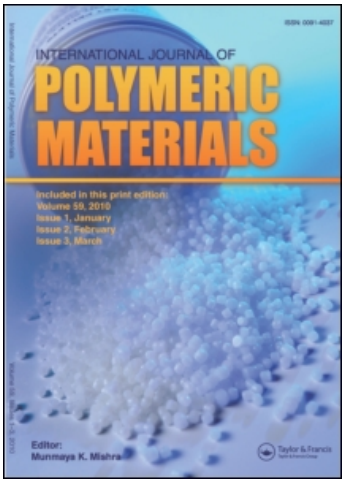

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Enzyme-catalyzed Polyester Synthesis

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Enzyme-catalyzed polymer synthesis has gathered many scientists around a major topic such as the synthesis of polyesters by enzyme catalysis. Since the mid 80's indeed, it is commonly accepted and experimented that lipases in particular are able to catalyze reverse reactions when they are used in organic media (less data are reported on esterases or proteases). Transposed to polymer chemistry, these pioneering results led to the definition of numerous reaction systems leading to the formation of polyesters. Thus AA+BB- and AB+AB-type polycondensations as well as lactones and macrolides polymerizations have been achieved in various organic solvents. In this article, the creation of aliphatic, unsaturated and aromatic polyesters is reviewed with a special attention paid to the equilibria between linear chains and rings.

Keywords: Aliphatic polyesters; Unsaturated polyesters; Aromatic polyesters; Enzyme; Lipase; Ring-chain equilibrium

1. INTRODUCTION

The fundamental and applied research on enzymatic catalysis in non-aqueous media has seen an important development in the last twenty years and it is the subject of several reviews and books [1-4].

As any catalyst enzymes have no influence on the thermodynamics of the reaction. They decrease its activation enthalpy and are recovered at the end of the reaction. On the other hand they are specific

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to a reaction and to a substrate. Very sensitive to *pH*, *T*, pressure and solvent, these biocatalysts are used in mild conditions in most cases. Commonly used temperatures range from 20 to 60°C even though some experiments were carried out at 100°C, indeed 130°C.

Enzyme-catalyzed polycondensations in organic solvents were used to prepare polyesters [5–15], polysaccharides [16–20], aromatic polyethers [21–23], polyphenols [24–31], polyanilines [32,33] and less classical polycondensates such as polyimines [34].

Lipase-catalyzed polyester synthesis has gained much attention from industrial people. Baxenden chemicals Ltd., is indeed the first company that sells enzymatically prepared polyesters [35].

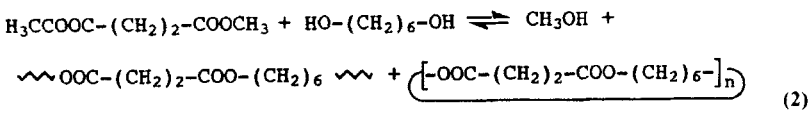
Polyesters are essentially prepared by ring opening-polymerization [36–46] or by polycondensation which are important topics of our group [7–12]. It will be the subject of this article. However our activity is not limited to the preparation of polyesters. It also covers monomer and polymer modification [47], hydrolysis of polymers [48–50] and enzyme-mediated initiation of radical polymerization [51–54].

2. ALIPHATIC POLYESTERS

Most polyesterifications and polytransesterifications are equilibria (Eq. (1)). The displacement of equilibrium 1 toward the formation of polyester raises difficult problems: most of enzyme-catalyzed reactions are carried out at nearly room temperature so that it is not possible to distill off the low molar mass compound (for instance water or methanol). The shifting can be obtained by scavenging methanol with a sieve [5,55] or eliminating it by a nitrogen stream [7]. Less often, selective solubilization or precipitation of one of the products is carried out.

$$R''COOC\text{-}R\text{-}COOR'' + HO\text{-}R'\text{-}OH \rightleftharpoons \sim\sim OOC\text{-}R\text{-}COO\text{-}R'\sim\sim + R''OH \quad (1)$$

Lalot *et al.* [7] studied the polyesterification of dimethyl succinate (S) with 1,6-hexanediol (H) catalyzed by Lipozyme (immobilized lipase from *Rhizomucor miehei*) or Novozyme (immobilized lipase from *Candida antarctica*) in toluene at 60°C. According to Eq. (2), poly(1,6-hexanediyl succinate) (PHS) was obtained.



(2)

Methanol was eliminated by a nitrogen stream bubbled through the reactive medium. The conversion at thermodynamic equilibrium ranges from 50 to 60%. When the flow of nitrogen is 50 ml · min^{-1} (low flow) the conversion reaches 100% after 20 days with Novozyme and 30 days with Lipozyme. On the other hand when it is 300 ml · min^{-1} (high flow) the same conversion is reached after only 8 hours.

The hydrophobicity of the solvent is essential. It is estimated by log P parameter where P is the partition coefficient defined as the ratio of the concentrations of an organic solvent when it is distributed between the two phases of an octanol-water mixture: $P = [\text{Solute}]_{\text{octanol}}/[\text{Solute}]_{\text{water}}$. When reaction 2 is carried out in the presence of Novozyme at 300 ml · min^{-1} nitrogen flow in toluene or ethylbenzene (log $P = 2.5$ and 3.0, respectively), the quantitative conversion is obtained after less than 1 day.

