Modeling of the kinetic for the acidolysis of different triacylglycerols and caprylic acid catalyzed by Lipozyme IM immobilized in packed bed reactor

Fernando Camacho\textsuperscript{b}, Alfonso Robles\textsuperscript{a,\,*}, Belén Camacho\textsuperscript{a}, Pedro A. González\textsuperscript{a}, Luis Esteban\textsuperscript{a}, Emilio Molina\textsuperscript{a}

\textsuperscript{a}Departamento de Ingeniería Química, Universidad de Almería, Spain
\textsuperscript{b}Departamento de Ingeniería Química, Universidad de Granada, Spain

Received 4 July 2006; received in revised form 2 March 2007; accepted 10 March 2007
Available online 14 March 2007

Abstract
This work proposes a lumped kinetic model for the acidolysis of a triacylglycerol (TAG) and an odd free fatty acid (FFA) in a non-aqueous medium, catalyzed by a 1,3 specific lipase immobilized on a solid support. This model is based on the mechanism of the acidolysis reaction by considering the following hypothesis: (1) only the fatty acids in positions 1 and 3 of TAG are exchanged and these two positions in the glycerol backbone are equivalent and (2) the only intermediate of appreciable lifespan in which the enzyme participates is the acyl-enzyme complex. The kinetic equation obtained for the rate of incorporation of an odd fatty acid to TAG has been applied to the results obtained in the acidolysis of three oils (commercial triolein, cod liver oil (CLO) and a commercial oil enriched in eicosapentaenoic acid (EPA), EPAX 4510TG) with caprylic acid (CA), catalyzed by the immobilized lipase Lipozyme IM contained in a packed bed reactor (PBR). The acidolysis has been carried out by recirculating the reaction mixture through the PBR until the reaction equilibrium was reached. In these conditions it has been proved that the PBR behaves as a perfect mixed dispersion reactor and the experimental results obtained at low TAG concentrations (< 100 mol/m\textsuperscript{3}) have been acceptably fitted to the kinetic expression obtained from the proposed model, with only two fitting parameters.
However, for TAG concentrations higher than 100 mol/m\textsuperscript{3}, an appreciable reduction of the reaction rate was observed. This result was due to the decrease of the effective diffusivity of reactants within the pores of the support where the lipase is immobilized, since the viscosity of the reaction mixture increases appreciably when the reactant concentration also does. When this phenomenon is included in the developed kinetic model, the experimental results obtained at high TAG concentrations could also be explained, even in absence of the organic solvent (\textit{n}-hexane). It is observed that the influence of diffusion into the pores increases with the degree of CA incorporation to TAG, which was due to the increase of TAG and native fatty acid concentrations in the particle pores, which determines a continuous decrease in the effective diffusivity of CA.

Keywords: Kinetic model; Acidolysis; Lipase-catalyzed reaction kinetics; Caprylic acid; Packed bed reactor; Immobilized lipase; Pore diffusion

1. Introduction
There is an increasing interest for clinical nutrition purposes in the production of structured triacylglycerols (ST) with medium-chain fatty acids (\textit{M}) located at positions 1 and 3 of the glycerol backbone and functional long-chain PUFAs (\textit{L}) at position 2 (MLM). ST have been used in absorption studies (Christensen et al., 1995; Carvajal et al., 2000) and for clinical nutrition (Lindgreen et al., 2001; Straarup and Hoy, 2001). The MLM structure of the ST facilitates their absorption because pancreatic lipase is 1,3 specific and hydrolyzes the ester bonds at positions 1 and 3 of triacylglycerols (TAG). Moreover, this lipase shows higher activity toward medium-chain than toward long-chain fatty acids, especially PUFAs (Mu and Porsgaard, 2005). The liberated medium-chain free fatty acids (FFAs) are directly absorbed into the portal vein and the 2-monoacylglycerols (2-MAG), with the essential long-chain
fatty acid, are well absorbed via the lymphatic route (Lien et al., 1997).

The simplest and most direct route for the synthesis of MLM type ST is the acidolysis between long-chain TAG and medium-chain FFAs catalyzed with a 1,3-specific lipase (Xu, 2003; Nagao et al., 2003; Shimada et al., 1999; Fomuso and Akoh, 2002; Xu et al., 1998; Torres and Hill, 2002; Camacho Páez et al., 2002). Lipases offer high catalytic efficiency, specificity and selectivity by incorporation of the required acyl group into a specific position of the native TAG. Moreover, the process can be carried out in an enzymatic packed bed reactor (PBR) where the lipase is immobilized, for example, within the column (González Moreno et al., 2004).

Most of the research into the synthesis of ST is directed toward studying the effect of variables such as the type of solvent, temperature, substrate mol ratio (Fomuso and Akoh, 2002), substrate concentrations, water content (Torres and Hill, 2002) and operational mode (Xu, 2003; Shimada et al., 1999; Xu et al., 1998). However, little has been reported on the kinetics of the lipase-catalyzed acidolysis between FFAs and heterogeneous TAG and on the formulation of rate equations that describe the performance of the lipase, optimize the control and allow a more rational design of reactors. Among the scarce works related with acidolysis kinetic, Reyes and Hill (1994) carried out a study of the mechanism of the acidolysis between FFA and a heterogeneous TAG, such as olive oil and milk fat, and they proposed a kinetic model that accounts for the effect of the concentration of all chemical species participating in the reaction. These authors propose a ping-pong mechanism where the products are released between the addition of two substrates. Also Yadav and Lathi (2005) propose a similar mechanism for the transesterification of methyl acetoacetate with n-butanol for obtaining keto esters, using Novozym 435. However, in the case of a simple esterification reaction, it seems much more appropriate to consider an ordered bi-bi mechanism, in which first the reactants bind with the lipase and then the products are released (García et al., 1999). Recently, we proposed a simple kinetic model based on first order rate equations with respect to each reactant to describe the acidolysis of triolein and caprylic acid (CA) (Camacho Páez et al., 2003). Also little information has been reported on the magnitude of rate constants in the esterification reaction. However, this information is essential for understanding how reaction variables affect the reaction rates and for a rational design and scaling up of the acidolysis reactors. In a previous paper (Camacho Páez et al., 2002), we calculated average reaction rates and kinetic constants of exchange of CA and native fatty acids of cod liver oil by assuming that the rate of incorporation of a fatty acid into a TAG per unit of amount of enzyme is proportional to the separation from the reaction equilibrium. Thus, a simple equation was proposed for predicting the fatty acid composition of ST at the exit of a PBR where the lipase was immobilized. For the acidolysis reaction in the continuous PBR the lipase amount/(flow rate × substrate concentration) ratio could be considered as the intensive variable of the process for use in scale up of the PBR (Camacho Páez et al., 2002).

This work describes a kinetic model for the acidolysis reaction focusing on the development of procedures for the synthesis of structured TAG.

2. Experimental

2.1. Chemicals and materials

Lipozyme® IM (donated by Novo Nordisk A/S, Bagsvaerd, Denmark) was supplied immobilized on a macro porous anion exchange resin containing 3–5% water, determined by Karl-Fischer titration (Compact titrator microKF 2026, Crimson, Alella, Spain). This enzyme showed 1,3-positional specificity. Analytical-grade CA and hexane were obtained from Sigma Aldrich (St. Louis, MO). Table 1 shows the fatty acid compositions of the oils used: commercial triolein, cod liver oil (CLO) and a commercial oil highly rich in eicosapentaenoic acid (EPAX4510TG, Pronova Biocare, Norway). The average molecular weights calculated for triolein, CLO and EPAX4510TG were 865.1, 910.0 and 924.3 Da, respectively.

