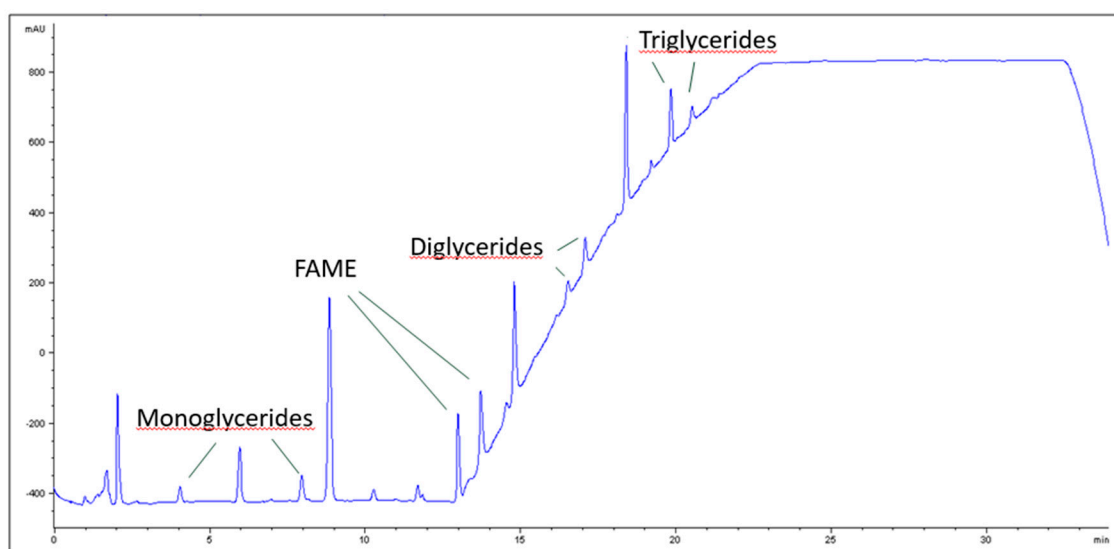


## Supplementary Material

*Paper: Lipozyme® TL IM biocatalyst for castor oil FAME and triacetin production by interesterification: activity, stability and kinetics*

*Analytical technique details: RP-HPLC*

**Figure S1** shows a typical chromatogram obtained from the analysis of the samples. The retention times for each of the compounds were clearly appreciated. The method of analysis chosen is so selective that it detects each of the triglycerides (ricinoleic, linoleic, oleic, stearic, linolenic) as well as their different diglycerides, which is why numerous peaks were observed. The total amount of diglycerides and triglycerides was calculated from the sum of the peaks that constitute these compounds.



**Figure S1. Typical RP-HPLC chromatogram for the castor oil – triacetin interesterification**

To determine the concentrations it is necessary to perform a calibration for each compound in the reaction. For this purpose, 2000, 4000, 6000, 8000 and 10000 ppm standards were prepared for all compounds. The obtained calibrations are shown in Figure S2.

With the equations obtained in the calibrations, with excellent fits (very high regression coefficient values:  $R^2 \geq 0.996$  in all cases), and making use of the material balance to calculate the concentration of the by-product triacetin (TAC), the concentrations of reactants, products and intermediate compounds can be known. Knowing the volumes involved, the moles at each instant are calculated by means of **equations S1-S6**. Since there is no internal standard to minimize experimental errors; the results are weighted based on the mass balance.

