counterpart.[23]

All this evidence points to a definite change in the basic structure of the polyesters derived from enzymatic and conventional procedures, supporting the theory of a fundamental change in the architectural build-up of the two types of polyester.

Table 2 Comparison of enzyme-derived polymer with Dynacol™

Analysis	Enzyme-derived polymer	Dynacol™ 7360
Acid number (mg KOH per g)	0.66	<2
Water content (%)	0.019	<0.05
Viscosity at 80 °C (Pa s)	2.26	<2
GPC M_n	8869	6025
GPC M_w	16408	11655
GPC Polydispersity	1.85	1.93

Conclusion

The above studies indicated that poly(hexane-1,6-diyl adipate) could be produced economically. Moreover the material had a constitution and associated physical properties that made it more attractive for various end-uses than the conventionally derived material. Consequently the preparation of poly(hexane-1,6-diyl adipate) was successfully scaled-up to multi-kilo, pilot plant 0.5 tonne and 2.0 tonne level without undue problems. Constant removal of water from the enzyme-catalysed polymerization process is crucial since it is apparent that, without the consequent shift in equilibrium, the reaction simply will not work.[24]

Experimental

Hexane-1,6-diol was purchased from BASF; Novozyme 435™ (batch reference number LC20003, activity 11950 propyl laurate units g^{-1}) was purchased from Novo Nordisk. The process was run using a 500 litre vessel, equipped with an oil jacket and heating coils; vacuum application was by means of a water-ring pump. A nitrogen sparge could be applied through a dip-tube.

Poly(hexamethylene) adipate

Hexanediol (115.7 kg) and deionized water (4.5 kg) were charged to the reaction vessel. The mixture was stirred at 60 °C until molten; adipic acid (134.3 kg) was added and the mixture was stirred at 60 °C for 18 h. Novozyme 435™ (1.3 kg) was added and vacuum (60 mbar) was applied. After 18 h the mixture was filtered through a nylon cloth (74 μm mesh size). (The immobilized enzyme was recovered and subsequently used in a second run without purification.)

The filtrate was returned to the reaction vessel. The mixture was heated for 48 h at 100 °C under vacuum (40 mbar) with a nitrogen sparge (10 L min^{-1}). The characteristics of the product were compared to Dynacol™ 7360, a material prepared by a conventional polymerization process (Table 2).

Acknowledgements

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