When the solvent is acetonitrile (log $P = -0.33$) the same result is obtained after 9 days. The evolution of the extent of reaction (p) with

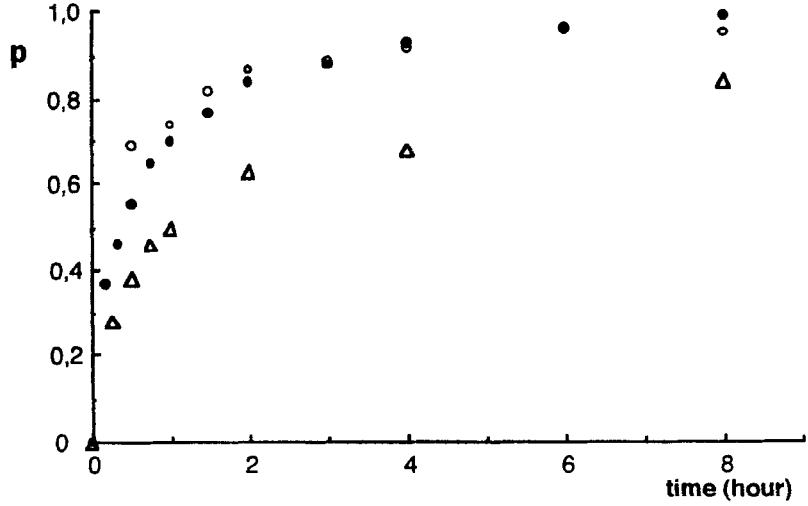


FIGURE 1 Enzyme-catalyzed PHS synthesis in (Δ) acetonitrile, (•) toluene, and (○) ethylbenzene at 60°C under N_2 (300 mL/min). Variation of p *versus* time [7].

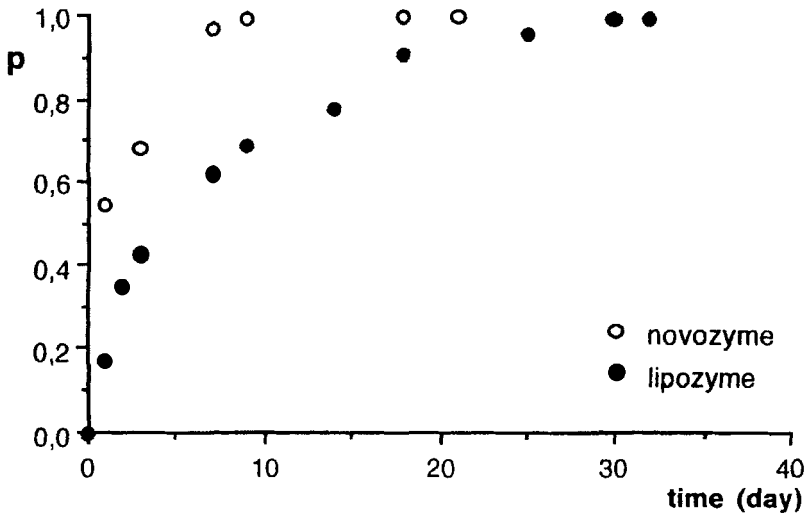


FIGURE 2 Enzyme-catalyzed PHS synthesis in toluene at 60°C under N_2 (50 mL/min). Variation of *p versus* time [7].

respect to time is given by Figures 1 and 2 which clearly show the influence of the enzyme and the solvent nature.

Experimental molar masses $M_{n,exp}$ of the polyesters are far below theoretical values $M_{n,th}$ (Tab. I). The crude product was extracted by methanol leading to two fractions (Tab. II). The insoluble one is

TABLE I Polytransesterification of dimethyl succinate with 1,6-hexanediol in toluene at 60°C in the presence of lipases: conversion, theoretical and experimental average molecular weights ($M_{n,th}$ and $M_{n,exp}$ respectively)

Enzyme	*Conversion*	$M_{n,th}$[a]	$M_{n,exp}$[a]
Novozyme	> 0.99	> 20000	2360
Lipozyme	> 0.99	> 20000	2450

[a] Average molecular weights in polystyrene equivalent.

TABLE II Polytransesterification of dimethyl succinate with 1,6-hexanediol in toluene at 60°C in the presence of Novozyme. Linear and ringed species contents

Components	*wt-%*	DP_n
Linear Polyester	60–65	2–22
Cycles	35–40	2–10

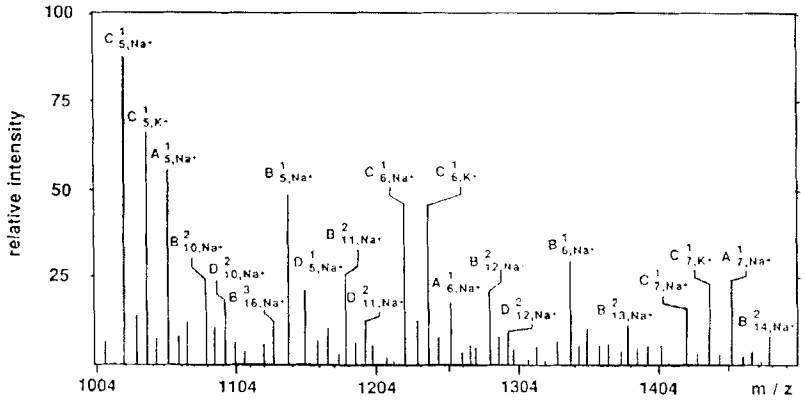


FIGURE 3 Mass spectrum of PHS sample synthesized by Lipozyme catalysis (extension from m/z = 1004 to 1504). The peaks are designated by $X^{a}_{n,i}$. X is relative to the nature of end groups: A, B, C and D correspond to α-methylester-ω-hydroxy, α, ω-dihydroxy, cyclic and α, ω-dimethylester chains respectively. The charge and the motif number of the chains correspond to subscripts a and n respectively. The cation nature (Na^+ or K^+) corresponds to *i* [7].

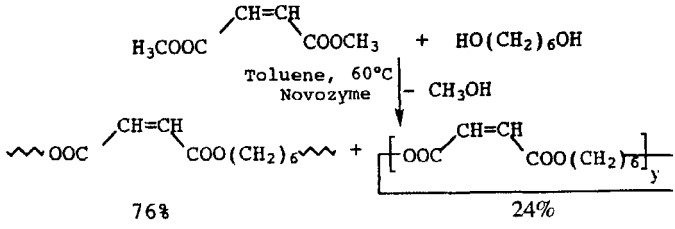
essentially made of linear chains and its $M_{n,exp}$ is close to 12000. The soluble phase mainly contains cycles and a few short linear chains. ^{1}H-NMR showed that the end group content is low which confirms the presence of cycles. The mass spectrometry analysis facilitated the identification of chain end groups and to determine the DP_n of the cycles (Fig. 3). It ranges from 2 to 10. The monomeric cycle ($n = 1$) was not observed even when Li^+ was used as a complexing cation.