$$C(FAME) = 0.0002 \cdot A(FAME)^2 + 2.6801 \cdot A(FAME) \quad (S1)$$

$$C(TG) = 0.0001 \cdot A(TG)^2 + 1.8031 \cdot A(TG) \quad (S2)$$

$$C(DG) = 0.0002 \cdot A(DG)^2 + 1.6315 \cdot A(DG) \quad (S3)$$

$$C(MG) = 0.0002 \cdot A(MG)^2 + 1.4558 \cdot A(MG) \quad (S4)$$

$$C(TAC) = C_0(TG) - C(TG) - C(DG) - C(MG) \quad (S5)$$

$$C(ACT) = (FAME) \cdot C_0(TG) - C(FAME) \quad (S6)$$

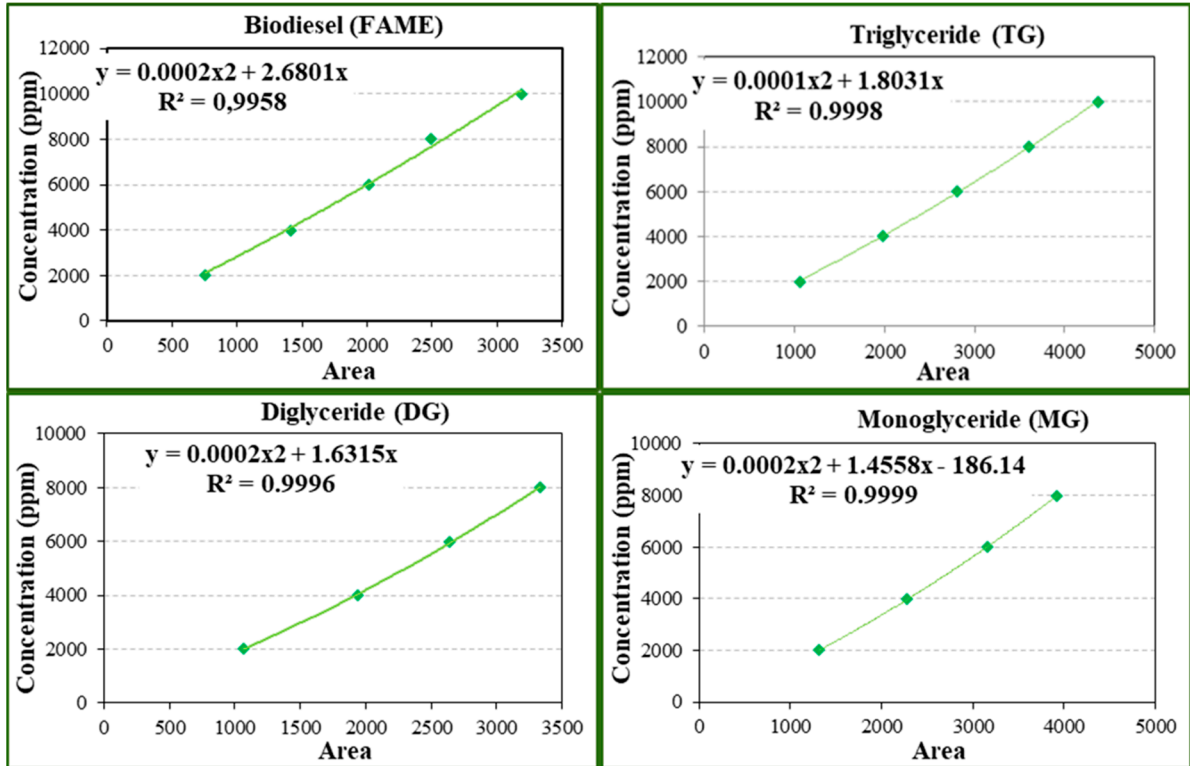


Figure S2. Biodiesel (FAME), Triglycerides (TG), Diglycerides (DG) and Monoglycerides (MG) Calibration Graphs

Data of kinetic runs in the L-L-S triphasic system glycerol-(toluene+ibuprofen)-  
Novozym®435

**Table S1.** Kinetic runs performed for the anhydrous triphasic system

Run	C <sub>w</sub> (% v/v)	T (°C)	C <sub>I0</sub> (g·L <sup>-1</sup> )
S4E1	0	50	20
S4E2	0	50	60
S4E3	0	50	100
S4E4	0	60	20
S4E5	0	60	60
S4E6	0	60	100
S4E7	0	70	20
S4E8	0	70	60
S4E9	0	70	100
S4E10	0	80	20
S4E11	0	80	60
S4E12	0	80	100

**Table S2.** Experimental results of the enzymatic esterification of glycerin with ibuprofen immobilized enzyme N435 at T=50 °C for the anhydrous system.

