0273-2289/90/2425—0375$02.40

Enzymatic Synthesis of Neopentylpolyol Esters in Organic Media

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ABSTRACT

Utilization of lipases for synthesis of esters of hydrophilic polyols has been investigated. The choice of a suitable solvent is crucial in this type of reaction. An interesting case is fatty acid esters from neopentylpolyols, such as trimethylolpropane, which are of great interest as high temperature lubricants. Enzymatic synthesis of trimethylolpropane tricaprylate was studied as an alternative to chemical manufacturing. Triester production occurred only if the water produced by esterification was continuously removed from the medium. In these condition, kinetics of appearance and transformation of mono-, di- and triesters were determined in order to define optimal conditions.

Index Entries: Polyol esters; lipases, esterification by; organic medium; trimethylolpropane esters.

INTRODUCTION

Recent studies have shown that enzymatic catalysis can occur in organic media instead of water (*1*). Main applications have been focused on esterification by lipases because they require the use of nonaqueous media and because of the particular mode of action of lipases (*2*). Most organic solvents suitable for enzymatic catalysis are hydrophobic, probably because they maintain an essential water layer around the enzyme to prevent its inactivation (*1,3*). Yet, with hydrophilic substrates, such as diols (*4*), sugars (*5,6*) and glycerol (*7*), a compromise has to be found

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between the insolubility of these substrates in apolar solvents and the denaturation of enzymes in polar solvents.

Esters of fatty acids (C_6–C_{10}) derived from neopentylpolyols, such as neopentylglycol, trimethylolpropane ($C_2H_5C(CH_2OH)_3$), or pentaerythritol, are thermally stable and not sensitive to oxidation, and their viscosity is not greatly affected by increase in temperature, conferring on them excellent lubricating properties (*8*). The preparation of these esters by conventional chemical methods has been reported as giving rise to by-products (*9*). The enzymatic synthesis of trimethylolpropane (TMP) triesters has been investigated as an interesting model for enzymatic catalysis in organic solvents using hydrophilic polyols and as an alternative way to their chemical manufacturing. The kinetics of multiple esterification of TMP with caprylic acid by lipases in organic media have been studied, and a preliminary optimization of this synthesis has been carried out.

MATERIALS AND METHODS

Enzymes

Most esterifications have been carried out using lipases from *Mucor miehei*. This enzyme has been used either in a free form as a powder (Esterase 30,000 from Gist Brocades, Seclin, France) or immobilized on a macroporous anion exchange resin (Lipozyme IM 20 from Novo Industries A/S, Bagsvaerd, Denmark). Two preparations of lipozyme have been utilized in this study: batch 1, 50 U/g; batch 2, 30 U/g. The water contents of esterase and lipozyme were 5 and 10% (w/w), respectively.

Reagents

Trimethylolpropane was purchased from Prolabo (Paris, France). Caprylic acid was from Sigma (St. Louis, MO, USA). Trimethylolpropane tricaprylate was from Ciba Geigy, Basel, Switzerland (Reolube LT 2400) and distilled before use as a chromatography standard. Molecular sieve type 3 Å (sodium and potassium alumino-silicate) was obtained from Union Carbide (Danbury, CT, USA). All other reagents were of analytical grade (purity, 99%).

Analyses

Esters of trimethylolpropane were quantified by capillary gas chromatography. Before injection, samples were diluted in *n*-nonane, and 0.5 μL samples were injected in a fused silica capillary J.W. DB1 (30 m×0.31 mm) column with a 0.25 μm film thickness. The chromatograph was a Varian 3500 equipped with a Varian 8035 autosampler. Injector temperature was programmed from 80 to 310°C; column temperature was increased from 80 to 300°C at a rate of 30°C/min and detector temperature was

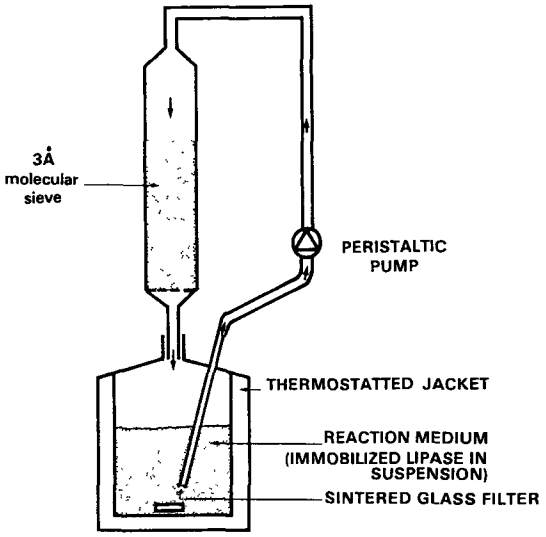

Fig. 1. Experimental set-up designed for continuous removal of water (used in procedure 2).

