DEVELOPMENT AND VALIDATION OF A GAS CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS DETERMINATION OF MULTICOMPONENTS DURING MONOGLYCERIDE SYNTHESIS BY GLYCEROLYSIS OF METHYL OLEATE: APPLICATION TO HOMOGENEOUS AND HETEROGENEOUS CATALYSIS.

Cristián A. Ferretti, Carlos R. Apesteguía, and J. Isabel di Cosimo[1],♥

Catalysis Science and Engineering Research Group (GICIC), Instituto de Investigaciones en Catálisis y Petroquímica -INCAPE-(UNL-CONICET), Santiago del Estero 2654, (3000).

Santa Fe, Argentina

_____

Abstract

A gas chromatography (GC) method for the simultaneous determination of glycerol, methyl oleate, monoglycerides (α- and β-glyceryl monooleates), diglycerides (1,2- and 1,3-glyceryl dioleates), and triglycerides (glyceryl trioleate) is postulated and validated to monitor the reaction product evolution during the catalytic synthesis of monoglycerides by glycerolysis of methyl oleate. The method is suitable for quantitative analysis of the multiphase reaction system, either under liquid or solid catalyzed conditions in a wide concentration range. Excellent separation of all the analytes was achieved with a 15 m-capillary column of 100 % methyl polysiloxane in 35 minutes. A discussion

♥ Corresponding author: e-mail: dicosimo@fiq.unl.edu.ar

on different solvents (toluene and n-hexane) and derivatization procedures is presented. A proper and unique combination of silylating agents for the trimethylsilylation of the free hydroxyl groups of glycerol, mono- and diglycerides is proposed as well as two internal standards, n-hexadecane and cholesterol. The method accuracy was evaluated from recovery experiments and the precision by repeated analysis of each sample. Relative recoveries of the analytes ranged from 92-105 % and relative standard deviations from 4.8 to 13.0 %. Good linearity of calibration curves was obtained in the investigated concentration ranges with high correlation coefficients ($r^2 \geq 0.992$) for all the analytes.

Key words: glycerolysis; monoglycerides; gas chromatography

Resumen

Se desarrolló y validó un método de cromatografía gaseosa para la determinación simultánea de glicerol, oleato de metilo, monoglicéridos (α- and β-monoleatos de glicerilo), diglicéridos (1,2- and 1,3-dioleato de glicerilo) y triglicéridos (trioleato de glicerilo) para monitorear la evolución de los productos de reacción durante la síntesis catalítica de monoglicéridos por glicerólisis de oleato de metilo. El método es adecuado para realizar el análisis cuantitativo de un sistema de reacción multifásico, ya sea cuando se emplean catalizadores líquidos o sólidos en un amplio rango de concentraciones. Una excelente separación de todos los analitos fue obtenida con una columna capilar de 15 m de 100 % metil-polisiloxano en 35 minutos. Se presenta la evaluación de los procedimientos de derivatización y el uso de diferentes solventes (tolueno y n-hexano). Se propone una adecuada y única combinación de agentes sililantes para la trimetilsililación de los grupos hidroxilos libres del glicerol, mono- y diglicéridos, así como también el empleo de dos estándares internos, n-hexano y colesterol. Se evaluó la exactitud del método a partir de ensayos de recuperación, y la precisión por repetición de análisis de cada muestra. El rango de recuperación relativa de los analitos fue del 92-105 % y la desviación estándar relativa de 4.8 a 13.0 %. Una buena linealidad en las curvas de calibración fue obtenida en los rangos de concentraciones investigados con elevados coeficientes de correlación ($r^2 \geq 0.992$) para todos los analitos.

Palabras clave: glicerólisis; monoglicéridos; cromatografía gaseosa.

Introduction

Biodiesel synthesis produces about 10% of glycerol (Gly) as byproduct [1]. The increasing production of low-cost Gly due to current and future biodiesel demands causes environmental and economical concerns. There is a need, therefore, to develop novel catalytic processes to convert Gly into valuable chemicals. The synthesis of monoglycerides (MG) via glycerolysis of fatty acid methyl esters (FAME) is an interesting option to obtain fine chemicals from bio-resources [2].

Monoglycerides are widely used as emulsifiers in food, pharmaceutical, cosmetic, and detergent industries [3]. The current technology for MG synthesis involves the use of corrosive liquid base catalysts and several neutralization and separation steps [4]. Moreover, the process is not selective and forms a mixture of mono-, di- (DG), and triglycerides (TG). The use of heterogeneous catalysis may certainly improve this technology, not only increasing the selectivity to MG but also because solid catalysts can be easily separated from the reaction media and are often reusable.

Glycerolysis of FAME forms not only MG, DG and TG, but also isomers and condensation products. This is a complex mixture of components having a considerable overlap of physical/chemical and thus chromatographic properties.

A number of techniques have been employed to identify and quantify glycerides and FAME, such as thin layer chromatography (TLC)**,** high-performance liquid chromatography (HPLC) coupled with other detectors such as refractive index (RI) detector, flame ionization detector (FID), density detector, evaporative light scattering detector (ELSD), and mass spectrometer detector [5,6]. However, all these techniques require long analytical procedures [7] and in some cases coelution of components is often verified, such as in mixtures containing low-molecular weight TG and high-molecular weight DG [5].