Time (min)	S4E1 C <sub>I0</sub> = 20 g·L <sup>-1</sup> T=50 °C		S4E2 C <sub>I0</sub> = 60 g·L <sup>-1</sup> T=50 °C		S4E3 C <sub>I0</sub> = 100 g·L <sup>-1</sup> T=50 °C	
	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )
0	0.000	0.000	0.000	0.000	0.000	0.000
10	0.080	0.008	0.030	0.009	0.020	0.010
20	0.140	0.013	0.050	0.014	0.030	0.014
30	0.190	0.018	0.080	0.023	0.050	0.024
40	0.231	0.022	0.110	0.032	0.070	0.034
60	0.312	0.030	0.160	0.046	0.090	0.043
90	0.412	0.040	0.220	0.063	0.150	0.072
120	0.489	0.047	0.270	0.078	0.190	0.091
150	0.531	0.051	0.320	0.092	0.240	0.115
180	0.584	0.056	0.380	0.109	0.270	0.130
240	0.662	0.064	0.450	0.130	0.320	0.154
300	0.741	0.071	0.520	0.150	0.380	0.182
360	0.775	0.074	0.560	0.161	0.410	0.197
400	0.794	0.076	0.590	0.170	0.430	0.206
500	0.845	0.081	0.620	0.179	0.470	0.226
600	0.874	0.084	0.660	0.190	0.510	0.245
700	0.889	0.085	0.690	0.199	0.550	0.264
800	0.905	0.087	0.720	0.207	0.580	0.278
900	0.924	0.089	0.740	0.213	0.610	0.293
1000	0.931	0.089	0.750	0.216	0.630	0.302
1100	0.934	0.090	0.770	0.222	0.650	0.312
1200	0.934	0.090	0.790	0.228	0.680	0.326
1300	0.938	0.090	0.810	0.233	0.700	0.336
1400	0.941	0.090	0.820	0.236	0.720	0.346
1500	0.942	0.090	0.830	0.239	0.735	0.353

**Table S3.** Experimental results of the enzymatic esterification of glycerin with ibuprofen immobilized enzyme N435 at T=60 °C for the anhydrous system.

Time (min)	S4E4 C <sub>IO</sub> = 20 g·L <sup>-1</sup> T=60 °C		S4E5 C <sub>IO</sub> = 60 g·L <sup>-1</sup> T=60 °C		S4E6 C <sub>IO</sub> = 100 g·L <sup>-1</sup> T=60 °C	
	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )
0	0.000	0.000	0.000	0.000	0.000	0.000
10	0.110	0.011	0.048	0.014	0.035	0.017
20	0.183	0.018	0.099	0.029	0.061	0.029
30	0.257	0.025	0.153	0.044	0.099	0.048
40	0.301	0.029	0.205	0.059	0.125	0.060
60	0.401	0.039	0.280	0.081	0.179	0.086
90	0.502	0.048	0.378	0.109	0.231	0.111
120	0.584	0.056	0.455	0.131	0.290	0.139
150	0.651	0.063	0.491	0.141	0.336	0.161
180	0.704	0.068	0.532	0.153	0.367	0.176
240	0.754	0.072	0.591	0.170	0.410	0.197
300	0.808	0.078	0.631	0.182	0.445	0.214
360	0.841	0.081	0.671	0.193	0.503	0.242
400	0.867	0.083	0.692	0.199	0.530	0.254
500	0.912	0.088	0.735	0.212	0.586	0.281
600	0.927	0.089	0.766	0.221	0.630	0.302
700	0.934	0.090	0.790	0.228	0.665	0.319
800	0.941	0.090	0.809	0.233	0.695	0.334
900	0.943	0.091	0.825	0.238	0.720	0.346
1000	0.948	0.091	0.838	0.241	0.741	0.356
1100	0.951	0.091	0.848	0.244	0.759	0.364
1200	0.952	0.091	0.858	0.247	0.775	0.372
1300	0.953	0.092	0.866	0.249	0.789	0.379
1400	0.954	0.092	0.872	0.251	0.802	0.385
1500	0.954	0.092	0.879	0.253	0.813	0.390

**Table S4.** Experimental results of the enzymatic esterification of glycerin with ibuprofen immobilized enzyme N435 at T=70 °C for the anhydrous system.

Time (min)	S4E7 C <sub>10</sub> = 20 g·L <sup>-1</sup> T=70 °C		S4E8 C <sub>10</sub> = 60 g·L <sup>-1</sup> T=70 °C		S4E9 C <sub>10</sub> = 100 g·L <sup>-1</sup> T=70 °C	
	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )
0	0.000	0.000	0.000	0.000	0.000	0.000
10	0.111	0.011	0.068	0.020	0.033	0.016
20	0.208	0.020	0.127	0.037	0.063	0.030
30	0.293	0.028	0.179	0.052	0.092	0.044
40	0.365	0.035	0.225	0.065	0.119	0.057
60	0.488	0.047	0.303	0.087	0.169	0.081
90	0.602	0.058	0.394	0.114	0.234	0.112
120	0.673	0.065	0.464	0.134	0.289	0.139
150	0.742	0.071	0.519	0.150	0.338	0.162
180	0.785	0.075	0.563	0.162	0.380	0.182
240	0.867	0.083	0.631	0.182	0.450	0.216
300	0.912	0.088	0.679	0.196	0.506	0.243
360	0.944	0.091	0.717	0.207	0.553	0.266
400	0.951	0.091	0.737	0.212	0.579	0.278
500	0.954	0.092	0.776	0.224	0.633	0.304
600	0.955	0.092	0.804	0.232	0.675	0.324
700	0.956	0.092	0.826	0.238	0.709	0.340
800	0.957	0.092	0.843	0.243	0.737	0.354
900	0.958	0.092	0.857	0.247	0.760	0.365
1000	0.959	0.092	0.868	0.250	0.779	0.374
1100	0.960	0.092	0.877	0.253	0.796	0.382
1200	0.961	0.092	0.885	0.255	0.810	0.389
1300	0.962	0.092	0.892	0.257	0.823	0.395
1400	0.962	0.092	0.898	0.259	0.834	0.400
1500	0.964	0.093	0.904	0.260	0.844	0.405