350°C. he water content was determined by automatic Karl Fischer analysissis with a DL 18 Mettler titration system. Hydrolysis rates of tributyrin, trimethylolpropane tricaprylate, or other esters were determined according to the Novo method (*10*).

Reaction Mixtures

Three different procedures were used: in the first one, esterifications were carried out in flasks containing 30 mL of solvent, substrates at indicated concentrations, and 5 g/L of lipase in suspension. Flasks were incubated at 30°C in a rotary shaker (New Brunswick Scientific Co., Edison, NJ, USA). Before use, solvents were dehydrated on 3 Å molecular sieve previously activated at 350°C.

In the second procedure, an experimental set-up was devised for continuous removal of water from the medium. It consisted of a thermostatted, stirred reactor (200 mL) containing the immobilized enzyme (lipozyme) in suspension in the reaction medium (120 mL). The medium was drawn off the reactor through a sintered-glass filter by means of a peristaltic pump, and percolated on a glass column containing 25 g of 3 Å molecular sieve before being recycled into the reactor (Fig. 1). The use of an immobilized lipase and a sintered-glass filter was made in order to keep the enzyme inside the reactor to prevent it from being inactivated by contact with the molecular sieve. The solvent was butyl ether, and the reaction temperature was generally 40°C. Initial concentrations of TMP and caprylic acid were 20 and 120 m*M*, respectively. The lipozyme concentration was 20 g/L when not indicated.

In the third procedure, continuous esterification was performed in a fixed-bed reactor consisting of a water-jacketed column (13×255 mm) con-

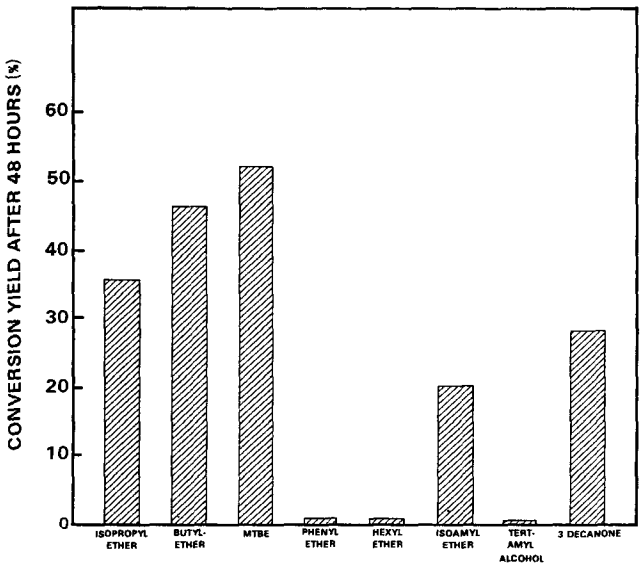

Fig. 2. Effect of the nature of the solvent on the transesterification yield (methyl caprylate, 50 m*M*; TMP, 50 m*M*; enzyme, esterase; all other conditions according to procedure 1).

taining seven successive layers of lipozyme and molecular sieve separated by cotton wool. The feed medium contained 20 m*M* trimethylolpropane and 100 m*M* caprylic acid in di-*n*-butyl ether. It was injected at the bottom of the reactor by means of a P 500 Pharmacia pump. The flow rate was 0.6 mL/h, i.e., a dilution rate of 0.018/h. The total amounts of lipozyme and molecular sieve in the column were 3.7 and 9.5 g, respectively. The column was thermostatted at 40°C.

The conversion yield is expressed as the ratio of the number of esterified groups upon the number of functions of the limiting reagent available for esterification. Generally a molar excess of acid/TMP greater than 3 was used. In this case, the conversion yield was equal to [1 × (mol of monoester) + 2 × (mol of diester)] + 3 × (mol of triester)]/3 × (mol of TMP).