Capillary GC has been widely studied in the quality control of FAME used as biodiesels [8,9]. Biodiesels contain essentially FAME and trace amounts of glycerides as impurities, while in the glycerolysis of FAME the main components of the reaction mixture are monoglycerides and diglycerides, and minor amounts of FAME, glycerol and triglycerides. Due to the differences of component concentration and chemical properties, the conventional silylating agents and the silylation procedure itself used for chemical analysis of biodiesels via GC are not appropriate for monitoring the time evolution of the reaction mixture composition during FAME glycerolysis and therefore a new method has to be developed.

In theory, the component mixture may be analyzed without derivatization on highly inert columns coated with polar stationary phases, but the inertness of the column cannot be easily maintained in routine analysis. Thus, a trimethylsilylation procedure of the free hydroxyl groups of Gly, MG and DG is required to drastically improve the analytical method sensitivity and the component chromatographic properties, in particular volatility and thermal stability. Analysis of different lipid compounds such as sterols, fatty acids, and glycerides has been achieved by using the trimethylsilylation method [10].

In this paper, we postulate and validate a new, rapid and efficient GC method for the routine analysis and simultaneous quantification of Gly, methyl oleate, MG, DG, and TG during the glycerolysis of methyl oleate. Methyl oleate (MO), the methyl ester of oleic acid, is therefore taken as a model FAME compound. All the mixture analytes are quantified after sample silylation. This reliable single method is suitable for a wide range of component compositions such as those present at the beginning of the reaction (high MO and low MG, DG and TG concentrations) and at the end (low Gly and MO concentration and high MG, DG and TG concentrations). A discussion on different silylation procedures and solvents is presented as well as a study of the method suitability not only when a liquid catalyst is used but also for the solid catalyzed reaction in which one more phase is present.

Experimental

Reagents and chemicals

All the reactants were analytical grade compounds. Hexamethyldisilazane (HMDS, Fluka, > 98.0 %), chlorotrimethylsilane (TMCS, Fluka, > 98.0 %), and N,O-Bis(trimethylsilyl)-acetamide (BSA, Sigma, > 95.0 %), were used as silylation reagents. Gly (Aldrich, 99.0 %), MO (methyl oleate, Fluka, 75 %), TG (glyceryl trioleate, Sigma, 65.0 %) and a commercial mixture containing 71.5 % MG (glyceryl monooleate, Fluka) and 25.3 % DG (glyceryl dioleate, Fluka) were used as analytical standards. Cholesterol (Aldrich, 95.0 %) and n-hexadecane (Sigma, > 99.0 %) were the internal standards, while toluene, n-hexane, and pyridine were used as solvents.

Magnesium oxide was prepared as described previously to obtain high-surface area MgO (150 m^2/g). The 25 % sodium methoxide (NaMeO) solution was prepared by reacting NaOH with methanol under stirring [12].

Catalytic reaction

The transesterification of Gly with MO was carried out in a stirred glass batch reactor equipped with a condenser system to continuously remove methanol formed during the reaction.

Standard reaction tests using MgO were performed at 220 ºC. The Gly/MO mixture (Gly/MO = 2.0 molar ratio) was stirred (700 rpm) and heated to 220 ºC in N^2 (35 cm^3/min). Then, the catalyst was loaded (W_{cat}/n^0_{MO} = 30 g/mol, where W_{cat} is the catalyst weight and n^0_{MO} is the initial molar amount of MO) and the 8-h reaction was started.

The homogeneously-catalyzed reaction was carried out at 135 ºC using a Gly/MO molar ratio of 2 and a stirring rate of 550 rpm. The liquid catalyst (sodium methoxide, NaMeO) was loaded keeping a $W_{cat}/n^0_{MO} = 5.5$ g/mol ratio.

In all the catalytic tests, samples were periodically withdrawn from the reactor for analysis. Main reaction products were α- and β-glyceryl monooleates (MG), 1,2- and 1,3-glyceryl dioleates (DG) and glyceryl trioleate (TG).

Preparation of samples and standard solutions

Stock solutions of Gly, (60.0 mg/mL), MO (90.0 mg/mL), MG (90.0 mg/mL), DG (30.0 mg/mL), TG (70.0 mg/mL), and internal standards of n-hexadecane (IS_1, 70.0 mg/mL), and cholesterol (IS_2, 10.0 mg/mL) in pyridine were used to prepare standard solutions at five concentration levels. Appropriate amounts of these stock solutions together with 50 μL of n-hexadecane and 50 μL of cholesterol solutions were transferred to glass vials; then, 200 μL of BSA and 100 μL of TMCS were added to these standard solutions. After 60 min at 90ºC, the silylated mixtures were cooled down to room temperature and diluted with 5 mL of n-hexane. The final volume of the silylation solutions was 5.5 mL. The concentration of the analytes in the standard solutions varied from 0.06 to 0.30 mg/mL for Gly; from 0.08 to 0.50 mg/mL for MO and MG; from 0.05 to 0.25 mg/mL for DG, and from 0.40 to 1.30 mg/mL for TG. In all the standard solutions the concentration of internal standard was 0.65 mg/mL for n-hexadecane and 0.09 mg/mL for cholesterol.