2.2. Acidolysis reaction in the PBR

Fig. 1 shows a diagram of the reaction system. Table 2 shows the experimental conditions for the acidolysis between TAG and CA. The immobilized lipase of 2–3 g was packed into a glass column of 6.6 mm i.d. × 250 mm length covered with aluminium foil to prevent photo-induced oxidation. The experiment carried out with CLO and 6 g of lipase (Table 2) was carried out in a column of 1.5 cm i.d. × 15 cm length. The enzyme bed was held between two mobile perforated disks. The porosity in this confined bed was 0.46 and the bed density of the catalyst was 0.36 g/cm³ (Table 3; Camacho Páez et al., 2002). The substrate mixture was kept in a reservoir submerged in a thermostated water bath. The column was jacketed to maintain the reaction temperature constant at 30°C. The mixture consisted of oil, 10–20 g; CA, 10–20 g; and hexane, 0–300 mL. The molar ratio of CA/oil, $m_a$, was kept around 6 in all the experiments (Table 2). The initial oil concentration ranged from 33.6 to 487.3 mol/m³, the latter corresponding to the experiment conducted without solvent. The reaction mixture was pumped up through the column by a peristaltic pump (Fig. 1) at flow rates between 30 and 200 mL/h.

The acidolysis was carried out by recirculation of the reaction mixture through the PBR and the reaction was monitored by sampling at different times (between 1.0 and 175 h) in the substrate reservoir. The content of the reservoir bottle was continuously agitated at 200 rpm during the reaction by a magnetic bar. The samples were stored at −20 °C until analysis. All analyses were carried out in triplicate. The standard deviation was always below 6%.

2.3. Identification of fatty acids and estimation of the molar fraction of fatty acids in the triacylglycerols

Hexane was removed from the samples of product mixtures in a vacuum evaporator. Acylglycerols (monoacylglycerols,
Table 1
Fatty acid composition (%mol) of the commercial triolein, cod liver oil (CLO) and EPAX4510TG oil \((F_{X0})\) and the structured TAG produced once equilibrium of the acidolysis reaction was reached \((F_{Xe})\)

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Triolein ((m_0 = 5.95^a))</th>
<th>CLO ((m_0 = 6.13^a))</th>
<th>EPAX 4510 ((m_0 = 6.41^a))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(8:0)</td>
<td>52.0</td>
<td>56.8</td>
<td>59.5</td>
</tr>
<tr>
<td>(12:0)</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(14:0)</td>
<td>3.2</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>(16:0)</td>
<td>6.4</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>(16:1n7)</td>
<td>4.6</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>(16:3n4)</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(18:0)</td>
<td>1.9</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>(18:1n9)</td>
<td>68.1</td>
<td>35.8</td>
<td></td>
</tr>
<tr>
<td>(18:1n7)</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(18:2n6)</td>
<td>10.8</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>(18:2n4)</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(18:4n3)</td>
<td></td>
<td>2.3</td>
<td>4.0</td>
</tr>
<tr>
<td>(20:1n9)</td>
<td>12.0</td>
<td>3.5</td>
<td>3.4</td>
</tr>
<tr>
<td>(20:4n6)</td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>(20:5n3)</td>
<td>9.2</td>
<td>5.1</td>
<td>40.4</td>
</tr>
<tr>
<td>(22:1n7)</td>
<td>8.7</td>
<td>2.9</td>
<td>4.3</td>
</tr>
<tr>
<td>(22:5n3)</td>
<td>1.9</td>
<td>0.8</td>
<td>2.7</td>
</tr>
<tr>
<td>(22:6n3)</td>
<td>12.8</td>
<td>10.0</td>
<td>7.7</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Temperature 30°C.

\(a\) CA/TAG molar ratio.

Lepage and Roy (1984). These methyl esters were analyzed by capillary GC following the procedure described in Camacho Páez et al. (2002).

3. The acidolysis model

This model is based on the following hypothesis: (1) due to the 1,3 positional specificity of lipases, only the fatty acids in positions 1 and 3 of the TAG participate in the reaction; (2) the exchange in position 1 does not depend on the nature of the fatty acid in position 3 and vice versa, i.e., these positions are equivalent; (3) the approximation to the stationary state for the diacylglycerol (DAG) concentration is applied; the concentration of DAG is low because we operated in absence of water and with a high CA/TAG molar ratio; (4) the only intermediates of appreciable lifespan in which the enzyme participates are the acyl-enzyme complexes and these are in equilibrium with the FFAs, i.e.:

\[ E + L \rightleftharpoons EL + W, \quad K_L' = \frac{[EL][W]}{[E][L]}, \quad K_L = \frac{[EL]}{[E][L]} \quad (1) \]

\[ E + M \rightleftharpoons EM + W, \quad K_M' = \frac{[EM][W]}{[E][M]}, \quad K_M = \frac{[EM]}{[E][M]} \quad (2) \]

where \(E\) is the free enzyme, \(L\) the FFAs liberated from the original TAG, \(M\) the odd fatty acid, \(W\) the water and \(EL\) and \(EM\) are the acyl-enzyme complex. Because of the high FFAs/TAG molar ratios \((m_0)\) and the low water content, it can be considered that most of the enzyme is in the acyl-enzyme form, \(EL\) or \(EM\). According to the definition of \(K_L\) and \(K_M\), there is
Table 2
Experimental conditions used in the acidolysis between TAG (triolein, CLO and EPAX4510TG) and CA with the lipase immobilized in a packed bed reactor (PBR)

<table>
<thead>
<tr>
<th>Oil</th>
<th>( m_{E}^a ) (g)</th>
<th>( V^b ) (m³)</th>
<th>[TAG]c (mol/m³)</th>
<th>( m_0^d )</th>
<th>( q^e ) (mL/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triolein</td>
<td>2.0</td>
<td>0.000322</td>
<td>35.9</td>
<td>5.88</td>
<td>132</td>
</tr>
<tr>
<td>Triolein</td>
<td>2.1</td>
<td>0.0001722</td>
<td>67.1</td>
<td>6.00</td>
<td>132</td>
</tr>
<tr>
<td>CLO</td>
<td>6.0</td>
<td>0.000322</td>
<td>34.1</td>
<td>6.13</td>
<td>63</td>
</tr>
<tr>
<td>EPAX4510TG</td>
<td>2.0</td>
<td>0.000322</td>
<td>33.6</td>
<td>6.28</td>
<td>74</td>
</tr>
<tr>
<td>EPAX4510TG</td>
<td>2.2</td>
<td>0.0003222</td>
<td>33.6</td>
<td>6.41</td>
<td>196</td>
</tr>
<tr>
<td>EPAX4510TG</td>
<td>2.5</td>
<td>0.0002444</td>
<td>88.5</td>
<td>6.41</td>
<td>200</td>
</tr>
<tr>
<td>EPAX4510TG</td>
<td>2.5</td>
<td>0.0001444</td>
<td>149.8</td>
<td>6.41</td>
<td>200</td>
</tr>
<tr>
<td>EPAX4510TG</td>
<td>3.0</td>
<td>0.0000444</td>
<td>487.3</td>
<td>6.41</td>
<td>30</td>
</tr>
</tbody>
</table>

\( ^a \) Lipase amount immobilized in the PBR.
\( ^b \) Reaction mixture volume.
\( ^c \) Initial concentration of TAG.
\( ^d \) CA/TAG molar ratio.
\( ^e \) Reaction mixture flow rate through the PBR.