3. UNSATURATED POLYESTERS

The classical preparation of unsaturated polyesters is the melting of maleic anhydride with a diol. The main reaction is accompanied by several side reactions such as the cis to trans isomerization and the formation of cyclic end groups or side chains by addition of hydroxy groups to double bonds belonging to the same chain or to another one [56–58].

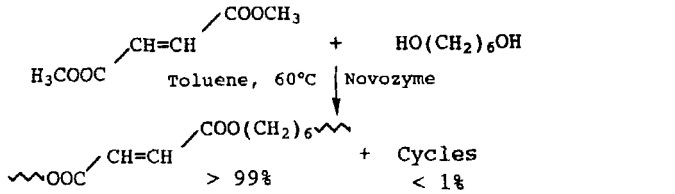
On the other hand Lalot *et al.* [8] polycondensed dimethyl maleate with 1,6-hexanediol (PHM) in the presence of Novozyme and did not observe the formation of the side structures which are characteristic of the chemical process. However the polyester contains macrocycles

(Eq. (3)).



(3)

The same observations hold for the 1,6-hexanediol/dimethyl fumarate system (PHF) (Eq. (4)) but only a very few cycles are formed (less than 1%). This almost complete absence of cycles is due to the geometry of fumaric unit which is very unfavourable to cyclization.



(4)

These structures were analyzed by ^{1}H-NMR and by mass spectrometry. The positive ion MALDI-TOF mass spectrum of cyclo[poly(1, 6-hexanediyl maleate) gives interesting information (Fig. 4).

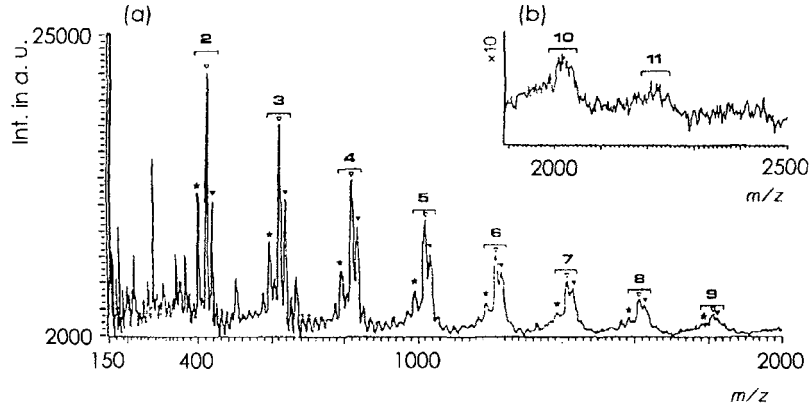


FIGURE 4 Positive ion MALDI-TOF mass spectrum of cyclo[poly(1,6-hexanediyl maleate) (matrix 2.5-DHB; 200 ml/pulse). n values are assignated above each signal. (⋆) M_y^+H (or M_y^+), (Δ) M_yNa^+, (▼) M_yK^+ [8].

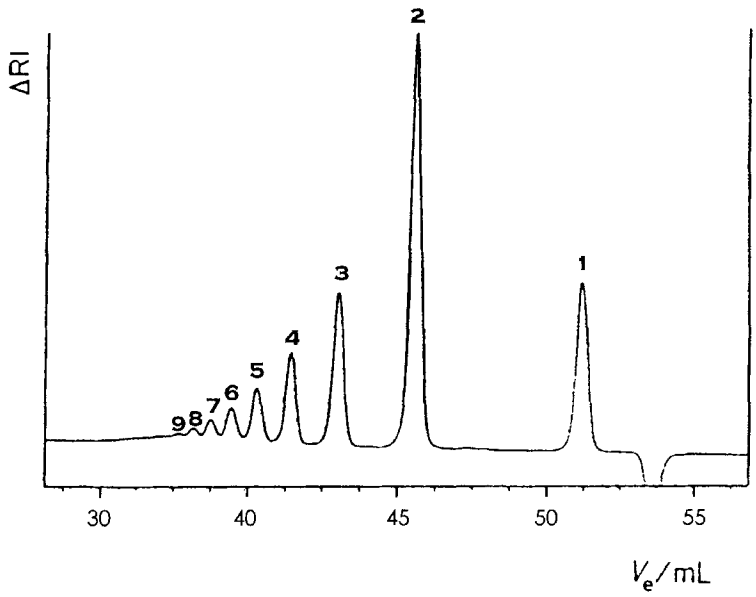


FIGURE 5 SEC Chromatogram of cyclo[poly(1,6-hexanediyl maleate); n values are assigned above each peak [8].

Each signal is made of three peaks corresponding to M_yH^+, M_yNa^+ and M_yK^+. The signal corresponding to $n = 1$ is not identified because it interfers with matrix signals. SEC chromatogram of the cyclic fraction particularly shows the abnormal intensity of peak 1 (Fig. 5). This distribution will be studied in parts 5 and 6.

4. AROMATIC POLYESTERS

Park *et al.* [59] prepared several aromatic polyesters by polycondensing a diester of terephthalic acid with 1,4-butanediol. The reaction was catalyzed by a commercial protease from *Bacillus lichenformis* in THF. The corresponding polyesters have molar mass ranging from 400 to 1000 with a dispersity index of 1.2. Unfortunately SEC chromatograms are not given which prevents us from knowing whether the products contain cycles or not.

The dimethyl esters of *o*-, *m*- and *p*-phthalic acids were polycondensed with 1,6-hexanediol in the presence of Novozyme [10]. The

contents of linear and cyclic polyesters are given in Table III. It shows that the relative positions of the esters have a drastic influence on the results.

Dimethyl *o*-phthalate and 1,6-hexanediol do not react because the vicinity of the ester functions forbids the enzymatic intervention. When they are in *meta* position the product contains 90% of linear chains and 10% of cycles which could be expected. Terephthalate leads to linear polyester with almost no cyclic species which fits the results of the industrial polycondensation of dimethyl terephthalate with ethanediol: 1 to 2% of cycles (mainly trimer).