**Table S5.** Experimental results of the enzymatic esterification of glycerin with ibuprofen immobilized enzyme N435 at T=80 °C for the anhydrous system.

Time (min)	S4E10 C <sub>I0</sub> = 20 g·L <sup>-1</sup> T=80 °C		S4E11 C <sub>I0</sub> = 60 g·L <sup>-1</sup> T=80 °C		S4E12 C <sub>I0</sub> = 100 g·L <sup>-1</sup> T=80 °C	
	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )
0	0.000	0.000	0.000	0.000	0.000	0.000
10	0.170	0.016	0.080	0.023	0.070	0.034
20	0.280	0.027	0.140	0.040	0.130	0.062
30	0.350	0.034	0.200	0.058	0.170	0.082
40	0.410	0.039	0.250	0.072	0.210	0.101
60	0.550	0.053	0.330	0.095	0.270	0.130
90	0.660	0.063	0.410	0.118	0.330	0.158
120	0.730	0.070	0.480	0.138	0.370	0.178
150	0.790	0.076	0.520	0.150	0.400	0.192
180	0.860	0.083	0.560	0.161	0.420	0.202
240	0.910	0.087	0.620	0.179	0.450	0.216
300	0.950	0.091	0.650	0.187	0.480	0.230
360	0.960	0.092	0.680	0.196	0.490	0.235
400	0.970	0.093	0.710	0.205	0.500	0.240
500	0.970	0.093	0.720	0.207	0.510	0.245
600	0.980	0.094	0.750	0.216	0.530	0.254
700	0.980	0.094	0.760	0.219	0.530	0.254
800	0.980	0.094	0.780	0.225	0.540	0.259
900	0.980	0.094	0.790	0.228	0.550	0.264
1000	0.980	0.094	0.790	0.228	0.550	0.264
1100	0.980	0.094	0.800	0.230	0.550	0.264
1200	0.980	0.094	0.810	0.233	0.560	0.269
1300	0.980	0.094	0.810	0.233	0.560	0.269
1400	0.980	0.094	0.820	0.236	0.560	0.269
1500	0.980	0.094	0.820	0.236	0.560	0.269

Data of kinetic runs in the L-L-S triphasic system (glycerol+7.4 %v/v water)-  
(toluene+ibuprofen)- Novozym®435

**Table S6.** Kinetic runs performed for the hydrated triphasic system

<b>Run</b>	<b>C<sub>w</sub> (% v/v)</b>	<b>T (°C)</b>	<b>C<sub>10</sub> (g·L<sup>-1</sup>)</b>
S4E13	7.4	50	20
S4E14	7.4	50	60
S4E15	7.4	50	100
S4E16	7.4	60	20
S4E17	7.4	60	60
S4E18	7.4	60	100
S4E19	7.4	70	20
S43E20	7.4	70	60
S4E21	7.4	70	100
S4E22	7.4	80	20
S4E23	7.4	80	60
S4E24	7.4	80	100



**Table S7.** Experimental results of the enzymatic esterification of glycerin with ibuprofen immobilized enzyme N435 at T=50 °C for the system with added water.