Table 3
Characteristic values of the immobilized enzyme particles (determined by mercury porosimetry), bed porosity, molar volumes of saturated liquids, molecular diffusivity of CA and diffusivity ratios of the species implied in the acidolysis reaction of EPAX 4510 with respect to CA

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average particle radii, ( R_P )</td>
<td>0.0002 m</td>
<td>Camacho Páez et al. (2002)</td>
</tr>
<tr>
<td>Bulk particle density, ( \rho_P )</td>
<td>6.677 \times 10^5 g/m³</td>
<td></td>
</tr>
<tr>
<td>Particle porosity, ( \varepsilon_P )</td>
<td>0.453</td>
<td></td>
</tr>
<tr>
<td>Packed bed density, ( \rho_L )</td>
<td>0.36 g/cm³</td>
<td></td>
</tr>
<tr>
<td>Packed bed porosity, ( \varepsilon_L )</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Molar volume of the saturated liquid (( V_B ), cm³/mol)</td>
<td>201.6</td>
<td>Le Bas (Poling et al., 2001b)</td>
</tr>
<tr>
<td>Caprylic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exchanged native fatty acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original oil (EPAX4510TG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mono-substituted oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di-substituted oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular diffusivity of caprylic acid in hexane ( D_{M,H} ) (m²/h)</td>
<td>1.08 \times 10^{-5}</td>
<td>Eq. of Wilke and Chang (Poling et al., 2001a)</td>
</tr>
<tr>
<td>Diffusivity ratios</td>
<td></td>
<td>Eq. of Wilke and Chang (Poling et al., 2001a)</td>
</tr>
<tr>
<td>Exchanged native fatty acid/caprylic acid, ( \alpha )</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Original oil/CA, ( \beta )</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Mono-substituted oil/CA, ( \beta_1 )</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Di-substituted oil/CA, ( \beta_2 )</td>
<td>0.41</td>
<td></td>
</tr>
</tbody>
</table>

an inverse relationship between \( K_L \) (or \( K_M \)) and the free water concentration; therefore, when the water content increases the values of these constants decrease and the previous equilibrium moves towards the free enzyme formation. By making an enzyme balance:

\[
[E] + [EL] + [EM] = e_T, \quad [E] = \frac{e_T}{1 + K_L[L] + K_M[M]},
\]

\[
[EL] = \frac{K_L e_T [L]}{1 + K_A[L] + K_M[M]},
\]

\[
[EM] = \frac{K_M e_T [M]}{1 + K_L[L] + K_M[M]},
\]

where \( e_T \) represents the total active enzyme concentration per gram of catalyst particle.

For the first exchange to take place, the initial TAG must be hydrolyzed, forming the DAG, and this may happen in positions 1 or 3, forming, respectively, the diacylglycerols \( GL \) and \( LG \):

\[
E + LGL \xrightleftharpoons[k_{-1L}]{k_{1L}} EL + GL,
\]

\[
E + LG \xrightleftharpoons[k_{-3L}]{k_{3L}} EL + LG.
\]

These two DAG are different because the carbon 2 of glycerol may be quiral or pro-quiral, depending on whether the initial TAG is heterogeneous or homogeneous, respectively. Then, the esterification of DAG with the odd fatty acid (\( M \)) will take place forming TAG with only one odd fatty acid
incorporated:

\[ EM + GL \xrightleftharpoons[k_{M}]{k_{1M}} E + MGL, \]

\[ EM + LG \xrightleftharpoons[k_{M}]{k_{3M}} E + LGM. \] (5)

In these equations only the fatty acids that occupy positions 1 and 3 (that participate in the exchange of acyl groups) are explicitly indicated. The kinetic constants are designated with a subscript that indicates the exchanged acyl group (L or M), the implied position (1 or 3) and whether the acyl group leaves (negative sign) or enters the acyglycerol (positive sign). If we accept that the positions 1 and 3 are equivalent (second hypothesis of the model):

\[ k_{L} = k_{1L} = k_{3L}, \quad k_{-L} = k_{-1L} = k_{-3L}, \]

\[ k_{M} = k_{1M} = k_{3M}, \quad k_{-M} = k_{-1M} = k_{-3M}. \] (6)

Analysis by TLC has revealed the almost total absence of DAG in the reaction products. This is logical since in absence of free water the concentration of DAG remains too low. It is therefore acceptable to apply the approximation to the stationary state to the DAG concentration (third hypothesis of the model), \( \text{d}[GL]/\text{d}t = \text{d}[LG]/\text{d}t = 0 \), which leads to:

\[ [GL] = \frac{k_{-L}[LGL] + k_{M}[MGL]}{k_{L}[EL] + k_{M}[EM]} [E], \]

\[ [LG] = \frac{k_{-L}[LGL] + k_{M}[LGM]}{k_{L}[EL] + k_{M}[EM]} [E]. \] (7)

Furthermore, if positions 1 and 3 are equivalent, the concentrations of LGM and MGL will remain the same during the exchange and the concentrations of TAG may be expressed in the form:

\[ [LGL] = [GL_{2}], \quad [MGL] = [LGM] = \frac{1}{2}[GLM], \]

\[ [MGM] = [GM_{2}]. \] (8)

Reactions (4) and (5) occur in series and at the same rate, due to the stationary state hypothesis for DAG. Therefore, the rate of the first incorporation (reaction (5)) will be

\[ r_{1} = k_{M}[EM][GL] - [LG] - k_{-M}[E][MGL] + [LGM]. \] (9)

By substituting (3), (7) and (8) into (9):

\[ r_{1} = \frac{k_{-L}eT (2[GL_{2}][M] - [LGL]([MGL] + [LGM]))}{(1 + K_{L}[L] + k_{M}[M]) ([k_{L}K_{L}/k_{M}K_{M}]L + [M])}. \] (10)

In the numerator of this equation the driving force of the reaction (11) corresponding to the first exchange appears, including positions 1 and 3:

\[ GL_{2} + M \leftrightarrow GLM + L, \] (11)

the equilibrium constant being:

\[ K_{e} = \frac{k_{-L}k_{M}K_{M}}{k_{L}k_{-M}K_{L}}. \] (12)

Similarly, for the second exchange:

\[ E + MGL \xrightleftharpoons[k_{L}]{k_{-L}} GL + MG, \]

\[ E + LGM \xrightleftharpoons[k_{L}]{k_{-L}} GL + GM, \] (13)

\[ EM + MG \xrightleftharpoons[k_{M}]{k_{-M}} E + MGM, \]

\[ EM + GM \xrightleftharpoons[k_{M}]{k_{-M}} E + MGL. \] (14)

Also, applying the stationary state approximation to DAG, MG and GM, the following equivalent equation is obtained:

\[ r_{2} = \frac{k_{-L}eT ([GLM][M] - (2/K_{e})[GM_{2}][L])}{(1 + K_{L}[L] + K_{M}[M]) ([k_{L}K_{L}/k_{M}K_{M}]L + [M])}. \] (15)

for the second exchange:

\[ GLM + M \leftrightarrow GM_{2} + L, \]

\[ LGL + M \leftrightarrow MGL + L, \]

\[ MGL + M \leftrightarrow MGM + L, \]

\[ LGM + M \leftrightarrow MGM + L. \] (17)

Since positions 1 and 3 are considered equivalent the equilibrium constant will be the same, i.e.:

\[ K_{e} = \frac{[MGL]_{e}[L]_{e}}{[LGL]_{e}[M]_{e}} = \frac{[LGM]_{e}[L]_{e}}{[GLM]_{e}[M]_{e}}. \] (18)

From (18) and (8) it can be deduced:

\[ [MGL]_{e} = [GM_{2}]_{e} = K_{e} [LGL]_{e} [M]_{e} [L]_{e}, \]

\[ [GLM]_{e} [L]_{e} = 2K_{e}, \] (19)
[MGL]_e = [LG]_e = [MGL]_e = [MGL]_e + [L]_e + 2[M]_e

\frac{[GM_2]_e[LG]}{[GLM]_e} = \frac{K_e}{2},

(20)

which agrees with the results obtained in the kinetic treatment (driving forces of Eqs. (10) and (15) equal to zero).