RESULTS AND DISCUSSION

Choice of a Suitable Solvent

Transesterification reaction between methylcaprylate and TMP was chosen for evaluation of solvents because methyl caprylate was easily determined and the reaction did not involve formation of water. All solvents used were dehydrated. TMP was soluble in polar solvents that inactivated lipase, such as DMSO or DMF, or in which lipase had a low activity, such as acetone (*11*). The solvents tested were ethers, a ketone, and a tertiary alcohol. The effect of the nature of the solvent on the transesterification yield is shown on Fig. 2. In certain ethers (methyl tert butyl

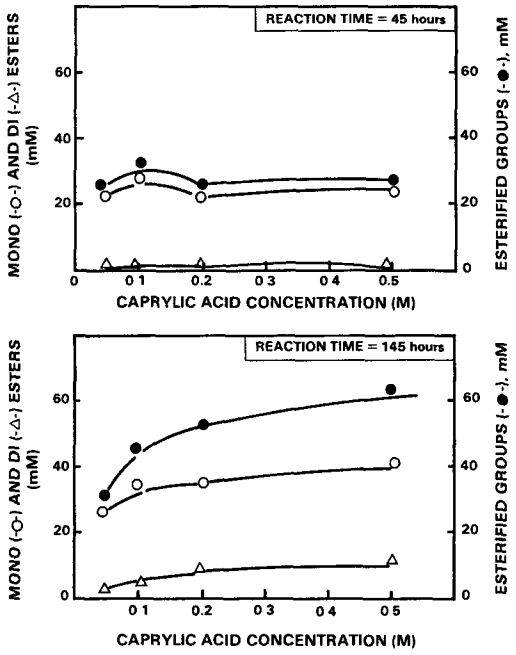

Fig. 3. Effect of initial caprylic acid concentration on TMP ester concentration (TMP, 50 m*M*; enzyme, esterase; solvent, MTBE; procedure 1).

ether, butyl ether, and di-isopropyl ether), substantial amounts of TMP were transesterified, albeit no triester was formed. These solvents are a little hydrophobic (3), and they constitute a good compromise between solubility of TMP and inactivation of the lipase. Lipases from other origins (*Candida cylindraceae* and *Pseudomonas fluorescens*) were tested using methyl tert butyl ether (MTBE) as a solvent, but no esterification activity was detected.

Effect of Initial Fatty Acid Concentration

While keeping the TMP concentration constant at 50 m*M*, the caprylic acid concentration was increased from 50 to 500 m*M* in order to define the optimal acid/TMP ratio. Assays were carried out using MTBE as solvent and esterase as enzyme. As shown on Fig. 3, esterification yield was low and only mono- and diesters were produced. Although a theoretical molar acid/TMP ratio of 3 would be sufficient to obtain the maximum yield, it seems that a larger excess of acid improved the final yield. Nevertheless, the esterification was far from being complete.

Effect of Initial Water Content

Different amounts of water were added to previously dehydrated MTBE that still contained 0.2 g/L of water and esterifications carried out using lipozyme. Esterification was favored by low initial water content,

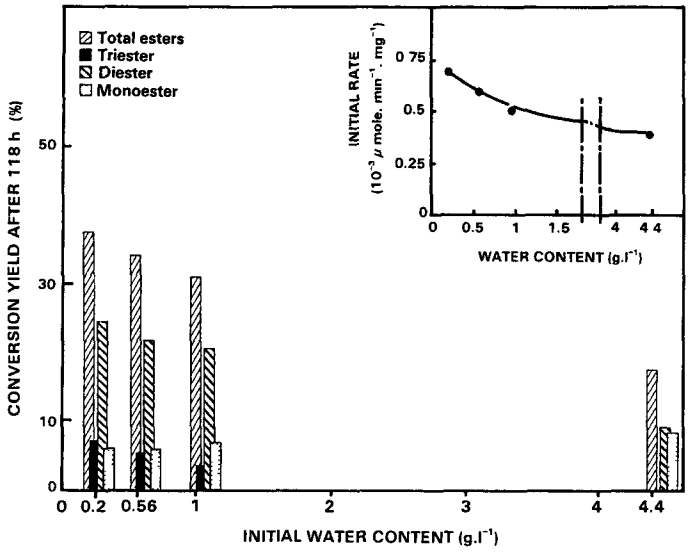

Fig. 4. Effect of initial water content on esterification yield (TMP, 20 m*M*; caprylic acid, 120 m*M*; solvent, MTBE; lipozyme batch 1, 20 g/L; procedure 1).

albeit the reaction still occurred at an initial water concentration of 4.4 g/L (Fig. 4). In the case of conversion carried out at an initial water content of 0.2 g/L (11.1 m*M*), the final water content was 55 m*M* and only 22.5 m*M* were owing to the esterification itself. That means that some water coming from the enzyme or the atmosphere got dissolved in the solvent during the assay.