For poly(1,6-hexanediyl *m*-phthalate), the presence of both linear and cyclic species is clearly shown by SEC analysis (Fig. 6). The chromatogram of the non-fractionated product is binodal and the large peak corresponds to linear polyesters. The methanol fractionation

TABLE III Polycondensation of phthalates with 1,6-hexanediol in the presence of Novozyme in toluene at 60°C. Linear and cyclic species content after reaction

	Linear %	*Cycle* %
o-phthalate	0	0
m-phthalate	90	10
p-phthalate	≈100	≈0

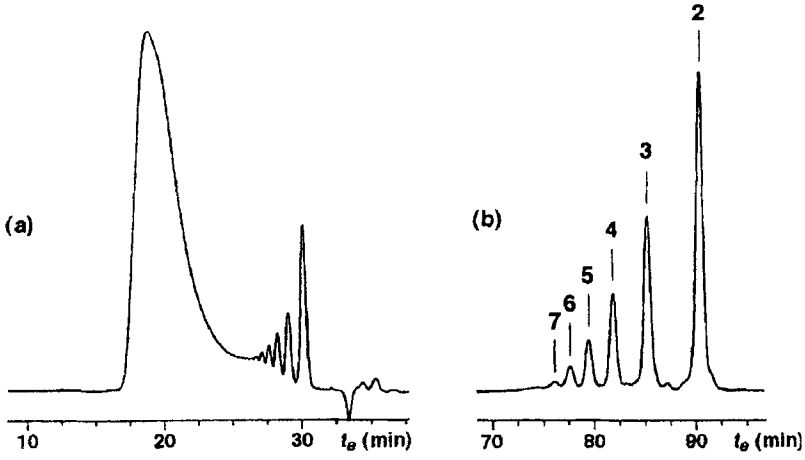


FIGURE 6 SEC Chromatograms of (a) unfractionated poly(*m*-hexanediyl phthalate) and (b) cyclo[poly(*m*-hexanediyl phthalate)]; n values are assigned above each peak [10].

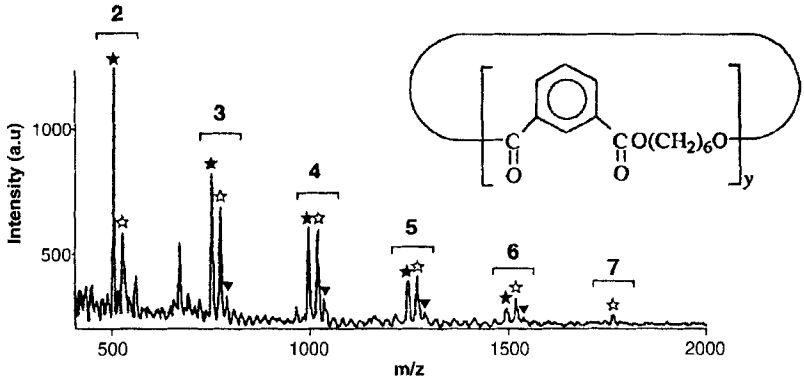


FIGURE 7 Positive ion MALDI-TOF mass spectrum of cyclo[poly(1,6-hexanediyl *m*-phthalate) (matrix 5: chlorosalysilic acid; 200 ml/pulse). n values are assigned above each signal. (⋆) M_y^+H (or M_y^+), (⋆) M_yNa^+, (▼) M_yK^+ [10].

leads to the cyclic fraction. Its mass spectrum shows the contribution of each cycle with the exception of the monomeric species (Fig. 7). This was already observed with cyclo[poly(1,6-hexanediyl maleate)] and will be discussed further.

5. RING-CHAIN EQUILIBRIUM

Formation of macrocycles is not specific to enzymatic catalysis. For instance Spassky *et al.* [60, 61] observed the same phenomenon when polycondensing α, ω-dibromoalkanes with alkaline salts of aliphatic diacids. This synthesis is a pure chemical process.

On the other hand the distribution of the cycles is specific to enzymatic catalysis. We carried out a thorough examination of this phenomenon. This study [11, 12] was made possible by a careful fractionation of the products which is not the case of many articles. However some authors carried out such separations by extraction or precipitation [62, 63].

To confirm that reaction 2 is an equilibrium, a cycle-free PHS sample was stirred in the presence of Novozyme in toluene at 60°C for one day. The SEC chromatograms of the sample before and after the enzymatic treatment clearly show the formation of rings from linear polyester (Fig. 8).

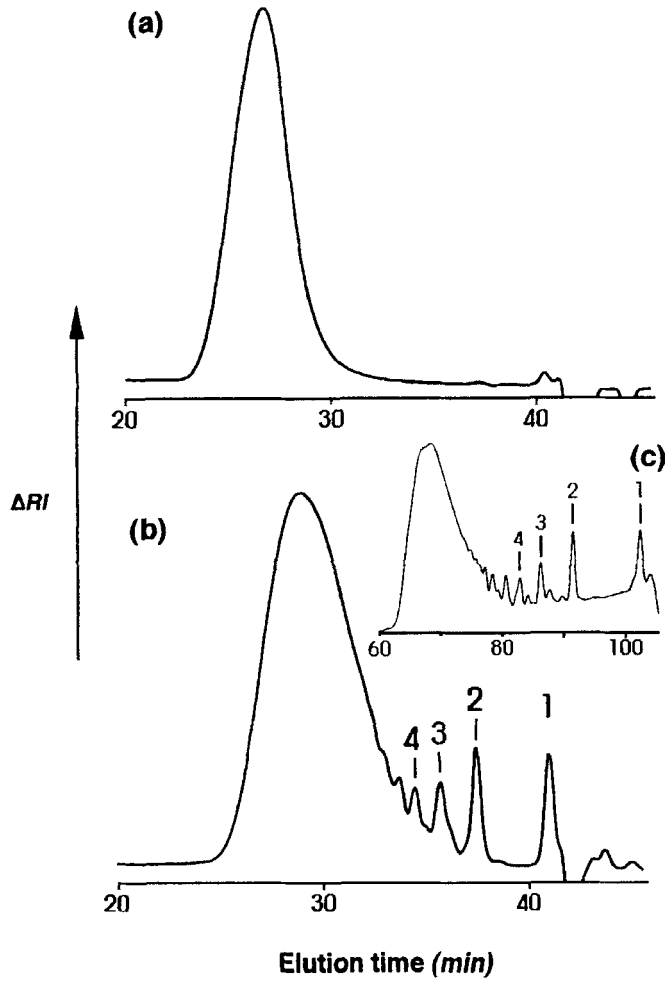


FIGURE 8 SEC profiles of PHS prepared by enzyme catalysis and obtained by pouring into methanol: (a) before and (b) after 24 h reaction in the presence of enzyme at 333 K in toluene [11].