A global treatment of the equilibrium can also be made. If the concentrations of ester groups at the extreme positions of the glycerol backbone, in which the odd (M) and the native (L) fatty acids participate, are represented, respectively, by [G − M]_e and [G − L]_e, the following equations will be fulfilled at equilibrium (taking into account (18)):

\[ [G − M]_e = [G]_e + [L]_e + 2[M]_e \]

\[ [G − L]_e = 2[L]_e + 2[M]_e \]

and therefore:

\[ [G − M]_e[LG] = \frac{K_e + \frac{(m_0)}{[L]_e}}{K_e([L]_e/[M]_e) + 1} = K_e. \]

(23)

This expression shows that \( K_e \) is the exchange equilibrium constant. By expressing this equation as a function of the molar fraction of M in the TAG at the equilibrium, \( F_{Me} \), and of the FFA/TAG molar ratio, \( m_0 \)

\[ [G − M]_e = 3[TG]_0 F_{Me}, \]

\[ [G − L]_e = 2[TG]_0 − 3[TG]_0 F_{Me}, \]

\[ [M]_e = m_0[TG]_0 − 3[TG]_0 F_{Me}, \]

\[ [L]_e = 3[TG]_0 F_{Me}, \]

\[ K_e = \frac{(3F_{Me})^2}{(2 − 3F_{Me}) (m_0 − 3F_{Me})}. \]

(25)

This equation allows the calculation of \( K_e \) from the molar fraction of CA experimentally obtained at equilibrium. Eq. (24) is also valid for no equilibrium conditions.

The form of the kinetic equations (10) and (15), with two factors in the denominator that include the FFA concentrations, allows us to explain that the incorporation in certain conditions and for a given time reaches a maximum when the molar ratio \( m_0 \) is varied. This effect has been experimentally observed elsewhere (Camacho Páez et al., 2003; Kuo and Parking, 1993) and it is generally attributed to a reduction of the enzymatic activity due to the acidification of the water film bound to the enzyme, when a great excess of FFAs is used.

The kinetic equations (10) and (15) can be simplified if the acyl-enzyme concentrations are large with respect to the free enzyme concentration. This is an acceptable hypothesis if the affinity of the enzyme for the fatty acids is large, the water content is small and the molar ratio \( m_0 \) is high, as is usually the case. By assuming this simplification, the 1 can be eliminated from the denominator in Eqs. (10) and (15), since this 1 represents the free enzyme concentration, [E] (see Eqs. (1)–(3)),

\[ r_1 = \frac{2(k_{−L} eT / K_m)([GL2]_e [M]_e (1/2 K_e))([M]_e(L)]_e + [M])((k_{L} K_L / K_m K_M [M])[L]_e + [M])}{((k_{L} K_L / K_M M)[L]_e + [M])}, \]

(26)

\[ r_2 = \frac{(k_{−L} eT / K_m)([GM2]_e [L]_e (2/Ke))([GM2]_e(L)]_e + [M])((k_{L} K_L / K_m K_M [M])[L]_e + [M])}{((k_{L} K_L / K_M M)[L]_e + [M])}. \]

(27)

In a perfect mixed dispersion reactor, if the influence of the diffusion within the pores is negligible, the rates of the first (disappearance of the original TAG) and of the second incorporation (appearance of the structured TAG, MLM) will be given by

\[ r_1 = \frac{V}{m_{F}} \left( -\frac{d[GL2]}{dt} \right), \quad r_2 = \frac{V}{m_{F}} \left( \frac{d[GM2]}{dt} \right) \]

(28)

both expressed as moles of the fatty acid M incorporated into the TAG per unit of time and unit of enzyme mass. As the formation of DAG is negligible, the molar fraction of the odd fatty acid incorporated into TAG (\( F_M \)), which is determined experimentally, is related with the GLM and GM2 concentrations by the expression:

\[ F_M = \frac{[GLM]_e + 2[GM2]_e}{3[TG]_0}. \]

(29)

The concentrations of the different types of TAG are related as follows:

\[ [GLM] = [TG]_0 − [GL2] − [GM2]. \]

(30)

Therefore, considering the relationships between the TAG and FFA concentrations (Eqs. (21), (22) and (24)) and those between the \( F_M \) and TAG concentrations (Eqs. (29) and (30)), rates \( r_1 \) and \( r_2 \), corresponding to the first and second incorporation, can be expressed as a function of \( F_M \), i.e., as the total incorporation rate of CA into the TAG:

\[ \frac{\partial F_M}{\partial \theta} = \frac{(k_{−L} eT / 3K_m)((2−3F_{Me}) (m_0−3F_{Me})−(1/Ke)(3F_{Me})^2)}{((k_{L} K_L / K_m K_M [M])[3F_{Me}] + (m_0−3F_{Me}))((k_{L} K_L / K_m K_M [M])[3F_{Me}])}. \]

\[ \theta = 0, \quad F_M = 0. \]

(31)

Eq. (31) has also been generalized by introducing a new variable which characterizes the treatment intensity:

\[ \theta = \frac{m_{F}}{V[TG]_0}. \]

(32)

In Eq. (31) it can be observed that the resulting driving force is consistent with the previously deduced Eq. (25). The integration of Eqs. (31), by giving values to the three kinetic parameters \( (k_{−L} eT / K_m, K_L / K_M \) and \( k_{L} / K_m \)), and its comparison with the experimental results, allows us to verify its applicability and deduce the best values of these parameters. For this integration the equilibrium constant \( (K_e) \) must be previously calculated from the value of \( F_M \) at equilibrium (Eq. (25)).
Eq. (25) indicates that the incorporation at equilibrium \((F_{Me})\) increases with the molar ratio \((m_0)\). However, Eq. (31) indicates that the reaction rate decreases with this variable. These two opposite effects, kinetic and equilibrium, can determine that the degree of incorporation in certain conditions, in which the equilibrium is not reached, passes through a maximum when increasing the molar ratio, \(m_0\). As commented above, this result was observed in a previous work \((\text{Camacho Páez et al., 2003})\).

4. Results and discussion

4.1. Equilibrium and kinetic

Table 1 shows the equilibrium compositions of the structured TAG (ST) obtained from the oils used in this work. The incorporation of CA was important for the three oils used (an average value of 56%), bearing in mind that the acidolysis reaction is reversible and that the highest incorporation of CA that can be attained is 66.7% (only positions 1 and 3 participate in the exchange). By using the molar fractions of CA in the ST at the equilibrium \((F_{Me})\), the equilibrium constants \((K_e)\) were calculated by Eq. (25). The \(K_e\) value (Table 4) is higher for most of the unsaturated oils. This may mean that the unsaturated fatty acids are more displaced from positions 1 and 3 than the saturated ones.

Fig. 2 shows that the incorporation rate of CA at flow rates of 74 and 196 mL/h were almost the same, indicating that, under these conditions, this operational variable does not affect the external mass transfer around the immobilized lipase particles. It follows, therefore, that the PBR behaves as a differential reactor, and that this system is equivalent to a perfectly mixed batch reactor (perfect mixing hypothesis).