In order to know whether the cessation of the esterification was owing to the water concentration, a final reaction mixture was filtered, dehydrated on a molecular sieve, and incubated again with lipase. A greater amount of triester was produced, suggesting that it was the water concentration (or activity) that governed the reaction equilibrium.

Since continuous removal of water was necessary to shift the equilibrium toward esterification, all other batch experiments were done using procedure 2 and the reactor described in Fig. 1. The solvent was butyl ether because of evaporation and safety problems with MTBE.

Kinetics of Enzymatic Esterification of TMP

A typical time course of an esterification of TMP with caprylic acid in the above-described reactor is shown in Fig. 5. TMP tricaprylate production occurred at a much greater extent with continuous removal of water. Because of the successive esterifications, complete reaction was slow; nevertheless, after 180 h, the conversion yield was 90%, with very low amounts of mono- and diesters. A maximal diester conversion yield of about 45% was attained after 20 h, with low proportions of mono- and triesters. A more detailed study was undertaken to precisely evaluate initial esterification rates. As shown in Table 1, mono- and diester formation

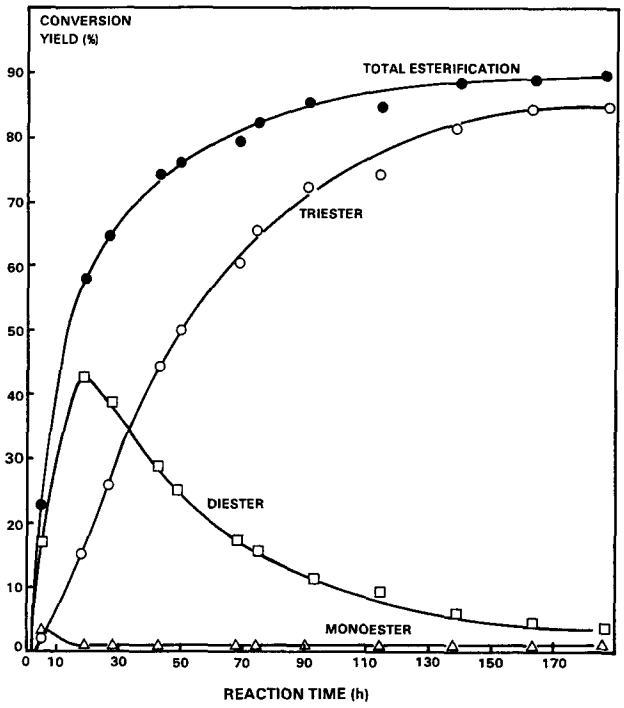

Fig. 5. Time course of esterification of TMP with caprylic acid using procedure 2 (all conditions of procedure 2; recirculation flow rate, 375 mL/h; enzyme, lipozyme batch 1).

Table 1
Maximum Esterification Rate During Appearance of TMP Mono-, Di- and Tricaprylate (Using Procedure 2 and Lipozyme Batch 2)

	Monoester	Diester	Triester
Esterification rate, μmol/min·mg	1.04×10^{-3}	1.14×10^{-3}	0.45×10^{-3}

rates were equivalent, but triester production rate was lower, probably because of a more difficult accessibility of TMP dicaprylate to *Mucor miehei* lipase. Although the main goal of this study was to produce triester, it is interesting to note that it was possible to selectively obtain di-or triesters according to the reaction time and reaction rates. Selective monoester production was not achieved because it was readily converted into diester.

Other experiments were carried out to determine the hydrolysis and synthesis rates of different esters: tricaprylin, butyl stearate, and trimethylolpropane tricaprylate (Table 2). The results show that the esterification reaction is slower than hydrolysis. The ratios of the hydrolysis/esterification rates have been found equal to 22.2 for TMP tricaprylate with lipo-

Table 2
Initial Hydrolysis and Synthesis Rate
of Different Esters Using Lipozyme or Esterase

	Initial reaction rate, μmol/min·mg			
	Lipozyme		Esterase	
Substrate	Hydrolysis	Synthesis	Hydrolysis	Synthesis
Tricaprylin	0.87	ND	13.07	ND
TMP tricaprylate	0.01	0.45×10^{-3}[2]	0.05	ND
Butyl stearate	ND	0.157[b]	1.69	0.23[b]

[a]With continuous removal of water.
[b]In butyl ether.