Reaction samples were separated from the catalyst by centrifugation at room temperature at 3000 rpm for 15 min. Samples of 10 μL of the reactor upper layer (MO-rich phase) were separated and weighed in a 10 mL glass vial; then, 50 μL of stock solution IS_1 and 50 μL of stock solution IS_2 were added to the glass vial. Finally, samples were silylated using the same procedure described above for the standard solutions.

Instrumentation and chromatographic conditions

The GC analyses were performed with a SRI 8610C gas chromatograph from SRI Instruments, equipped with an on-column injector and a flame ionization detector. A 15 m x 0.32 mm ID fused silica capillary column of 100 % methyl polysiloxane (HP-1 Agilent Technologies) was used, connected in series with a 0.53 mm ID high-temperature guard column of deactivated fused silica. Nitrogen at 5 mL/min was used as carrier gas (inlet pressure, 10 psi). The chromatographic conditions were the following: The inlet temperature was 50 ºC. The column oven was held 1 min at 50 ºC and then heated to 100 ºC at 50 ºC/min, and held 1 min at 100 °C. Then, it was consecutively heated to 130 ºC at 20 ºC/min, to 170 ºC at 6 ºC/min, to 190 ºC at 20 ºC/min, to 250 ºC at 10 ºC/min, to 285 ºC at 35 ºC/min, to 310 ºC at 20 ºC/min and finally to 350 ºC at 4 ºC/min. The column temperature was maintained at 350 ºC till elution of all the components. The total length of the chromatographic run was 35 min. The detector temperature was 350 ºC and the injection volume 0.6 μL. Data were collected using Peak Simple 2000 analysis software.

Evaluation of the analytical procedure

The analysis specificity, linearity, quantification and detection limits, accuracy and precision were evaluated using solutions of calibrated concentrations. Similar procedure was used for the study of the effect of different dilution solvents.

Equations for calculation of catalyst activity and selectivity

MO conversion (X_{MO}), selectivities (S_j, mol of product j/mol of MO reacted) and yields (Y_j, mol of product j/mol of MO fed) were calculated through the following equations:

$$X_{MO} = \frac{n_{MG} + 2n_{DG} + 3n_{TG}}{n_{MG} + 2n_{DG} + 3n_{TG} + n_{MO}}$$

$$S_{MG} = \frac{n_{MG}}{n_{MG} + 2n_{DG} + 3n_{TG}}$$

$$S_{DG} = \frac{2n_{DG}}{n_{MG} + 2n_{DG} + 3n_{TG}}$$

$$S_{TG} = \frac{3n_{DG}}{n_{MG} + 2n_{DG} + 3n_{TG}}$$

$$Y_j = X_{MO} \cdot S_j$$

where n_j, mol of product j.

Results and discussion

Different GC column heating ramp rates were initially employed to achieve a good separation of all the sample chromatographic peaks (Gly, MO, MG, DG, and TG). An optimum separation performance was obtained using the temperature program and the fused silica capillary column of 100 % methyl polysiloxane (non-polar stationary phase) detailed above. This column type allowed the compound separation following the carbon atom number. Besides, the column low film thickness (0.1 mm) and short length (15 m) improve the compound elution at low temperatures, thereby decreasing its retention time.

Derivatization

The following reaction conditions were evaluated to achieve complete trimethylsilylation of glycerides: (1) sample silylation with a HMDS/TMCS mixture in pyridine, and 15 min of reaction at room temperature; (2) sample silylation with a HMDS/TMCS mixture in pyridine, and 10 min of reaction at 70 ºC; (3) sample silylation with BSA in pyridine, and 60 min of reaction at 90 ºC; (4) sample silylation with a BSA/TMCS mixture in pyridine, and 60 min of reaction at 90 ºC. Pyridine was used as solvent in all the cases due to its high solvation capacity and also because acts as an HCl acceptor when organochlorosilanes are present (silylation using TMCS). The cholesterol molecule (internal standard) contains a free OH group available for silylation. Because of the chemical nature and high molecular weight of cholesterol, derivatization of this molecule is kinetically slower than those of sample analytes. Then, complete silylation of cholesterol, as denoted by the shape and size of the cholesterol chromatographic peak, is taken as an indication of complete sample derivatization. Complete silylation of Gly and glycerides was obtained using reaction procedures (1), (2), and (4). It seems that addition of TMCS is required in order to improve the silylation capacity of HMDS and BSA and to attain the complete sample silylation. However,

continuous precipitation of ammonium chloride took place in methods (1) and (2) causing poor sample injection reproducibility. In contrast, method (4) showed excellent reproducibility and was then selected for sample derivatization in this work.