This equilibrium obeys the classical laws which rule the formation of rings and particularly it is favored by dilution (Fig. 9). However the peak of cycle 1 has an unusual intensity.

To clarify this point we compared the experimental values to the theoretical ones calculated in the frame of Jacobson-Stockmayer theory [64]. It takes into account three different systems: (I) all monomers have identical units with two identical functional groups

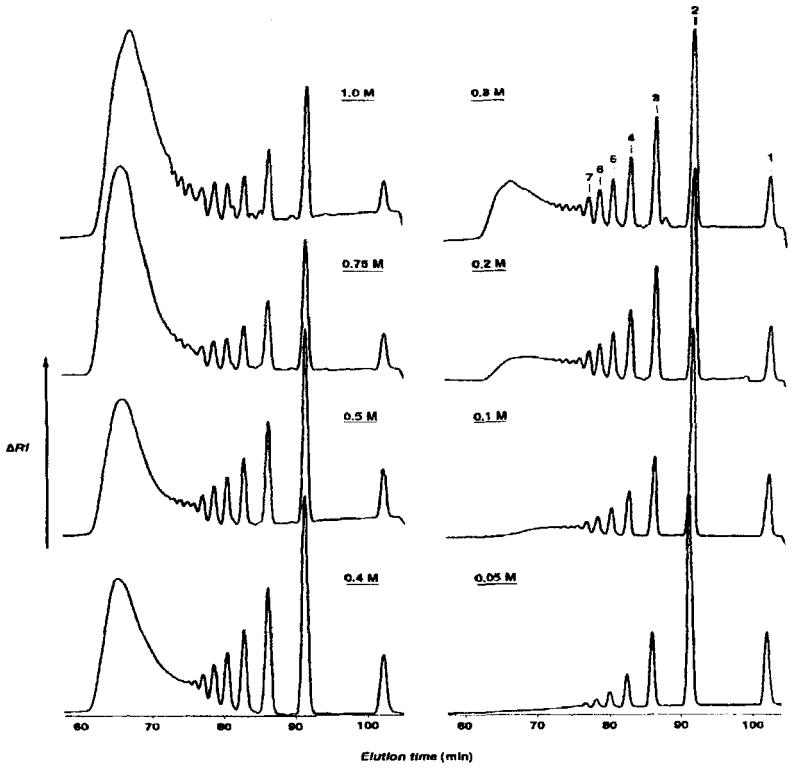


FIGURE 9 SEC chromatograms of unfractionated PHS synthesized from 1,6-hexanediol and dimethyl succinate in stoichiometric ratio by enzymatic catalysis in toluene at 333 K at different initial concentrations of reactive functions [11].

(this is the case of polydimethylsiloxanes); (II) the systems contain only one type of monomer with two different functional groups and (III) the systems are made of two different kinds of monomer but the functional groups of each monomer are identical. The systems studied in this article belong to case III.

Reaction 5 describes the reversible cleavage of a linear polymer ($L_{n+m,n+s}$) cleaved into a shorter one ($L_{m,s}$) and a cycle (R_{nn}). K is molar cyclization constant (Eq. 6).

$$L_{n+m,n+s} \underset{}{\overset{K}{\rightleftharpoons}} L_{ms} + R_{nn} \qquad s = m \text{ or } m+1 \tag{5}$$

$$K = [R_{nn}]/x^n y^n \approx [R_{nn}]/p^2 \tag{6}$$

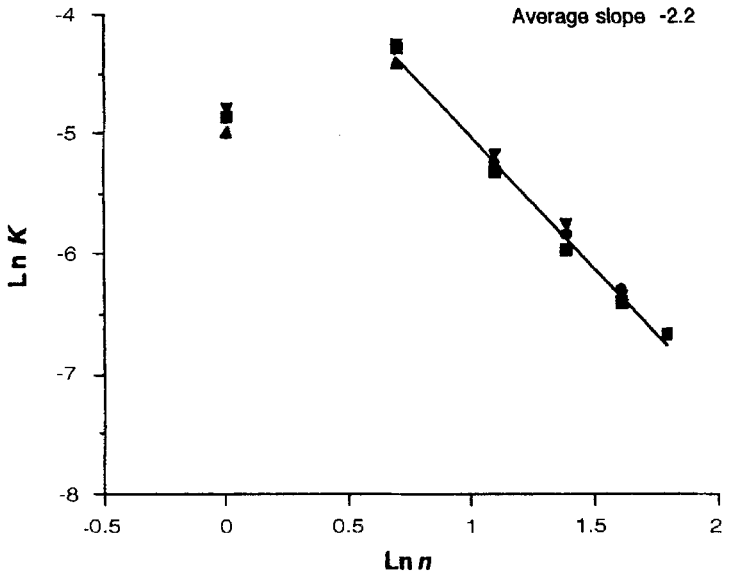


FIGURE 10 Experimental molar cyclization equilibrium constants $K(\text{mol} \cdot \text{L}^{-1})$ for cyclics in the PHS toluene solution at 333 K as a function of the repeating unit number in the ring n. LnK *versus* Lnn for different initial concentrations of the reactive functions: $C_0 = 1.0$ (▼), 0.75 (▲), 0.5 (■) and 0.4 mol · L^{-1} (•) [11].

