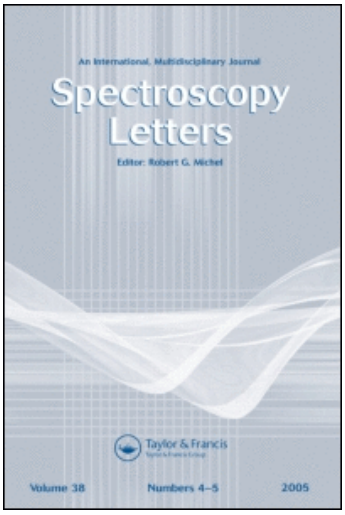

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Use of Multivariate Curve Resolution to Monitor an Esterification Reaction by Near-Infrared Spectroscopy

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Abstract: Multivariate curve resolution–alternating least squares (MCR-ALS) methodology was applied to near-infrared (NIR) spectra with view to estimating the reaction profile for the esterification of a mixture of caprylic and capric acids with glycerol. The reaction was conducted in excess glycerol, so the major products were the monoglyceride and diglyceride; the triglyceride was obtained in considerably lower proportions. Esterification processes were performed in a laboratory-scale reactor from which samples were withdrawn to record NIR spectra. Some samples were also analyzed by gas chromatography and acid-base titration in order to determine the composition of the reaction mixture. Concentration and spectral profiles were obtained by using the MCR-ALS algorithm. Subsequently, concentration values and the pure spectra of reagents and the triglyceride were used to refine the models. The spectra obtained were processed with MCR-ALS in new esterification batches to obtain their concentration profiles.

Keywords: Esterification reaction, MCR-ALS, NIR spectroscopy, reaction profiles

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INTRODUCTION

The esterification between glycerol and middle-chain fatty acids represents a reaction of high industrial interest for the production of esters used by the food, cosmetic, and pharmaceutical industries. The reaction starts with the formation of a monoglyceride that is further esterified to a diglyceride and then to a triglyceride; the process involves simultaneous transesterifications. The industrial process can be conducted by using variable initial proportions of the raw materials; the presence of excess acid leads preferentially to the triglyceride, whereas that of excess glycerol gives rise to the mono- and diglyceride as major products and the triglyceride in lower proportions. The process studied in this work was of the latter type: the use of excess glycerol and a mixture of caprylic and capric acids yielded mono-, di-, and triglycerides in addition to residual (excess) glycerol. The monoglyceride was a mixture of esters between glycerol and both acids at positions 1 or 2 (i.e., four different compounds) in variable proportions depending on the relative concentrations of the two acids. The diglyceride consisted of a mixture of glycerol esters at positions 1,1 or 1,2 of caprylic and/or capric acid (7 compounds in all) also in variable proportions. The monoglyceride was taken to be a component consisting of several (1 or 2 caprylic or capric) esters, and so was the diglyceride. In this work, multivariate curve resolution (MCR)[1] methodology was used to extract relevant information about the temporal evolution of the reaction products with a view to identifying the time at which the process should be stopped in order to obtain the desired ester mixture composition depending on its intended use.

As noted earlier, the fact that the chemical system involves a number of reacting chemical species makes it rather complex. Obtaining a single mixture of the mono- or diglyceride, or a sample consisting solely of monoglyceride (or diglyceride) in the same isomer ratio, is obviously impossible. This hinders the obtainment of the spectra for the two components, which would be highly useful with a view to calculating the concentration profiles by Multivariate curve resolution–alternating least squares (MCR-ALS). The high correlation between the NIR spectra for the compounds formed in the course of the reaction, the rank deficiency of the spectral data matrix, and rotational and intensity ambiguities restrict the applicability of MCR-ALS here. In the absence of noise and other variability sources such as temperature and the acid concentration ratio, the rank of the spectral data matrix should coincide with the number of chemical species involved.[2] This assumption fails with the studied system, where two of the reaction products (the mono-and diglycerides) exhibit a high spectral correlation and similar concentration profiles. The rank of the matrix for this system is lower than the number of components involved in the reaction. This problem was circumvented by the following methods: augmented matrices,[3] the NIR spectra available for the pure components, and the concentrations provided by reference methods as MCR-ALS constraints. Whereas the first methodology gave poor results, the latter two

provided useful spectral information for the compounds involved in the process. This methodology allowed us to estimate the near-infrared (NIR) spectra for the mono- and diglyceride. Such spectra were in turn used to determine the concentration profiles for the components involved in new reaction batches.