Method validation: Specificity, linearity, quantification and detection limits, accuracy and precision

We investigated the specificity of the method developed here by analyzing a typical sample of the catalytic glycerolysis of methyl oleate. Chromatographic elution peaks were identified using reference solutions of the analytes.

Figure 1 shows a typical chromatogram of a silylated sample of reactants and products after the reaction between glycerol and MO, using cholesterol and n-hexadecane as internal standards. In the chromatogram, the peaks with retention times (t_R) below 3.5 min correspond to the dilution solvent and silylation reactants. Then, the elution of Gly (t_R = 4.2 min), n-hexadecane (t_R = 6.3 min), MO (t_R = 11.6 min), β- MG (t_R = 15.8 min), α- MG (t_R = 16.6 min), cholesterol (t_R = 18.3 min), 1,2- DG (t_R = 21.8 min), 1,3- DG (t_R = 22.2 min) and three peaks attributed to TG (t_R = 26.5, 27.5 and 28.4 min) are observed. The other peaks are impurities of the MO reactant, mainly the saturated myristic acid and palmitic acid methyl esters.

It is observed that there are no overlapping peaks in the analyte elution region and that the different fatty acid methyl esters and glycerides are separated according to carbon numbers (CN), defined as the total carbon atom number in the acyl chain. Gly and n-hexadecane elute at column temperatures lower than 150 ºC while the other components appear at temperatures higher than 170 ºC.
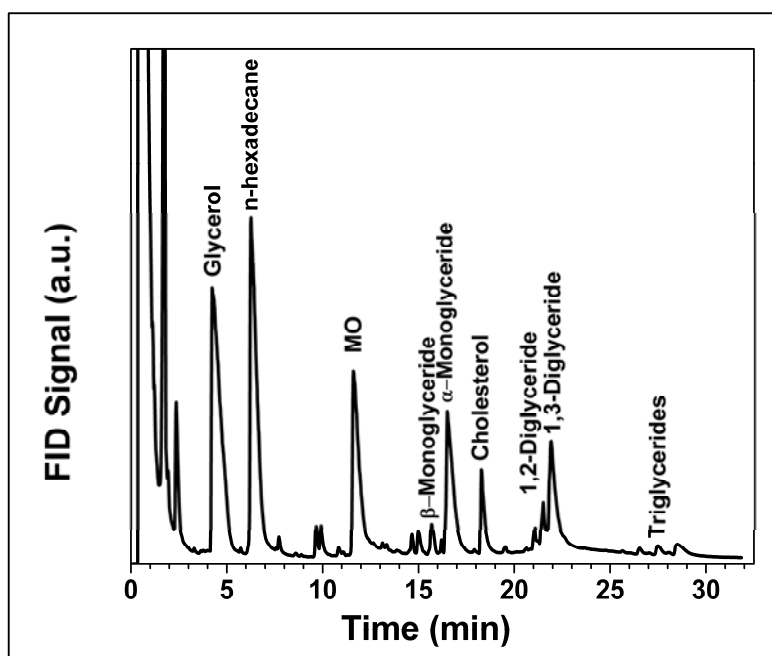

Figure 1. Typical chromatogram of a silylated sample using n-hexadecane and cholesterol as internal standards.

The Gly, MO, MG and DG retention times of the chromatographic peaks were referenced to the retention time corresponding to n-hexadecane, whereas TG peaks were referenced to the

cholesterol retention time. Therefore, relative retention times (RRT) were determined for each analyte. The lightest compounds, Gly and MO presented RRT values of 0.67 and 1.84, respectively, whereas for monoglycerides RRT of 2.51 and 2.63 were determined for the β- and α-MG isomers of CN=18, respectively, which correspond to the signals of monoolein, monolinolein and monolinolenin that only differ in the number of C=C double bonds. Diglycerides appear in a group of peaks corresponding to 1,2- and 1,3-isomers. Then, RRT of 3.46 and 3.52 were assigned respectively, to the 1,2- and 1,3-DG isomers with CN=36. Finally, triglycerides appear in a group of three peaks that when referenced to cholesterol presented a calculated RRT of 1.45, 1.50 and 1.55 that are attributed to TG with CN of 50 (from myristic acid methyl ester, CN = 14), 52 (from palmitic acid methyl ester, CN = 16) and 54 (from MO, CN = 18), respectively.

A calibration using the reference substances was carried out for quantitative determination of sample components. Standard solutions containing known amounts of Gly, MO, MG, DG and TG, and both internal standards at 5 concentration levels were analyzed by capillary GC. The resulting calibration curves were referenced to the respective internal standards. The obtained a and b values and the regression data are shown in Table 1. High correlation coefficient values ($r^2 \geq$ 0.992) for all the analytes indicate excellent linearity in a wide compositional range. The data used in the calibration were analyzed using lack of fit test to determine whether the linear model is suitable to describe the observed data. For all the compounds, the linear model satisfactory described the observed data.

Table 1. Calibration equation for Gly, MO, MG, DG and TG: $A_j/A_{IS} = a + b\,(W_j/W_{IS})$.