	S4E16		S4E17		S4E18	
	$C_{I0} = 20 \text{ g}\cdot\text{L}^{-1}\text{T}=50 \text{ }^{\circ}\text{C}$		$C_{I0} = 60 \text{ g}\cdot\text{L}^{-1}\text{T}=50 \text{ }^{\circ}\text{C}$		$C_{I0} = 100 \text{ g}\cdot\text{L}^{-1}\text{T}=50 \text{ }^{\circ}\text{C}$	
Time (min)	$X_{MG}$	$C_{MG} \text{ (mol L}^{-1}\text{)}$	$X_{MG}$	$C_{MG} \text{ (mol L}^{-1}\text{)}$	$X_{MG}$	$C_{MG} \text{ (mol L}^{-1}\text{)}$
0	0.000	0.000	0.000	0.000	0.000	0.000
10	0.085	0.008	0.047	0.014	0.035	0.017
20	0.157	0.015	0.089	0.026	0.068	0.033
30	0.217	0.021	0.129	0.038	0.099	0.048
40	0.270	0.026	0.165	0.048	0.128	0.062
60	0.355	0.034	0.229	0.067	0.180	0.087
90	0.450	0.044	0.308	0.090	0.249	0.121
120	0.520	0.050	0.374	0.109	0.308	0.149
150	0.573	0.056	0.428	0.124	0.358	0.174
180	0.615	0.060	0.474	0.138	0.402	0.195
240	0.677	0.066	0.548	0.159	0.475	0.230
300	0.721	0.070	0.605	0.176	0.533	0.258
360	0.753	0.073	0.649	0.189	0.580	0.281
400	0.770	0.075	0.674	0.196	0.607	0.294
500	0.803	0.078	0.724	0.211	0.663	0.321
600	0.827	0.080	0.761	0.221	0.705	0.342
700	0.845	0.082	0.791	0.230	0.740	0.359
800	0.859	0.083	0.814	0.237	0.767	0.372
900	0.870	0.084	0.833	0.242	0.791	0.383
1000	0.879	0.085	0.849	0.247	0.810	0.393
1100	0.887	0.086	0.863	0.251	0.827	0.401
1200	0.893	0.087	0.875	0.255	0.841	0.408
1300	0.899	0.087	0.885	0.257	0.854	0.414
1400	0.903	0.088	0.894	0.260	0.865	0.419
1500	0.907	0.088	0.901	0.262	0.875	0.424

**Table S8.** Experimental results of the enzymatic esterification of glycerin with ibuprofen immobilized enzyme N435 at T=60 °C for the system with added water.

Time (min)	S4E19 C <sub>I0</sub> = 20 g·L <sup>-1</sup> T=60 °C		S4E20 C <sub>I0</sub> = 60 g·L <sup>-1</sup> T=60 °C		S4E21 C <sub>I0</sub> = 100 g·L <sup>-1</sup> T=60 °C	
	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )
0	0.000	0.000	0.000	0.000	0.000	0.000
10	0.103	0.010	0.061	0.018	0.042	0.020
20	0.187	0.018	0.115	0.033	0.080	0.039
30	0.256	0.025	0.163	0.047	0.115	0.056
40	0.314	0.030	0.207	0.060	0.148	0.072
60	0.406	0.039	0.281	0.082	0.207	0.100
90	0.505	0.049	0.371	0.108	0.282	0.137
120	0.575	0.056	0.441	0.128	0.345	0.167
150	0.627	0.061	0.497	0.145	0.397	0.192
180	0.667	0.065	0.544	0.158	0.442	0.214
240	0.726	0.070	0.615	0.179	0.515	0.250
300	0.766	0.074	0.668	0.194	0.572	0.277
360	0.795	0.077	0.709	0.206	0.617	0.299
400	0.811	0.079	0.731	0.213	0.642	0.311
500	0.840	0.081	0.775	0.225	0.694	0.336
600	0.861	0.083	0.807	0.235	0.733	0.355
700	0.877	0.085	0.831	0.242	0.764	0.370
800	0.889	0.086	0.851	0.248	0.788	0.382
900	0.899	0.087	0.867	0.252	0.809	0.392
1000	0.906	0.088	0.880	0.256	0.826	0.400
1100	0.913	0.089	0.891	0.259	0.840	0.407
1200	0.919	0.089	0.900	0.262	0.853	0.414
1300	0.923	0.089	0.908	0.264	0.864	0.419
1400	0.927	0.090	0.915	0.266	0.873	0.423
1500	0.931	0.090	0.921	0.268	0.882	0.428

**Table S9.** Experimental results of the enzymatic esterification of glycerin with ibuprofen immobilized enzyme N435 at T=70 °C for the system with added water.