THEORY

Multivariate curve resolution (MCR) methodology assumes the data fit a linear pattern conforming to Beer's law, based on the following expression (1):

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{\mathbf{T}} + \mathbf{E} \tag{1}$$

where $\mathbf{D}$ ($I \times J$) is the experimental data matrix, the rows of which represent spectra in a time sequence; each element d_{ij} in $\mathbf{D}$ is corresponds to the absorbance at wavelength j for the sample withdrawn at time i. $\mathbf{C}$ ($I \times N$) contains the concentration profiles for the absorbing species involved in the reaction; each element c_{in} represents the concentration of analyte n in the sample at time i. $\mathbf{S}^{\mathbf{T}}$ ($N \times J$) contains the spectra for the absorbing species involved in the reaction; each element s_{nj} in it corresponds to the absorbance of analyte n at wavelength j. $\mathbf{E}$ ($I \times J$) is the residuals matrix, which contains all information not explained by matrices $\mathbf{C}$ and $\mathbf{S}^{\mathbf{T}}$, and is included in $\mathbf{D}$. When the linear model is obeyed, d_{ij} is the combined absorbance at wavelength j of the N species present in sample i:

$$d_{ij} = \sum_{n} c_{in} s_{nj} + e_{ij} \tag{2}$$

The equation system (1) can be solved by iterative process of the ALS (alternating least squares) algorithm. Although the system has no unique solution, only a few chemically acceptable solutions exist where $\mathbf{S}^{\mathbf{T}}$ corresponds to the spectra for the analytes and $\mathbf{C}$ to their concentration profiles.

MCR-ALS methodology is subject to two types of ambiguity, *viz.* intensity ambiguity,[4] which means that the estimated profiles may be scaled by some unknown factor, and rotational ambiguity,[4] which means that the estimated spectrum for any of the components may be an unknown linear combination of the real components. The introduction of the iterative process of the spectra for absorbing species involved in the reaction helps suppress rotational ambiguity[4,5] arising from the absence of selective spectral regions—a frequent occurrence in NIR spectroscopy.[6] The introduction of quantitative values in the iterations provides accurately scaled concentration profiles and suppresses intensity ambiguity.[4,5] A set of feasible solutions can be obtained by using alternating least squares with a series of constraints including unimodality (when the concentration profiles possess a single maximum value), closure (when the sum of all selected components

should equal a constant value) and spectral and concentration non-negativity (when neither the concentrations nor the spectra can take negative values).

The procedure used to solve Eq. (1) therefore started with the determination of the number of components present in **D** and with an estimation of the $\mathbf{S^T}$ or **C** matrix. There are different tools to estimate the number of components in a mixture, such as PCA, EFA, FSMW-EFA, or singular value decomposition (SVD). These techniques are widely commented in the references.[4,7–9] EFA is also a useful technique in order to calculate the initial estimates of matrices **C** or $\mathbf{S}^{\mathrm{T}}$ necessary to start the iteration process. The use of a matrix **D** containing the spectra for more than one process, an augmented data matrix (5), can help solve the problem posed by a rank deficiency.[3] An $\mathbf{S^T}$—or the spectra for the pure components, if available—or **C** initial estimate is used to start the iterative process in order to calculate new **C** and $\mathbf{S^T}$ estimates in each cycle, and simultaneously, the above-described constraints being applied in accordance with the chemical information available. Iterations where stopped when matrix **E** was minimized, that is, when the difference between the root mean squared error (RMS) between two consecutive iterations fell below a preset threshold.

$$RMS = \sqrt{\frac{\sum_i \sum_j e_{ij}^2}{n}} \tag{3}$$

The goodness of fit of the model thus obtained was assessed in terms of the percent-calculated variance:

$$\%Var = \frac{\sum_i \sum_j \hat{d}_{ij}^2}{\sum_i \sum_j d_{ij}^2} \times 100 \tag{4}$$

Finally, the quality of the MCR-ALS estimated spectra was evaluated from the similarity criterion:

$$r = \cos\gamma = \frac{S_i^T \hat{S}_i}{\|S_i\| \cdot \|\hat{S}_i\|} \tag{5}$$

EXPERIMENTAL

Apparatus and Software

Esterification processes were conducted in a 1 liter LabMax laboratory reactor from Mettler Toledo.