Compound	a $\left(\dfrac{\%Area_j}{\%Area_{IS}}\right)$	b $\left(\dfrac{\%Area_j.mg_{IS}}{\%Area_{IS}.mg_j}\right)$	Standard error, σ $\left(\dfrac{\%Area_j}{\%Area_{IS}}\right)$	r^2	Test range (mg/mL)
Gly	0.077 ± 0.009	1.851 ± 0.030	0.018	0.997	0.06-0.30
MO	0.015 ± 0.003	0.721 ± 0.007	0.007	0.999	0.08-0.50
MG	0.022 ± 0.003	0.540 ± 0.006	0.006	0.998	0.08-0.50
DG	-0.033 ± 0.001	0.473 ± 0.006	0.002	0.998	0.05-0.25
TG	-0.011 ± 0.026	0.107 ± 0.003	0.035	0.992	0.40-1.30

A_j: compound *j* peak area; A_{IS}: peak area of internal standard; W_j: compound *j* weight; W_{IS}: weight of internal standard; r: correlation coefficient.

The quantification limit (*QL*) is defined as the lowest analyte concentration that can be determined with accuracy and precision in a sample [13]. The quantification limit for component *j* was calculated from Eq. (1):

$$QL_j = 10\sigma_0 \qquad (1)$$

where σ_0 is the standard error predicted for a blank sample [14,15]. We determined the following *QL* values: Gly, 0.048 mg/mL; MO, 0.046 mg/mL; MG, 0.049 mg/mL; DG, 0.022 mg/mL; TG, 0.206 mg/mL.

The detection limit (*DL*) is defined as the lowest analyte concentration that can be identified in a sample, but not necessarily quantified, under the stated experimental conditions [13]. The detection limit for component *j* was calculated from Eq. (2):

$$DL_j = 3.3\sigma_0 \qquad (2)$$

The *DL* values were: Gly, 0.016 mg/mL; MO, 0.015 mg/mL; MG, 0.016 mg/mL; DG, 0.007 mg/mL; TG, 0.068 mg/mL.

These *QL* and *DL* values were analytically confirmed by properly diluting stock solutions of the corresponding compounds. The highest *QL* and *DL* value were determined, as expected, for TG taking into account that the triglycerides are molecules of high molecular weight and boiling point [16].

Sample quantification was performed through the calibration curves. The concentration of the MG isomers was determined by integration of the corresponding chromatographic peaks and using the MG calibration curve. The total MG concentration was calculated by addition of individual MG concentrations. DG and TG quantification was similarly achieved.

The method accuracy was evaluated from recovery (R) experiments using reference samples containing known amounts of standard substances. The recovery percentage for each analyte was calculated from Eq. (3):

$$R_j(\%) = \frac{\overline{x_{obs_j}}}{\overline{x_{ref_j}}} 100 \qquad (3)$$

where $\overline{x_{obs_j}}$ is the average concentration of compound j determined from GC analysis and the actual concentration of j in standard samples. For these experiments, the criterion for a suitable recovery was R values in the range of 90-107% [17]. The obtained R (%) values are given in Table 2. Small deviations were observed for MG at concentration levels below the quantification limit. The recovery values were tested statistically using the method of the averaged recovery [17], proving that the method is accurate.

The method precision was evaluated by consecutively analyzing a sample 6 times. Data of standard deviations and relative standard deviations (*RSD*) for Gly, MO, MG, DG and TG compounds in Table 3 show a good repeatability of quantitative results. The *RSD* values were in all the cases lower than 10 %, except for TG (*RSD* = 13.0 %).

Table 2. Recovery experiments for Gly, MO, MG, DG and TG using three reference samples.

Compound	Reference sample 1		Reference sample 2		Reference sample 3	
	Concentration (mg/mL)	Recovery (%)	Concentration (mg/mL)	Recovery (%)	Concentration (mg/mL)	Recovery (%)
Gly	0.000	-	0.350	100	0.350	105
MO	0.000	-	0.500	92	0.500	100
MG	0.004	125[a]	0.500	100	0.600	104
DG	0.300	103	0.200	99	0.400	102
TG	0.700	98	0.000	-	0.250	92

[a] MG concentration in Reference sample 1 was below the quantification limit.

Table 3. Method precision: quantitative results for Gly, MO, MG, DG, and TG obtained by repeated analysis (n = 6).

Sample	Concentration (weight %)				
	Gly	MO	MG	DG	TG
1	15.468	25.111	16.145	13.157	10.094
2	13.659	26.091	15.640	13.525	9.500
3	14.568	25.811	16.022	12.529	10.306
4	14.754	26.131	14.270	11.195	7.783
5	13.677	29.815	12.636	13.901	8.273
6	14.475	28.002	15.957	14.288	7.745
$\bar{x}$ [a]	14.434	26.827	15.112	13.099	8.950
SD [b]	0.688	1.750	1.395	1.113	1.113
RSD [c] (%)	4.8	6.5	9.2	8.5	13.0

[a] Average   [b] Standard deviation   [c] Relative standard deviation

Effect of solvent on sample quantification

Toluene and n-hexane were selected for diluting the silylated samples because all the sample components are extensively soluble in both solvents. To establish the solvent effect on sample quantification, a silylated sample was prepared and diluted with 5 mL of toluene (five samples) and 5 mL of n-hexane (other five samples). Results were analyzed statistically by comparing both the averages and the standard deviations, proving that the use of different solvents did not affect the sample quantification process.