Figs. 3, 4 and 5 show the variation of the molar fraction of CA in the TAG \((F_M)\) according to the treatment intensity \(\theta\) (Eq. (32)). These experimental results were fitted to Eq. (31), by determining the kinetic parameters \((k_{-LeT}/K_M, K_L/K_M\) and \(k_L/k_M\) that minimized the sum of the squared differences (SSD) between the experimental results and those predicted by the model:

\[
\text{SSD} = \sum_i (F_M \text{calculated} - F_M \text{experimental})^2.
\]

This fit has been carried out using a non-linear regression program in MatLab 7.0. The results obtained in a first fitting showed that the parameter \(k_L/k_M\) is practically equal to one for the three systems. This result indicates that the reaction between the DAG and the acyl-enzyme is very fast and that it is determined to a greater extent by the probability of contact between the DAG and the acyl-enzyme than by the fatty acid type (reactions and Eqs. (4)–(6), (13) and (14)). This fact may explain why the DAG concentrations are negligible, as it has been observed experimentally. By introducing this result in Eq. (31), the number of parameters in the model is reduced to only two (since the equilibrium constant is determined independently), and the kinetic equation which gives the rate of incorporation of CA into the TAG is

\[
\frac{dF_M}{d\theta} = \frac{\left(k_{-LeT}/3K_M\right)((2-3F_M)(m_0-3F_M)-(1/K_e)(3F_M)^2)}{\left(\left(K_L/K_M\right)(3F_M)+(m_0-3F_M)\right)^2},
\]

\(\theta = 0, \quad F_M = 0.\) (34)

![Fig. 2. Acidolysis of EPAX4510TG and CA: influence of time and reaction mixture flow rate through the PBR on the mass fraction of CA \((F_M)\) in the structured TAG.](image)

<table>
<thead>
<tr>
<th>Oil</th>
<th>([\text{TG}]_0) (mol/m³)</th>
<th>(K_e)</th>
<th>(k_{-LeT}/K_M) (mol/(g h))</th>
<th>(K_L/K_M)</th>
<th>DCM^a</th>
<th>(R_0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triolein</td>
<td>35.9–67.1</td>
<td>1.26</td>
<td>0.00782</td>
<td>2.17</td>
<td>0.012</td>
<td>0.003</td>
</tr>
<tr>
<td>AHB</td>
<td>34.1</td>
<td>2.22</td>
<td>0.000742</td>
<td>1.36</td>
<td>0.044</td>
<td>0.005</td>
</tr>
<tr>
<td>EPAX4510TG</td>
<td>33.6–88.5</td>
<td>3.20</td>
<td>0.00516</td>
<td>1.96</td>
<td>0.011</td>
<td>0.001–0.003</td>
</tr>
<tr>
<td>EPAX4510TG</td>
<td>149.8</td>
<td>3.20</td>
<td>0.00465</td>
<td>15.0</td>
<td>0.016</td>
<td>0.001</td>
</tr>
<tr>
<td>EPAX4510TG</td>
<td>487.3</td>
<td>3.20</td>
<td>0.000732</td>
<td>10.2</td>
<td>0.015</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^a\text{Calculated by } DCM = \sqrt{\sum_{i=1}^{n} (\text{Experimental} - \text{Predicted})^2/\text{Predicted}}.\)
This equation should fit the experimental results for the acidolysis of each TAG tested with the same parameters, i.e., these parameters should not depend on the TAG concentration but only on the TAG type. Indeed, this was obtained with the two experiments carried out with different initial concentrations of triolein (Fig. 3) and with CLO (Fig. 4). Table 4 shows the kinetic parameters obtained, which were used to draw the continuous lines in Figs. 3 and 4. Also, the results obtained with EPAX4510TG at concentrations of 33.6 and 88.5 mol/m³ were acceptably fitted with the same parameter value for these two lowest concentrations (Table 4 and Fig. 5). However, the results obtained at the two highest concentrations showed appreciably lower reaction rates than were predicted by the model (Fig. 5 and values of the parameter $k_{-L} e_T/K_M$ in Table 4). Nevertheless, Figs. 3–5 show that the fit of the experimental results to Eq. (34) is good in all cases. The goodness of the fit is also shown by the average deviation values (ADV) of the experimental data shown in Table 4.

The kinetic parameters shown in Table 4 allow us to determine the initial reaction rate in each experiment by Eq. (31), by making $F_M = 0$. The average residence time of the reaction mixture in the PBR is

$$t_R = \frac{\varepsilon_L V_L}{q} = \frac{\varepsilon_L m_E}{p_L q}, \quad (35)$$

where $\varepsilon_L$, $V_L$, and $p_L$ are the porosity, volume and density of the packed bed, respectively (Table 3). The product (residence time $\times$ initial reaction rate) allows estimation of the maximum incorporation of CA to TAG per passage through the PBR ($R_0$):

$$R_0 = \frac{2}{3m_0} K_{L} \frac{K_M}{K_M} \left( \frac{m_E}{V[TG]_0} \right) \left( \frac{m_E}{p_L q} \right). \quad (36)$$

Table 4 shows the range of $R_0$ values (0.001 – 0.005). This result again confirms the hypothesis that the PBR behaves as a differential reactor because the obtained values are below or around 0.5%. The experimental system used is, therefore, equivalent to a well mixed dispersion reactor.

Comparing the kinetic and equilibrium parameters obtained at concentrations below 100 mol/m³ (Table 4), it is observed that the CA exchange rate with CLO (a natural TAG) is one order of magnitude lower than with TO and EPAX4510TG (artificial TAG, synthesized by interesterification reactions). The values of the $K_L/K_M$ ratio in these experiments are higher than one, which indicates a greater affinity of the lipase for the native fatty acids than for CA.

On the other hand, for the highest TAG concentrations used (149.8 and 487.3 mol/m³, in the experiment carried out in absence of hexane), the kinetic parameters depend on this variable, which reduces their usefulness for the reactor design. In these experiments the parameter $K_L/K_M$ is one order of magnitude higher (Table 4), which implies a certain inhibition by the product in the reaction, because it is elevated to the square in the denominator of Eq. (34) and is multiplied by the FFA concentration (Eqs. (26) and (27)).

The large reduction of the reaction rate when the concentration increases (Fig. 5) may be due to the influence of the
diffusion within the pores of the immobilized lipase particles, since it occurs when the viscosity of the reaction mixture increases appreciably. Indeed, at 30°C (the operational temperature) the viscosity of a diluted solution of reactants in n-hexane is approximately 0.3 mPa s, whereas in the absence of solvent the viscosity of an unsaturated oil-free fatty acid mixture is about 30 mPa s or higher. This great increase in viscosity causes a large reduction of the molecular and effective diffusivity of the different species through the pores of the immobilized lipase particles.

4.2. Influence of pore diffusion

The influence of pore diffusion on the kinetic of the interesterification reactions of TAG catalyzed by immobilized lipases on porous supports, in the absence of solvent, has already been shown by Ison et al. (1994). This influence can be included in the previously developed kinetic model by assuming that: (i) the catalyst is formed by spherical porous particles of radii \( R_P \) and apparent density \( \rho_P \) (Table 3), with the lipase adsorbed on the internal and external surface of the catalyst, together with a certain amount of water; (ii) these particles are disperse within a medium formed by reactants and products dissolved in an organic solvent; (iii) the independent reactions (1), (2), (4), (5), (13) and (14) occur in the enzymatic film that covers the catalytic surface; and (iv) there is no appreciable concentration gradient between the organic phase bulk and the external catalyst surface because the organic phase mixing is sufficiently intense, i.e., it is considered that the influence of the external mass transfer is negligible, as the results represented in Fig. 2 show. However, in the pores of the catalyst particles the reactants must be transferred into the interior by diffusion and the products must be transferred simultaneously in the opposite direction. In these conditions the continuity equation for each reactant (\( GL_2 \) and \( M \)) and product (\( GLM, GM_2 \) and \( L \)) in the particles must be written using the effective diffusivity concept \( (D_e) \). These equations are