Time (min)	S4E19 $C_{I0}= 20 \text{ g}\cdot\text{L}^{-1}\text{T}=70 \text{ }^{\circ}\text{C}$		S4E20 $C_{I0}= 20 \text{ g}\cdot\text{L}^{-1}\text{T}=70 \text{ }^{\circ}\text{C}$		S4E21 $C_{I0}= 20 \text{ g}\cdot\text{L}^{-1}\text{T}=70 \text{ }^{\circ}\text{C}$	
	$X_{MG}$	$C_{MG} \text{ (mol L}^{-1}\text{)}$	$X_{MG}$	$C_{MG} \text{ (mol L}^{-1}\text{)}$	$X_{MG}$	$C_{MG} \text{ (mol L}^{-1}\text{)}$
0	0.000	0.000	0.000	0.000	0.000	0.000
10	0.140	0.014	0.106	0.031	0.084	0.041
20	0.246	0.024	0.192	0.056	0.155	0.075
30	0.329	0.032	0.263	0.076	0.217	0.105
40	0.397	0.038	0.323	0.094	0.270	0.131
60	0.499	0.048	0.419	0.122	0.358	0.174
90	0.602	0.058	0.521	0.152	0.457	0.222
120	0.671	0.065	0.594	0.173	0.531	0.257
150	0.721	0.070	0.649	0.189	0.588	0.285
180	0.759	0.074	0.691	0.201	0.633	0.307
240	0.812	0.079	0.752	0.219	0.701	0.340
300	0.847	0.082	0.794	0.231	0.748	0.363
360	0.872	0.085	0.825	0.240	0.784	0.380
400	0.886	0.086	0.841	0.245	0.803	0.389
500	0.911	0.088	0.872	0.254	0.840	0.407
600	0.928	0.090	0.894	0.260	0.867	0.420
700	0.941	0.091	0.911	0.265	0.887	0.430
800	0.951	0.092	0.923	0.268	0.902	0.437
900	0.959	0.093	0.934	0.272	0.915	0.444
1000	0.965	0.094	0.942	0.274	0.925	0.448
1100	0.970	0.094	0.949	0.276	0.933	0.452
1200	0.975	0.095	0.955	0.278	0.941	0.456
1300	0.978	0.095	0.960	0.279	0.947	0.459
1400	0.982	0.095	0.964	0.280	0.952	0.462
1500	0.985	0.096	0.968	0.282	0.957	0.464

**Table S10.** Experimental results of the enzymatic esterification of glycerin with ibuprofen immobilized enzyme N435 at T=80 °C for the system with added water.

Time (min)	S4E22 C <sub>I0</sub> = 20 g·L <sup>-1</sup> T=80 °C		S4E22 C <sub>I0</sub> = 60 g·L <sup>-1</sup> T=80 °C		S4E24 C <sub>I0</sub> = 100 g·L <sup>-1</sup> T=80 °C	
	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )	X <sub>MG</sub>	C <sub>MG</sub> (mol L <sup>-1</sup> )
0	0.000	0.000	0.000	0.000	0.000	0.000
10	0.283	0.027	0.187	0.054	0.096	0.047
20	0.441	0.043	0.315	0.092	0.176	0.085
30	0.542	0.053	0.408	0.119	0.243	0.118
40	0.612	0.059	0.479	0.139	0.300	0.145
60	0.703	0.068	0.579	0.168	0.393	0.191
90	0.780	0.076	0.674	0.196	0.495	0.240
120	0.826	0.080	0.734	0.213	0.569	0.276
150	0.856	0.083	0.775	0.225	0.624	0.303
180	0.877	0.085	0.805	0.234	0.668	0.324
240	0.905	0.088	0.846	0.246	0.732	0.355
300	0.922	0.089	0.873	0.254	0.777	0.377
360	0.934	0.091	0.892	0.259	0.810	0.393
400	0.943	0.091	0.906	0.264	0.835	0.405
500	0.950	0.092	0.917	0.267	0.855	0.414
600	0.960	0.093	0.932	0.271	0.885	0.429
700	0.965	0.094	0.942	0.274	0.903	0.438
800	0.970	0.094	0.948	0.276	0.917	0.445
900	0.973	0.094	0.954	0.277	0.928	0.450
1000	0.975	0.095	0.958	0.279	0.937	0.454
1100	0.978	0.095	0.962	0.280	0.945	0.458
1200	0.980	0.095	0.965	0.281	0.951	0.461
1300	0.981	0.095	0.968	0.282	0.956	0.463
1400	0.982	0.095	0.970	0.282	0.961	0.466
1500	0.984	0.095	0.972	0.283	0.965	0.468