Evaluation of homogeneous and heterogeneously catalyzed tests: Qualitative and quantitative analysis

The analytical method validation was performed to evaluate the synthesis of MG from Gly and MO by homogeneous and heterogeneous catalysis. In both cases the reaction system is complex because of the formation of different phases with compositions changing during the progress of the reaction. Three phases are formed when the reaction is carried out using liquid catalysts (homogeneous catalysis): i) a gaseous phase containing methanol that is highly volatile at the reaction temperature and is continuously eliminated from the reaction system using an inert gas stream; ii) two liquid phases containing Gly and MO, respectively. Gly is barely soluble in the MO phase and MO is insoluble in the Gly phase. The liquid catalyst, NaMeO, is distributed in both phases. Thus, the reaction takes place only in the MO phase where both reactants coexist. In the case of heterogeneous catalysis, the solid MgO catalyst is the fourth phase that is also distributed in both liquid phases.

Liquid samples withdrawn from the reactor during the homogeneous catalytic tests showed that the glycerol phase contained MO soaps, probably because the Na ions of the NaMeO catalyst react with MO and form products soluble in the Gly phase. In contrast, no soap formation was

observed during the heterogeneous catalytic tests. Analysis of the MO phase during the homogeneously catalyzed reaction showed formation of MG, DG and TG. Contrarily, no formation of TG was detected when the catalyst was MgO. In both catalytic tests, the Gly concentration in the MO phase was initially negligible but then increased with the reaction time. These results suggest that the solubility of Gly in the MO phase increases as the MG concentration in this phase increases with the progress of the reaction. This is explained by the surfactant properties of MG. Figure 2 shows typical chromatograms of MO phase samples obtained before the reaction (Figure. 2a) and during the homogeneously and heterogeneously catalyzed tests (Figures 2b and 2c, respectively).
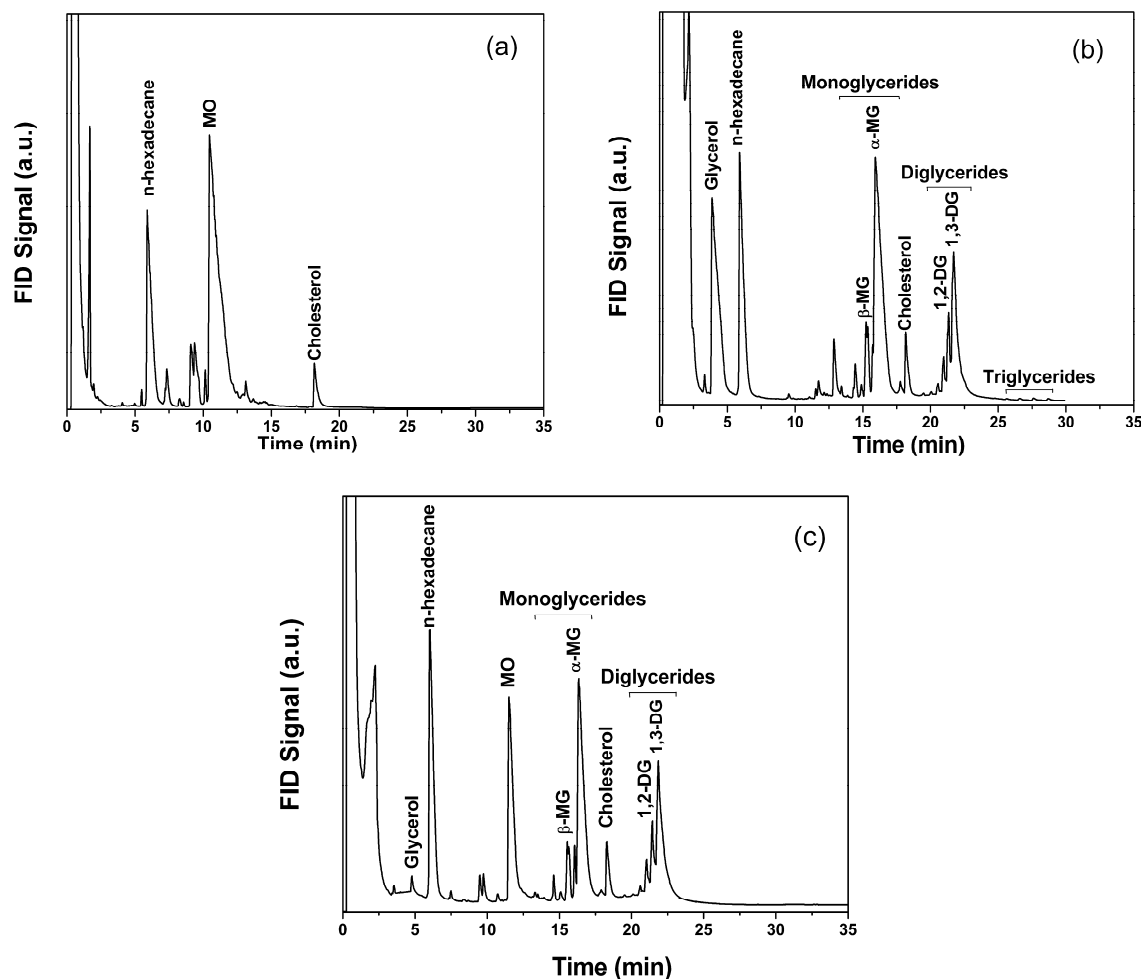