\[
\frac{D_eM}{r^2} \frac{d}{dr} \left( r^2 \frac{d[M]}{dr} \right) - \rho_P (r_1 + r_2) = 0, \\
\frac{D_eL}{r^2} \frac{d}{dr} \left( r^2 \frac{d[L]}{dr} \right) + \rho_P (r_1 + r_2) = 0, \\
\frac{D_eGL_2}{r^2} \frac{d}{dr} \left( r^2 \frac{d[GL_2]}{dr} \right) - \rho_P r_1 = 0, \\
\frac{D_eGLM}{r^2} \frac{d}{dr} \left( r^2 \frac{d[GLM]}{dr} \right) + \rho_P (r_1 - r_2) = 0, \\
\frac{D_eGM_2}{r^2} \frac{d}{dr} \left( r^2 \frac{d[GM_2]}{dr} \right) + \rho_P r_2 = 0
\]

along with the boundary conditions:

\[ r = R_P, \; [M] = [M]_S, \; [L] = [L]_S, \]

\[ [GL_2] = [GL_2]_S, \; [GLM] = [GLM]_S, \; [GM_2] = [GM_2]_S \]

and

\[ r = 0, \; \frac{d[M]}{dr} = 0, \; \frac{d[L]}{dr} = 0, \]

\[ \frac{d[GL_2]}{dr} = 0, \; \frac{d[GLM]}{dr} = 0, \; \frac{d[GM_2]}{dr} = 0. \]  

Eqs. (37)–(41) represent mass balances where a pseudostationary state has been supposed. This assumption can be considered when the diffusion and reaction processes within the catalyst particles are much faster than the change of composition in the external liquid phase, which is continuously mixing with the liquid phase contained in the recirculation tank. This hypothesis will be demonstrated later by estimating the time constants of both processes.

Combining Eqs. (37)–(41) this system can be reduced to only two differential equations (which provide the variation of reactant concentrations, \([M] \) and \([GL_2] \), (37) and (39), respectively) and three algebraic equations, which allow us to express the concentrations of products \( L, GLM \) and \( GM_2 \) at any particle position as a function of \([M] \) and \([GL_2] \):

\[ [L] = [L]_S + \frac{D_eM}{D_eL} ([M]_S - [M]), \]  

\[ [GM_2] = [GM_2]_S + \frac{D_eM}{D_eGM_2} ([M]_S - [M]) - \frac{D_eGL_2}{D_eGM_2} ([GL_2]_S - [GL_2]), \]  

\[ [GLM] = [GLM]_S - \frac{D_eM}{D_eGLM} ([M]_S - [M]) + 2 \frac{D_eGL_2}{D_eGLM} ([GL_2]_S - [GL_2]). \]

Now by introducing kinetic equations (26) and (27), the average particle radii \( (R_P) \), the initial concentration of TAG \(([TG]_0)\) and the ratios between the effective diffusivities of the different species \( (L, GL_2, GLM \) and \( GM_2) \) and the effective diffusivity of CA, \( D_eM \), given by Eq. (47):

\[ \alpha = \frac{D_eL}{D_eM}, \; \beta = \frac{D_eGL_2}{D_eM}, \]  

\[ \beta_1 = \frac{D_eGLM}{D_eM}, \; \beta_2 = \frac{D_eGM_2}{D_eM} \]  

the differential equations (37) and (39) are converted into:

\[ \frac{R_P^2}{r^2[TG]_0} \frac{d}{dr} \left( r^2 \frac{d[M]}{dr} \right) - \rho_P R_P^2 \beta_1 \frac{k_{eT}}{D_eM[TG]_0} \left( 2[GL_2] + [GLM] \right) \frac{([M] - (1/K_0)K_1([GLM] + 2[GM_2])[L])}{([M] + K_1[L])^2} = 0, \]  

(48)
Replacing the expressions of the observable reaction rates in the particles, (52) and (53), in (54) and (55) we obtain:

\[
\frac{d[GL_2]_s}{dr} = -\frac{3βk_e_r T R_P}{\phi^2} \left( \frac{m_E}{V[TG]_0} \right) \left( \frac{d[GL_2]}{dr} \right)_{r=R_P}, \tag{56}
\]

\[
\frac{d[M]_s}{dr} = -\frac{3k_e_r T R_P}{\phi^2} \left( \frac{m_E}{V[TG]_0} \right) \left( \frac{d[M]}{dr} \right)_{r=R_P}. \tag{57}
\]

The integration of this equation system by finite increments, starting from the initial condition

\[ t = 0, \quad [GL_2]_s = [TG]_0; \quad [M]_s = m_0[TG]_0; \]

\[ [L]_s = 0; \quad [GLM]_s = 0; \quad [GM_2]_s = 0 \] (58)

allows us to calculate the variation of [GL2] and [M] with time. Choosing an increment of time, \( \Delta t \), and solving Eqs. (48) and (49) in the particle to obtain \( (d[GL_2]/dr)_{r=R_P} \) and \( (d[M]/dr)_{r=R_P} \), the concentration changes on the surface of the particles can be calculated by means of Eqs. (56) and (57) expressed as finite increments, i.e.,:

\[
\Delta[GL_2]_s = -\frac{3βk_e_r T R_P}{\phi^2} \left( \frac{m_E}{V[TG]_0} \right) \left( \frac{d[GL_2]}{dr} \right)_{r=R_P} \Delta t, \tag{59}
\]

\[
\Delta[M]_s = -\frac{3k_e_r T R_P}{\phi^2} \left( \frac{m_E}{V[TG]_0} \right) \left( \frac{d[M]}{dr} \right)_{r=R_P} \Delta t. \tag{60}
\]

and taking into account the stoichiometry of the two independent reactions taking place, (11) and (16), the changes in the superficial concentrations of the products will be

\[
\Delta[L]_s = -\Delta[M]_s, \quad \Delta[GLM]_s = -2\Delta[GL_2]_s + \Delta[M]_s, \quad \Delta[GM_2]_s = -\Delta[M]_s + \Delta[GL_2]_s. \tag{61}
\]

The process of calculation has to be repeated with decreasing increments of time until this increment does not influence the obtained results. The properties of the particles where the enzyme is immobilized are shown in Table 3. The molecular diffusivities of the chemical species involved in the acidolysis of EPAX4510TG and CA, \( D_B \), can be estimated by the equation of Wilke and Chang (Poling et al., 2001a):

\[
\frac{D_B \mu}{T} = 7.40 \times 10^{-8} (xPM_S)_{0.5}^{0.5} \frac{V_{B}^{0.5}}{V_{B}}, \tag{62}
\]

where PM_S is the molecular weight of the solvent, \( x \) its association degree (1 for the n-hexane), \( \mu \) the viscosity of the reaction mixture and \( V_B \) the molar volume of solute as saturated liquid. Although this equation is only approximate and valid for diluted solutions, it shows that the molecular diffusivity is inversely proportional to the viscosity of the liquid phase and that the ratios between the effective diffusivities depend only on the molar volumes of the species as saturated liquids. These volumes have been calculated by the atomic contribution method of Le Bas (Poling et al., 2001b) and the diffusivities ratios \( (x, \beta, \beta_1, \gamma, \beta_2) \) shown in Table 3 have been obtained. The effective diffusivity is related to the molecular diffusivity by the particle characteristics, which are the same for all the species. This
means that to simulate the results with the developed extended model, considering the influence of the pore diffusion, it is only necessary to assume the effective diffusivity of the CA and to calculate Thiele modulus (Eq. (51)), admitting that the kinetic parameter \(k_c\) previously determined for low concentrations is still valid (i.e., \(k_c = k_{L}e_T = (k_{L}/K_M)e_T\), Table 4).