zyme and 7.3 for butyl stearate with esterase, showing that, whatever the reason, for example diffusional limitations, the activity of lipases was decreased in nonaqueous media. Compared to other substrates, trimethylolpropane tricaprylate was very difficult to hydrolyze enzymatically. Furthermore, the initial rate of butyl stearate synthesis by lipozyme was about 350 times higher than the rate of synthesis of TMP tricaprylate and 140 times higher than the rate of synthesis of TMP mono- or dicaprylate. The same solvent (butyl ether) was used in both syntheses. Thus, it seems that the steric hindrance owing to the neopentyl group of TMP had a detrimental effect on the enzyme activity. It can also be remarked that the ratio of esterase and lipozyme activities toward tricaprylin hydrolysis was found to be 15.0, whereas the same ratio in the case of butyl stearate synthesis was 1.5, indicating that the two enzymes are different, albeit both produced by *Mucor miehei* (probably different strains).

Effect of Recirculation Flow Rate

The effect of the recirculation flow rate on conversion yield is presented in Fig. 6. Regarding triester production, the optimum recycling flow rate was at least 100 mL/h, i.e., the totality of the reaction medium passed through the molecular sieve in 1.2 h. At lower flow rates, the water production rate was probably higher than the dehydration rate. On the other hand, high recirculation flow rates had a deleterious effect on enzymatic esterification. Similar results were obtained with initial esterification rates.

Effect of Temperature

The effect of temperature on initial reaction rate and on conversion yield is shown in Fig. 7. The esterification rate was maximum at 70°C, but some inactivation of the enzyme occurred at this temperature and the conversion yield was affected. The optimum temperature was 60°C.

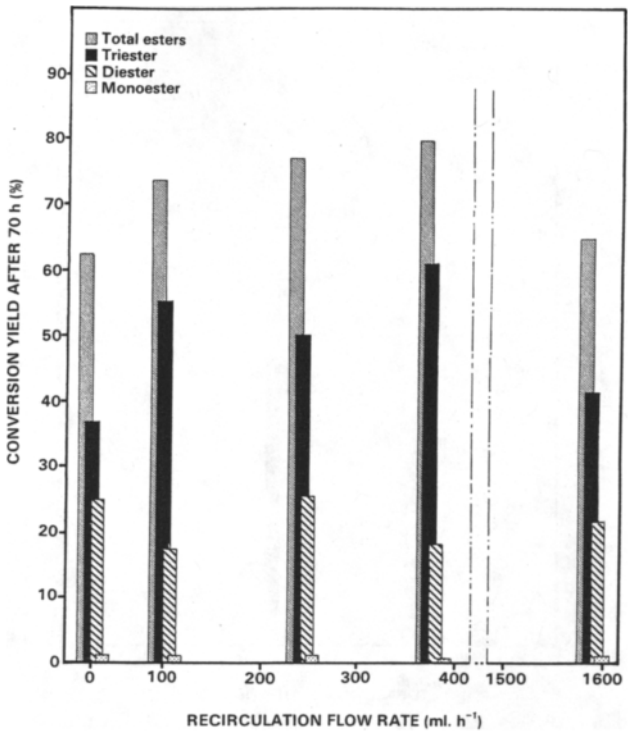

Fig. 6. Effect of recirculation flow rate on conversion yield (procedure 2; lipozyme batch 1).

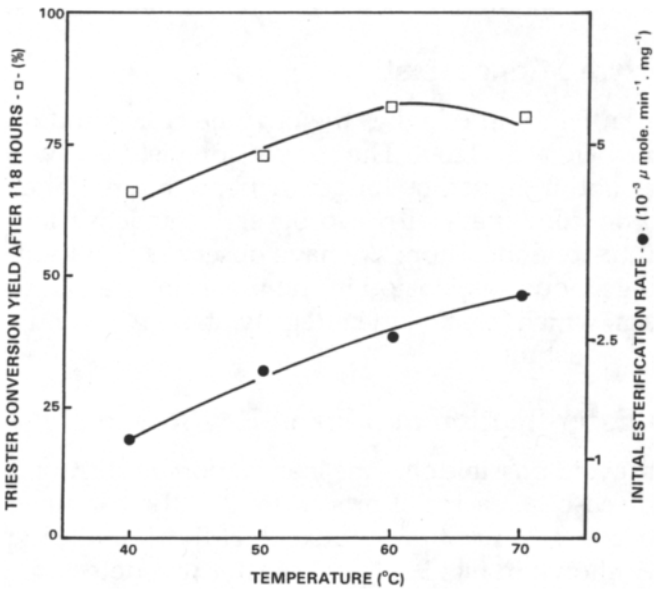

Fig. 7. Effect of temperature on conversion yield and initial esterification rate (procedure 2; lipozyme batch 1; recirculation flow rate, 240 mL/h).