Figure 2. Typical chromatograms of the MO-rich phase samples withdrawn from the reactor: (a) before introducing the catalyst; (b) during the homogeneously-catalyzed reaction test; (c) during the heterogeneously-catalyzed reaction test.

Calibration curves for all the reaction compounds were created to determine the relative response factors that were used to quantify the evolution of reactants and products during the reaction and to calculate the MO conversion (X_{MO}) as well as the yields to MG, DG and TG (Y_{MG}, Y_{DG} and Y_{TG}).

The relative response factors were determined with Eq. (4):

$$f_j = (W_{IS}/W_j).(A_j/A_{IS}) \qquad (4)$$

The concentration values resulting from Eq. (4) were plotted as a function of the concentration values predicted by the calibration equations of Table 1 and a statistical correlation analysis by linear regression was carried out. Then, the fj values were adjusted to achieve 95 % confidence for the slope [18].

The reaction pathways under homogeneous and heterogeneous catalyzed conditions can be elucidated from Figure 3. Figure 3a shows that in the homogeneously catalyzed test the MG yield (contribution of the α- and β- isomers) rapidly increased and then reached a maximum, thereby indicating that MG was converted to secondary products. The evolution of the yield curves in Figure 3a suggests that MG reacts with MO to consecutively form DG (1,2- and 1,3- isomers) and TG, as it is illustrated in Scheme 1. After 5 h of reaction, MO conversion reached 100 % and the yields to MG, DG and TG were 59 %, 24 % and 17 %, respectively. Then, the MG yield decreased in spite of that MO was completely converted, probably because MG is slowly transformed to higher glycerides and Gly via disproportion reactions.
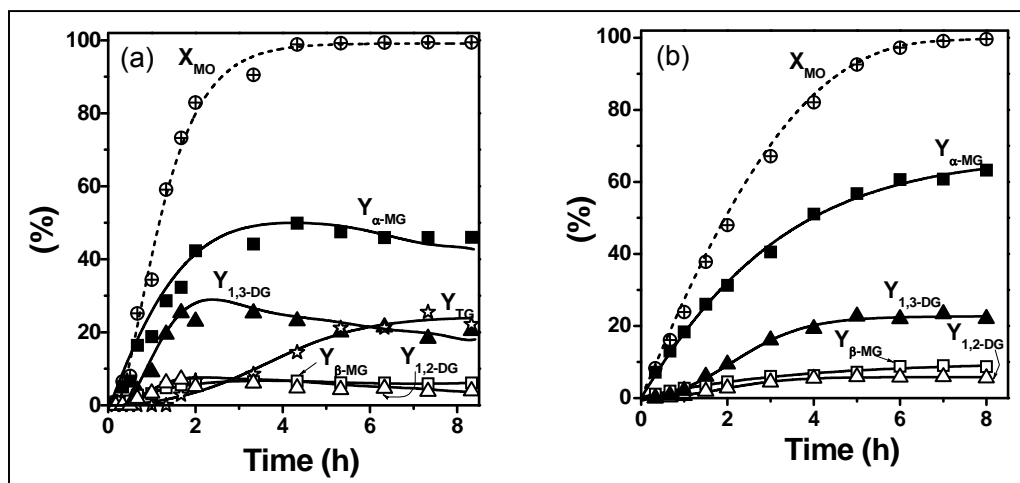

Figure 3. MO conversion and glyceride yields. (a) Catalyst: Na methoxide, T = 135°C, Gly/MO = 2; (b) Catalyst: MgO, T = 220°C, Gly/MO = 2.