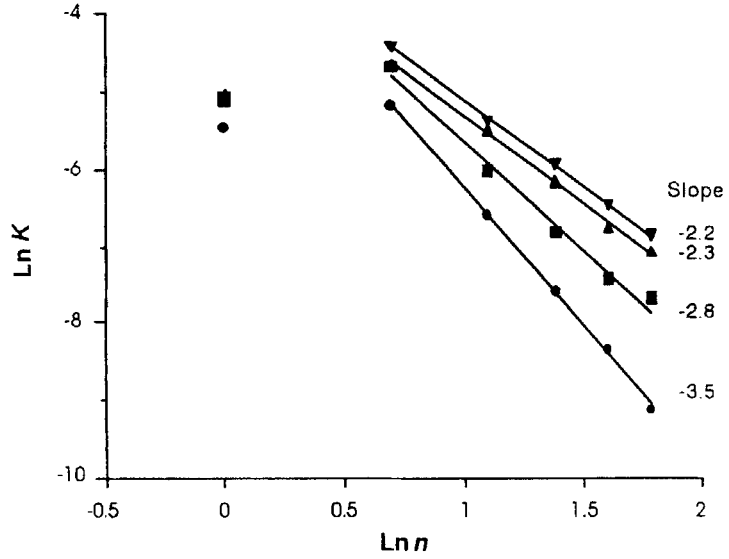


FIGURE 11 Experimental molar cyclization equilibrium constants K (mol · L^{-1}) for cyclics in the PHS toluene solution at 333 K as a function of the repeating unit number in the ring n. LnK *versus* Lnn for different initial concentrations of the reactive functions: $C_0 = 0.3$ (▼), 0.2 (▲), 0.1 (■) and 0.05 mol · L^{-1} (•) [11].

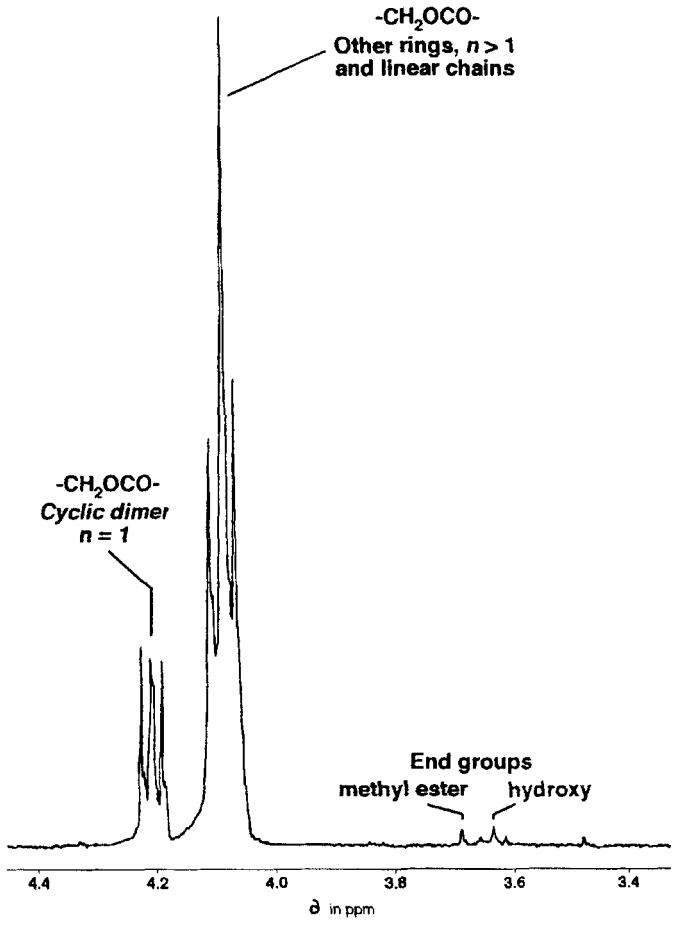


FIGURE 12 ^{1}H NMR spectrum of PHS. Pattern of group —$CH_2OC(O)$—. Part (a): $n > 1$; part (b): $n = 1$ [12].

Conversions can be identified to the extent of reaction (p) at least above a floor value depending on the system. LnK is given by relation 7.

$$\mathbf{Ln}K = \mathbf{Ln}B\text{-}5/2\mathbf{Ln}n \tag{7}$$

Its validity was shown by experiments relative to PHS. They were carried out with initial concentrations (C_0) ranging from 0.4 to 1 mol. L^{-1}. Figure 10 shows that the variation of LnK against Lnn is linear and the slopes of the straight lines (-2.2) are very close to the

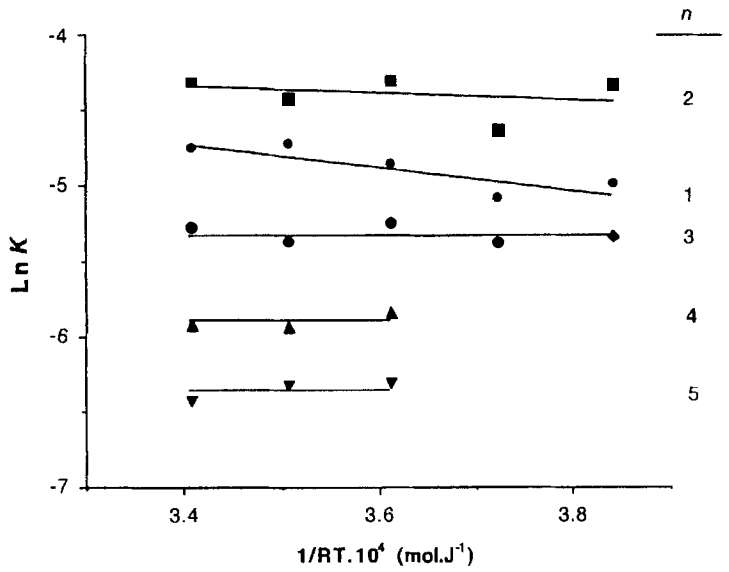


FIGURE 13 Plot of LnK against $1/T$ for PHS (toluene). $n = 1$ (•), 2 (■), 3 (◆), 4 (▲), and 5 (▼) [11].