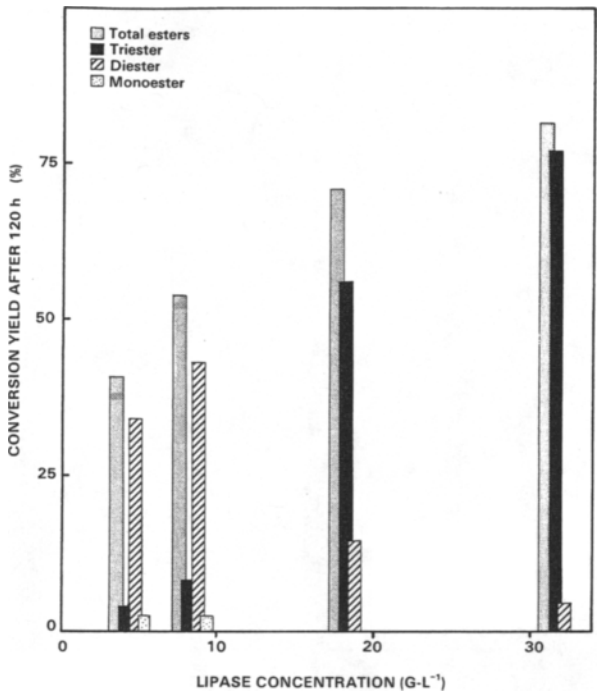

Fig. 8. Effect of enzyme concentration on conversion yield (procedure 2; lipozyme batch 2; recirculation flow rate, 180 mL/h).

Nevertheless, the increase of activity vs temperature was low, probably because of diffusional limitations with the immobilized lipase.

Effect of Enzyme Concentration

As shown in Fig. 8, the higher the enzyme concentration, the better the conversion yield after 120 h. The conversion yield at low enzyme concentration was not improved by longer incubation time. The slowness of the esterification combined with a progressive inactivation of enzyme could explain this phenomenon. We have observed that to maintain lipozyme in homogeneous suspension in the medium, we had to vigorously stir the medium, which affected the integrity of the resin and, thus, possibly inactivated lipozyme.

Continuous Esterification in a Fixed-Bed Reactor

In order to avoid possible enzyme inactivation by stirring, we switched from the fluidized-bed reactor of procedure 2 to the fixed-bed reactor described in procedure 3. The time course of TMP tri- and dicaprylate concentrations are shown in Fig. 9. No monoester was detected. Continuous feed at 0.018/h lasted 430 h. A progressive decrease of TMP tricaprylate

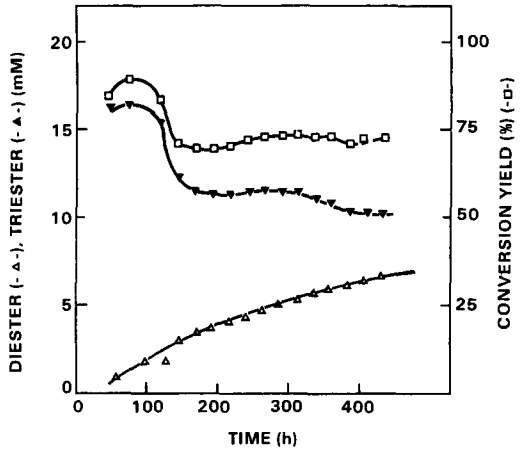

Fig. 9. Time course of continuous esterification in a fixed-bed reactor according to procedure 3 (enzyme, lipozyme batch 2).

concentration and a concomitant increase of TMP dicaprylate concentration was observed. This change may be owing to saturation of the molecular sieve by water.

Thus, the present study demonstrated the feasibility of an enzymatically-catalyzed synthesis of TMP tricaprylate with good conversion yield and few byproducts. Nevertheless, esterification rates were slow and the production of triester occurred late in the process. Comparison with the kinetics of hydrolysis and synthesis of other esters showed that TMP was not a good substrate for the lipases used. Since good synthesis rates were obtained for butyl stearate in butyl ether, and since other hydrophilic polyols, such as glycerol, are known to be good substrates for lipases, it appears that the low synthesis rates obtained for TMP tricaprylate are more related to the molecular geometry of TMP than to its hydrophilic character and the nature of the solvents that it entails.

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