Figure 3b shows the results obtained when the catalyst was MgO. The MG isomers were the only primary products of the Gly/MO glycerolysis, similarly to the results observed in the homogeneously catalyzed run in Figure 3a. After that, MG isomers were slowly converted to DG. MO was completely converted at the end of the 8-h catalytic run, yielding 73 % MG and 27% DG. MgO did not form TG and was therefore more selective for MG synthesis than the NaMeO catalyst used in the homogenously catalyzed reaction. In all the cases, α-glyceryl monooleate and 1,3-glyceryl dioleate were the main MG and DG isomers, respectively (Scheme 1).
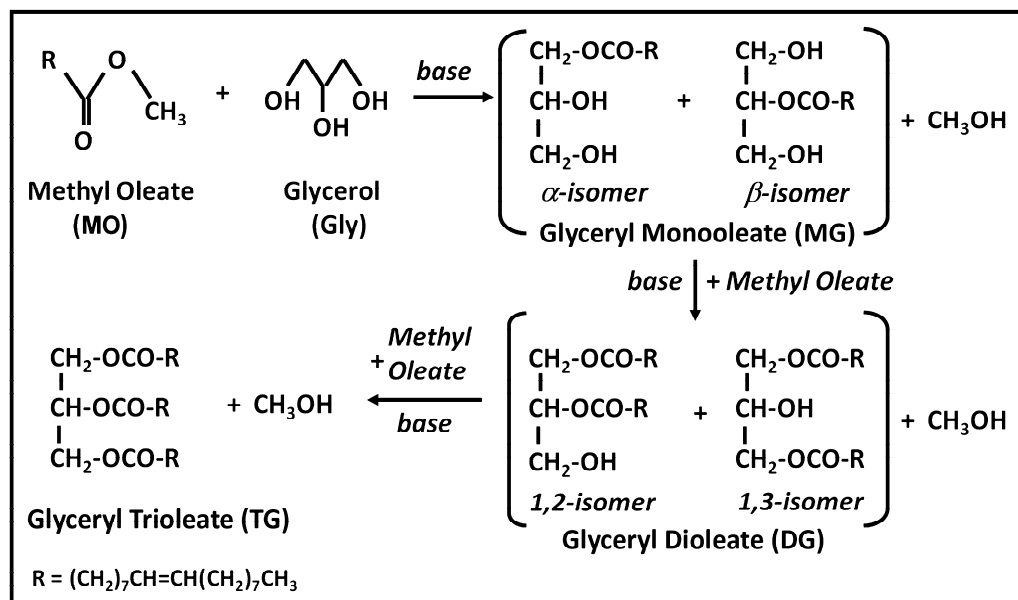
Scheme 1. Monoglyceride synthesis via transesterification (glycerolysis) of methyl oleate (MO) with glycerol (Gly) and consecutive reactions to diglycerides and triglycerides.

Product formation rates might be easily calculated from Figure 3 as the derivative of yield versus time curves and therefore not only reaction pathways but also valuable kinetic information can be obtained from these experiments.

Conclusions

A rapid, accurate and efficient analytical method is proposed and validated for the simultaneous determination of glycerol, methyl oleate, glyceryl monooleates (α and β isomers), glyceryl dioleates (1,2- and 1,3-isomers), and glyceryl trioleate by GC to follow the reaction product evolution during the monoglyceride synthesis by glycerolysis of methyl oleate.

The method affords complete qualitative and quantitative information about the reaction. Therefore, the complex reaction network involving not only formation of monoglycerides but also consecutive reaction pathways leading to diglycerides and triglycerides can be monitored as a function of time in a wide compositional range.

This reliable single method can be used either under homogeneously or heterogeneously catalyzed conditions to determine reaction pathways and kinetic parameters.

References

[1] G.W. Huber, S. Iborra, A. Corma, *Chem. Rev.*, **2006**, *106*, 4044.

[2] A. Corma, S. B. A. Hamid, S. Iborra, A. Velty, *J. Catal.*, **2005**, *234*, 340.

[3] Y. Zheng, X. Chen, Y. Shen, *Chem. Rev.*, **2008**, *108*, 5253.

[4] A. Corma, S. Iborra, S. Miquel, J. Primo, *J. Catal.,* **1998,** *173,* 315.

[5] P. Fagan, C. Wijesundera, P. Watkins, *J. Chromatogr. A,* **2004***, 1054*, 251.

[6] M. Holcapek, P. Jandera, J. Fischer, B. Prokes, *J. Chromatogr. A*, **1999**, *858*, 13.

[7] B. Freedman, E. H. Pryde, T. L. Mounts, *J. Am. Oil Chem. Soc.,* **1986**, *61*, 1638.

[8] B. Freedman, W. F. Kwolek, E. H. Pryde, *J. Am. Oil Chem. Soc.,* **1986**, *63*, 1370.

[9] C. Plank, E. Lorbeer, *J. Chromatogr. A*, **1995**, *697*, 461.

[10] W. Christie, Lipid Analysis, 3rd ed., The Oily Press, Bridgewater, England, 2003.

[11] J. I. Di Cosimo, V. Díez, C. R. Apesteguía, *Appl. Catal. A: General*, **1996**, *137*, 149.

[12] *United States Pharmacopeia*, *32-NF27*, 908, 2008.

[13] Q2 (R1), *Validation of analytical procedures: text and methodology,* International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Chicago, USA, 2005.

[14] L.A. Currie, *Anal. Chim. Acta*, **1999**, *391*, 127.

[15] K. Danzer, L.A. Currie, *Pure & Appl. Chem.*,**1998**, *70*, 993.

[16] A. S. Pereira, F. R. Aquino Neto, *Trends Anal. Chem.*, **1999**, *18*, 126.

[17] A. G. González, M. A. Herrador, A. G. Asuero, *Talanta*, **1999**, *48*, 729.

[18] J. C. Miller, J. N. Miller, Statistics for Analytical Chemistry, 2nd ed., Ellis Norwood, England, 1988.