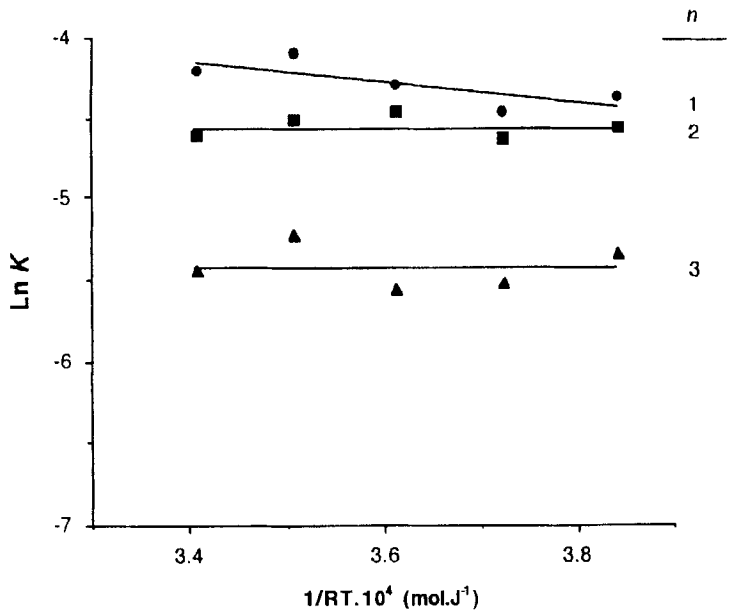


FIGURE 14 Plot of LnK against $1/T$ for POS (toluene). $n = 1$ (•), 2 (■), 3 (▲) [11].

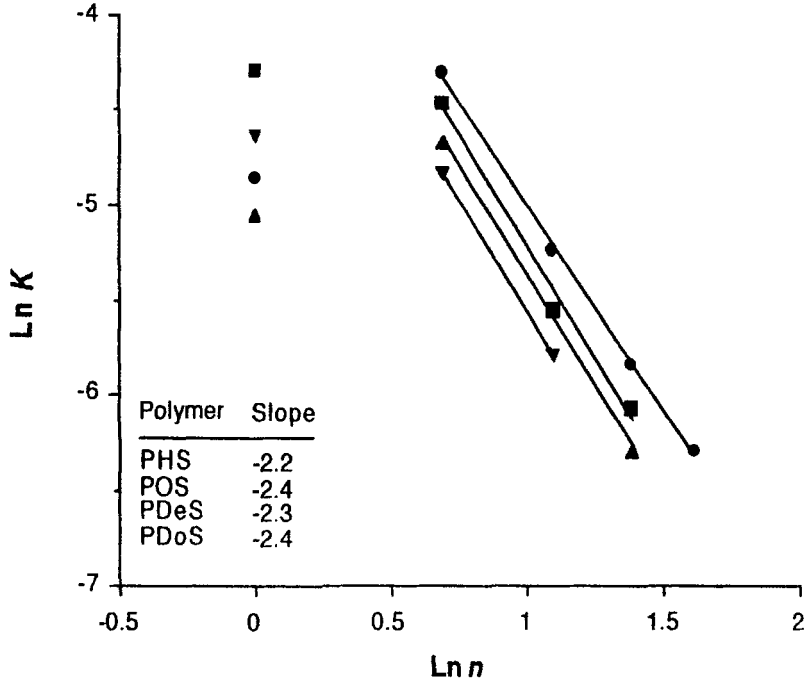


FIGURE 15 Equilibrium between linear and cyclic fraction of several polyesters, in the presence of Novozyme, in toluene at 333 K. Variation of K with n. PHS: poly(1,6-hexanediyl succinate) (•); POS: poly(1,8-octanediyl succinate) (■); PDeS: poly(1,10-decanediyl succinate) (▲); PDoS: poly(1,12-dodecanediyl succinate) (▼) [11].

theoretical value (-2.5). This confirms that linear chains and rings obey equilibrium 5.

On the other hand when the concentrations are below 0.4 mol · L^{-1}, there is no common straight line which would bring together all the results obtained for different concentrations (Fig. 11). This shows that the assimilation of x and y to p is no longer valid. The plot is formed of independent straight lines. Each of them corresponds to a specific concentration. Their slopes decrease with increasing dilution showing that the formation of the largest rings is not favored.

Figures 10 and 11 show that relation 7 does not hold for the monomeric ring ($n = 1$). In this respect, it should be mentioned that the ^1H NMR pattern of the $-CH_2OC(O)-$ group of the monomeric ring presents peculiar characteristics: the chemical shift and the

multiplicity are different from those of the same group in the larger rings (Fig. 12).

The variation of LnK with temperature obeys Arrhenius law whatever n. This is valid for both PHS and cyclo[poly(1,8-octanediyl succinate)] (POS) (Figs. 13 and 14). However the slope of the straight line corresponding to $n = 1$ is different from those of the other rings. LnK increases with increasing temperature whereas for the other rings LnK does not depend on temperature.

It was interesting to investigate the influence of the diol length keeping succinate as the diacid residue. Whatever the diol, the variation of LnK with Lnn is linear with the same slope (-2.2) (Fig. 15). K decreases with the number of skeletal atoms.

In the same way we compared polyesters prepared from 1,6-hexanediol and dimethyl diesters of succinic, maleic and isophthalic diacids (PHiP). The LnK *vs.* Lnn plots are straight lines with the