The application of this extended model by MatLab 7.0 leads to the results shown in Fig. 6. In this figure, the continuous upper lines indicate the variation of the CA incorporation degree with time calculated by using the kinetic parameters determined for low concentrations (no pore diffusion influence, Table 4), which practically coincide with the simulated results up to values of Thiele’s module of 2. Fig. 6a also shows the simulations corresponding to \(\Phi\) values of 10, 20 and 30, along with the experimental results corresponding to \([\text{TG}]_0 = 149.8\,\text{mol/m}^3\). Comparing the curves of the model with the experimental results, it can be assumed that the influence of the pore diffusion must be the fundamental reason for the reduction of the reaction rate at very high concentrations of reactants. Nevertheless, the experimental results indicate that this reduction increases with time, or with the degree of incorporation, more than is predicted by the model, which suggests that the effective diffusivity is not constant. Thus, it can be observed that up to 20 h the experimental data practically coincide with the line corresponding to \(\Phi = 10\), for higher reaction times the experimental data come successively closer to the lines corresponding to \(\Phi = 20\) and 30. A similar result was obtained in the experiment carried out in the absence of solvent (Fig. 6b), although in this case for higher values of \(\Phi\), which is logical given the strong increase in the liquid phase viscosity. It is also observed that when the incorporation of CA increases, the experimental data become increasingly closer to the lines corresponding to higher values of \(\Phi\).

Therefore, Fig. 6a and b indicate that the main reason for the decrease in the reaction rate when the TAG concentration increased above a certain value is the influence of the pore diffusion. Nevertheless, the effective diffusivity of CA changes with the composition, as generally occurs in concentrated solutions, diminishing as the degree of incorporation increases. For this reason the Thiele modulus increases, as does the influence of the pore diffusion.

To justify quantitatively this reduction in the CA effective diffusivity with the degree of incorporation, it has been taken into account that the CA effective diffusivity in the reaction mixture (a multi-component liquid phase in the immobilized enzyme particles) depends on the composition and the binary effective diffusivities of CA in each of the species present, as follows (Froment and Bischoff, 1990):

\[
\frac{1}{D_{eM}} = \sum_i \frac{1}{D_{eMi}} \left( \frac{y_i - N_i/N_M}{y_M} \right) = \frac{1}{D_{eMH}} \left( y_H - N_H/N_M y_M \right) + \frac{1}{D_{eMGL}} \left( y_{GL} - N_{GL}/N_M y_M \right) + \frac{1}{D_{eMGL}} \left( y_{GLM} - N_{GLM}/N_M y_M \right)
\]

\[
\]
where \( D_{eM0} \) (the effective diffusivity of CA in the initial reaction mixture) is divided by an expression that increases proportionally with the number of moles of CA incorporated to TAG per unit volume of reaction mixture. Therefore, the Thiele modulus increases continuously in direct proportion to the increase per unit volume of reaction mixture. Therefore, the Thiele modulus increases continuously in direct proportion to the increase of the incorporation degree. This can be seen in Fig. 6a and b.

For the experiments considered, the values of \( D_{eM0} \) were obtained by extrapolation of the results shown in Fig. 6a and b, to time zero. That is, by determination of the Thiele modulus which fits the experimental results at short times, and then by calculating the effective diffusivity from this Thiele modulus (Eq. (51)):

\[
\begin{align*}
[TG]_0 &= 149.8 \text{ mol/m}^3, \quad \phi_0 = 3, \\
D_{eM0} &= 1.01 \times 10^{-7} \text{ m}^2/\text{h}, \\
[TG]_0 &= 487.3 \text{ mol/m}^3, \\
\phi_0 &= 12, \quad D_{eM0} = 6.29 \times 10^{-9} \text{ m}^2/\text{h}.
\end{align*}
\]

To justify the stationary state hypothesis made in Eqs. (37)–(41) we can estimate and compare the characteristic diffusion time and the time constant for the change of composition in the external liquid phase. The characteristic diffusion times within the catalyst particles can be calculated from the previously diffusion coefficients in the form:

\[
\begin{align*}
\frac{[TG]_0}{D_{eM0}} &= 149.8 \text{ mol/m}^3, \quad \frac{t}{R_p^2/D_{eM0}} = 0.4 \text{ hours}, \\
\frac{[TG]_0}{D_{eM0}} &= 487.3 \text{ mol/m}^3, \quad \frac{t}{R_p^2/D_{eM0}} = 6.4 \text{ hours}.
\end{align*}
\]

The time constant for the change of composition of the external liquid phase can be estimated by fitting the changes of composition of the liquid phase for these two experiments (shown in Fig. 7) to the equation:

\[
\frac{dF_M}{dt} = k_{ap}(F_{Me} - F_M)
\]

and in this way it can be obtained:

\[
\begin{align*}
[TG]_0 &= 149.8 \text{ mol/m}^3, \quad k_{ap} = 0.024 \text{ h}^{-1}, \\
t &= 1/k_{ap} = 42 \text{ hours}, \\
[TG]_0 &= 487.3 \text{ mol/m}^3, \quad k_{ap} = 0.0125 \text{ h}^{-1}, \\
t &= 1/k_{ap} = 80 \text{ hours},
\end{align*}
\]

which can justify the pseudo-stationary state assumption. Eq. (65) was useful to fit experimental results with these and other similar systems (Camacho Páez et al., 2002; González Moreno et al., 2004).

The values of \( D_{eM0} \) calculated can be compared with the effective diffusivity in the pores that are full of hexane. The latter value can be estimated as described in Table 3:

\[
D_{eMH} = \frac{D_{MH} \phi_p}{\tau} = 1.63 \times 10^{-6} \text{ m}^2/\text{h},
\]

where the theoretical value of the tortuosity factor for a random pore distribution (= 3) was used. It is observed that there is a difference greater than two orders of magnitude. This difference is similar to the difference between the hexane and triolein viscosities in these conditions.

Eq. (64) contains only one fitting parameter \( \alpha \). As Fig. 7 shows, the experiments have been acceptably fitted for a value of \( \alpha = 0.41 \text{ m}^3/\text{mol} \).

5. Conclusions

1. By using a reactor where the lipase is immobilized, which permits an easy reutilization of the lipase, structured TAG of MLM type have been obtained by acidolysis of three oils (commercial triolein, cod liver oil and EPAX4510TG) with CA. A 56% average incorporation of CA to the structured TAG at equilibrium was attained (close to the maximum 66.7%).

2. The time course variation for the incorporation of CA into TAG (or with the treatment intensity) at low TAG and CA concentrations has been acceptably fitted to a kinetic model based on the reaction mechanism of acidolysis, which considers that the only intermediate of appreciable lifespan in which the enzyme participates is the acyl-enzyme complex. This model also considers the two steps of incorporation of odd fatty acids at positions 1 and 3 of the glycerol backbone, each divided into one step of hydrolysis and another of esterification.