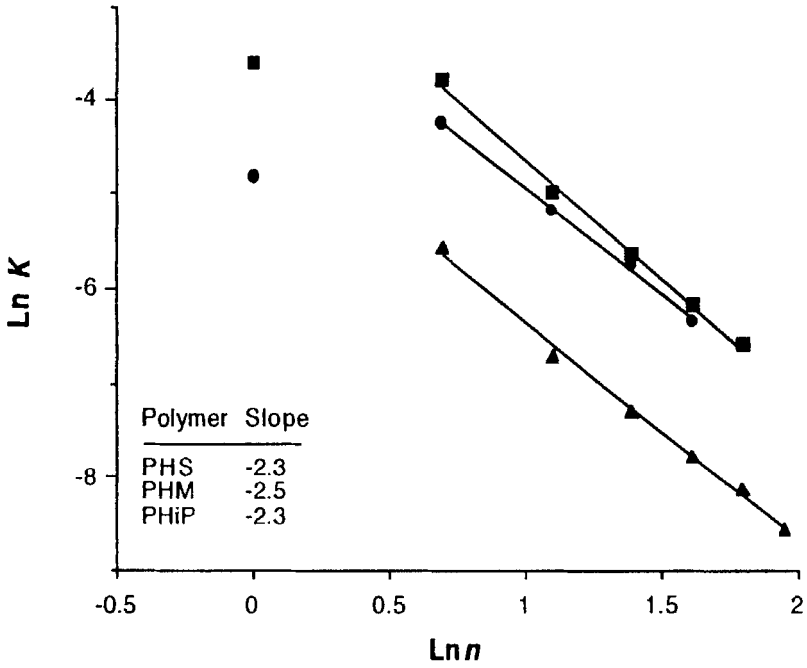


FIGURE 16 Equilibrium between linear and cyclic fraction of several polyesters, in the presence of Novozyme, in toluene at 333 K. Variation of LnK with Lnn. PHS (●), PHM (■), PHiP (▲) [11].

same slope (−2.2) (Fig. 16). K is almost the same for PHS and PHM but much lower in the case of PHiP.

6. GENERAL DISCUSSION

The different steps which take place in a lipase-catalyzed polyesterification are described by Eqs. (8)–(14). The global enzymatic process is classical (Eqs. (8) and (9)). The activated OH group of the enzyme reacts with ester R_1-COO-R_2 leading to R_2-OH and R_1COO-Enz. The latter reacts with alcohol R_3-OH leading to the ester R_2-COO-R_3.

$$R_1\text{-COO-}R_2 + \text{HO-}Enz \rightleftharpoons R_1\text{-COO-}Enz + R_2\text{-OH} \quad (8)$$

$$R_1\text{-COO-}Enz + R_3\text{-OH} \rightleftharpoons R_1\text{-COO-}R_3 + \text{HO-Enz} \quad (9)$$

In the case of the systems on study, the acylation step includes the following steps (Eqs. (10)–(13)): In the presence of free enzyme (II), an α, ω -dihydroxy-polyester gives an α, ω -dihydroxy-polyester (IV) and compound III. Compound III should be considered as an α-hydroxy-ω-acylenzyme-polyester. An α, ω-dimethylester-polyester (V) gives an α-hydroxy-ω-methylester-polyester (VII) and compound VI. As for compound III, compound VI should be considered as an α-methylester-ω-acylenzyme-polyester. An α-hydroxy-ω-ester-polyester (VIII) gives III or VI depending on the orientation of attack by II. To sum up, two kinds of acylenzyme are formed in the acylation step whatever the starting chain: an α-hydroxy-ω-acylenzyme-polyester (III) or an α-methylester-ω-acylenzyme-polyester (VI).

$$\underset{\text{I}}{\text{HO}\sim\text{C(O)O}\sim\text{OH}} + \underset{\text{II}}{\text{OH-}Enz} \rightleftharpoons \underset{\text{III}}{\text{HO}\sim\text{C(O)O-}Enz} + \underset{\text{IV}}{\text{HO}\sim\text{OH}} \quad (10)$$

$$\underset{\text{V}}{\text{CH}_3\text{OC(O)}\sim\text{C(O)O}\sim\text{C(O)OCH}_3} + \text{II} \rightleftharpoons \underset{\text{VI}}{\text{CH}_3\text{OC(O)}\sim\text{C(O)O-}Enz} + \underset{\text{VII}}{\text{HO}\sim\text{C(O)OCH}_3} \quad (11)$$

$$\underset{\text{VIII}}{\text{HO}\sim\text{C(O)O}\sim\text{C(O)OCH}_3} + \text{II} \rightleftharpoons \text{III} + \text{VII} \quad (12)$$

$$\text{CH}_3\text{OC(O)}\sim\text{C(O)O}\sim\text{OH} + \text{II} \rightleftharpoons \text{VI} + \text{IV} \quad (13)$$

Amongst the two acylenzyme intermediates, only compound III leads to the formation of cyclic species in the deacylation step. Reaction 14 takes place between the hydroxy group of compound III and its enzyme moiety. Both are the end groups of the chain.

$$HO\sim C(O)O\text{-}Enz \rightleftharpoons Ring + HO\text{-}Enz \quad (14)$$

The deacylation of compound VI cannot lead to ring formation because of the absence of free hydroxy group as an end group. Therefore deacylation is necessarily a bimolecular reaction. Only linear chains are obtained from compound VI.

When $n = 1$, the reaction between OH and COOEnz is hindered by their close proximity and the large volume of the enzyme. On the other hand this hindrance does not exist when $n > 1$. When the catalyst is not an enzyme but a protonic acid or a metallic derivative such as $Ti(OR)_4$, the contribution of the monomeric species obeys the rules which govern equilibrium.

The relative contribution of peak 1 is controlled by the kinetics of the process. The equilibrium which takes place between each cycle and the chains is specific with its own constant K_n. The experimental value of K is an average value of K_n. The ratio $k_{1,n}/k_{2,n}$ (equilibrium 2) can be considered as a good approximation of K. When $n = 1$, $k_{1,1}$ is below $k_{1,n}$ leading to an abnormal value of K_1 ($K_1 < K_n$) and showing that this equilibrium is kinetically controlled. When n is above 1, the contribution of each cycle decreases with increasing n which corresponds to a thermodynamic control confirmed by the independence of K with respect to temperature.

K_1 increases with temperature (Fig. 13) which could be expected since the internal movements of the chains relieve the strain due to the steric hindrance.

In the case of poly(1,6-hexanediyl isophthalate) two parameters must be considered: the hindrance responsible for the abnormal intensity of peak 1 and the rigidity of the chain which explains the low K value compared to those obtained for PHS or POS (Fig. 15).

On the other hand this steric hindrance prevents the contribution of side reactions in the polycondensation of a diol with dimethyl maleate as the attack of OH on the double bond is hindered by the bulkiness of the polyester chain and of the enzyme.

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