3. For the highest concentrations of TAG used the experimental results have been fitted to an extended model that takes into account the previously established intrinsic kinetics and the diffusion of reactants and products into the catalyst particle pores. This model justifies the reduction of the reaction rate because of the decrease in the effective diffusivity of the species during the reaction.
Notation

AVD average deviation of the experimental results with respect to the results predicted by the model (Table 4)
CA caprylic acid
CLO cod liver oil
DAG diacylglycerol
DB molecular diffusivity of the species involved in the acidolysis
Dei effective diffusivity coefficient of the specie i (L, long-chain fatty acids; M, medium-chain fatty acids; GL2, long-chain triacylglycerols; GLM, triacylglycerols with long-chain acyl groups in positions 1 or 3 and medium-chain acyl groups in positions 3 or 1; GM2, triacylglycerols with medium-chain acyl groups in positions 1 and 3) in the reaction mixture into the catalyst pores
eM0 effective diffusivity coefficient of the medium-chain free fatty acid, M, in a mixture with the specie i (H, hexane; L, long-chain fatty acid; GL2, long-chain triacylglycerols; GLM, triacylglycerols with long-chain acyl groups in positions 1 or 3 and medium-chain acyl groups in positions 3 or 1; GM2, triacylglycerols with medium-chain acyl groups in positions 1 and 3) in the initial reaction mixture (reaction time zero) (Eq. (63))
DMH molecular diffusivity of CA in hexane (Eq. (65), Table 3)
eT total active enzyme concentration per gram of catalyst particle
E free enzyme
[E] concentration of E per gram of catalyst particle
EL long-chain acyl-enzyme complex
[EL] concentration of EL per gram of catalyst particle
EM medium-chain acyl-enzyme complex
[EM] concentration of EM per gram of catalyst particle
EPA eicosapentaenoic acid (20:5n3)
FFA free fatty acid
FM molar fraction of medium-chain acyl group in the triacylglycerols
FMe molar fraction of medium-chain acyl group in the triacylglycerols at the equilibrium
Fxe percentage of a determined fatty acid in the triacylglycerols obtained at the acidolysis equilibrium (Table 1)
Fx0 percentage of a determined fatty acid in the oils used in this work (Table 1)
GC gas chromatography
GL diacylglycerol with a long-chain acyl group in the position 3 of the glycerol backbone (Eqs. (8), (16))
[GL] concentration of GL
[GL]e concentration of GL on the surface of the catalyst particle
GL2 triacylglycerol with long-chain acyl groups in the positions 1 and 3 of the glycerol backbone (Eq. (8))
[GL2] concentration of GL2
[GL2]e concentration of GL2 on the surface of the catalyst particle
GM diacylglycerol with a medium-chain acyl group in position 3 of the glycerol backbone
[G – M]e concentration of ester groups at positions 1 and 3 in which participate the medium-chain acyl group at the equilibrium (Eq. (21))
GM2 triacylglycerol with medium-chain acyl groups in the positions 1 and 3 of the glycerol backbone (Eqs. (8), (16))
[GM2] concentration of GM2
[GM2]e concentration of GM2 on the surface of the catalyst particle
kC k−L/KM ratio (Eq. (50))
kL kinetic constant for the incorporation of a long-chain acyl group to position 1 or 3 of a diacylglycerol GL, LG, GM or MG (Eqs. (4), (13))
k−M kinetic constant for the elimination of a long-chain acyl group from position 1 or 3 of a triacylglycerol LGL, MGL or LGM (Eqs. (4), (6), (13))
kM kinetic constant for the incorporation of a medium-chain acyl group in position 1 or 3 of diacylglycerols GL, LG, MG or GM (Eqs. (6), (14))
k−M kinetic constant for the elimination of a medium-chain acyl group from position 1 or 3 of triacylglycerols MGL, LGM or MGM (Eqs. (5) (6), (14))
k1L kinetic constant for the incorporation of a long-chain acyl group to position 1 of a diacylglycerol GL (Eq. (4))
k−1L kinetic constant for the elimination of a long-chain acyl group from position 1 of a triacylglycerol LGL (Eq. (4))
k1M kinetic constant for the incorporation of a medium-chain acyl group to position 1 of a diacylglycerol GL (Eq. (5))
k−1M kinetic constant for the elimination of a medium-chain acyl group from position 1 of a triacylglycerol MGL (Eq. (5))
k3L kinetic constant for the incorporation of a long-chain acyl group to position 3 of a diacylglycerol LG (Eq. (4))
k−3L kinetic constant for the elimination of a long-chain acyl group from position 3 of a triacylglycerol LG (Eq. (4))
kinetic constant for the incorporation of a medium-chain acyl group to position 3 of a diacylglycerol LG (Eq. (5))

kinetic constant for the elimination of a medium-chain acyl group from position 3 of a triacylglycerol LGM (Eq. (5))

exchange equilibrium constant of the four independent reactions that take place in the acidolysis (Eqs. (17), (18), (10), (15), (12), (23))

$K_i$  
$K_L$  
$K'_L$  
$K_M$  
$L$  
$L_e$  
$LGL$  
$LGM$  
$L_s$  
$m_E$  
$m_0$  
$M$  
$M_e$  
$MG$  
$MGL$  
$MGM$  
$MLM$  
$M_s$  

2-MAG  
$N_i$  
Polyunsaturated fatty acids

molar flow rate of specie $i$ ($M$, medium-chain fatty acid; $H$, hexane; $L$, long-chain fatty acid; $GL_2$, long-chain triacylglycerols; $GLM$, triacylglycerols with long-chain acyl groups in position 1 or 3 and medium-chain acyl groups in position 3 or 1; $GM_2$, triacylglycerols with medium-chain acyl groups in positions 1 and 3) in the catalyst pores (Eq. (63))

packed bed reactor

molecular weight of the solvent (Eq. (62))

polyunsaturated fatty acids

reaction mixture flow rate through the packed bed reactor (Table 2)

radial coordinate in the catalyst particle, $0 \leq r \leq R_P$

reaction rate of the first incorporation of $M$ in a DAG GL and LG (Eqs. (5) and (9))

observable disappearance rate for native triacylglycerols (Eqs. (52))

observable disappearance rate for medium-chain fatty acids (Eqs. (53))

reaction rate of the second incorporation of $M$ in a DAG GM and MG (Eqs. (13), (14), (15))

average radii of the catalyst particle (Table 3)

sum of the squared differences between the experimental results and those predicted by the model (Eq. (33))

structured triacylglycerol

reaction time

average residence time of the reaction mixture in the packed bed reactor

absolute temperature (Eq. (62))

triaclylglycerol

initial concentration of triacylglycerols

thin layer chromatography

reaction mixture volume (Table 2)

molar volume of solute as saturated liquid (Eq. (62), Table 3)

packed bed volume

water

concentration of water

association degree of solvent (Eq. (62))

molar fraction of specie $i$ ($M$, medium-chain fatty acid; $H$, hexane; $L$, long-chain fatty acid; $GL_2$, long-chain triacylglycerols; $GLM$, triacylglycerols with long-chain acyl group in position 1 or 3 and medium-chain acyl groups in position 3 or 1; $GM_2$, triacylglycerols with medium-chain acyl groups in positions 1 and 3) in the catalyst pores (Eq. (63))

Greek letters

$\alpha$  

$D_{el}/D_{eM}$ ratio (Eq. (47), Table 3)
\[ \beta = \frac{D_{eGL_z}}{D_{eM}} \text{ ratio (Eq. (47), Table 3)} \]
\[ \beta_1 = \frac{D_{eGLM}}{D_{eM}} \text{ ratio (Eq. (47), Table 3)} \]
\[ \beta_2 = \frac{D_{eGM_z}}{D_{eM}} \text{ ratio (Eq. (47), Table 3)} \]
\[ \gamma \] fitting constant (Eq. (64))
\[ \phi_P \] catalyst particle porosity (Eq. (65))
\[ \theta \] variable which characterizes the treatment intensity (Eq. (32))
\[ \mu \] viscosity of the reaction mixture (Eq. (62))
\[ \rho_L \] packed bed density (Table 3)
\[ \rho_P \] apparent density of the catalyst particles (Table 3)
\[ \tau \] tortuosity factor (Eq. (65))
\[ \Phi \] Thiele modulus (Eq. (51))
\[ \phi_0 \] initial Thiele modulus (reaction time zero)

Acknowledgements

This research was supported by Grants 1FD97-0731 and AGL2003-03335 from Ministerio de Educación y Cultura and Ministerio de Ciencia y Tecnología (Spain).

References