NIR transflectance spectra were recorded on a FOSS NIRSystems 6500 spectrophotometer equipped with a Rapid Content Analyzer (RCA) module. The instrument was controlled via the Vision v. 2.51 software package.

A Hewlett-Packard HP 5890 Series II gas chromatograph equipped with a flame ionization detector and furnished with a Supelco SPB-1701 fused silica capillary column (15 m × 0.25 mm, 0.25 μm film thickness) was used.

Multivariate curve resolution was used in conjunction with alternating least squares with penalty function, using the software GUIPRO (10) as implemented in Matlab.[11]

Reagents and Samples

Samples were collected from esterification batches containing glycerol and a mixture of caprylic and capric acids in a 95:5 ratio. A glycerol excess of 35% with respect to the amount theoretically required to obtain the monoglyceride was used. The reaction mixture was heated to 220 ± 5°C, which was held for about 6 hr, under a continuous stream of nitrogen. A distillate consisting of water and small amounts of acid was produced during the reaction. Samples from 3 batches, named as A, B, and C, were withdrawn from the reactor throughout the process after the first 40 min for the batches A and B and 80 min for batch C—the time needed for the reaction mixture to become homogeneous. The number of samples in each batch is 15 for batch A, 16 for batch B, and 13 for C.

NIR Spectra

NIR spectra for the reaction mixtures and pure products available were recorded in the transflectance mode at room temperature, using a quartz vessel and a gold reflector of 0.5-mm pathlength, which leads to absorbance units between 0 and 1.8 (inside the linear absorbance response of the instrument). Each individual spectrum was the average of 32 scans done at 2 nm intervals over the wavelength range 1100–2500 nm. Figure 1 shows the spectra for the compounds available (viz. glycerol, the acid mixture and the triglyceride).

Reference Methods

The samples withdrawn from one of the laboratory esterification batches were analyzed by using reference methods. The acid content was determined by titration with ethanolic KOH, using a 1:1 ethanol/xylene mixture as solvent.[12] The glycerol and glyceride contents were determined by gas chromatography, using methyl palmitate as internal standard and a temperature ramp of 7°C/min from 120°C to 270°C. The percent content in each compound was obtained by interpolation into a calibration plot of analyte-to-internal standard peak area as corrected for the response factor of each analyte. The response factors for the analytes available in pure form were determined by calibration from standard solutions; those analytes for which no standard was available (viz. caprylic–capric mixed glycerides) were

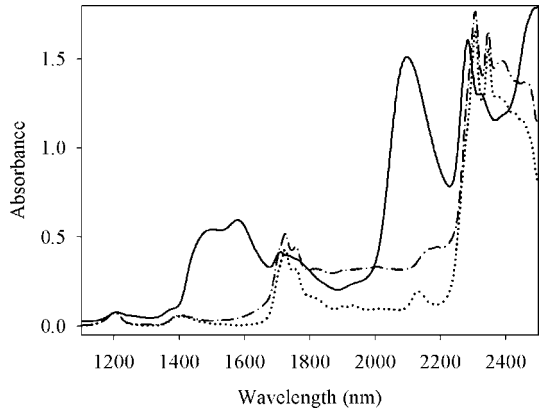

Figure 1. NIR spectra for glycerol (—), the mixture of caprylic and capric acids (- · -), and the triglyceride (···).

quantified by interpolation of their effective carbon number into the straight line obtained by plotting the response factors against the effective number of carbon atoms for the species available in pure form.[13]

Data Processing

Spectra data were subjected to the standard normal variate (SNV) treatment in order to suppress potential baseline shifts or scattering caused by some turbid samples. The regions of negative absorbance provided by the SNV treatment were removed by adding a scalar to all spectra in order to eliminate negative spectral values and facilitate application of the spectral non-negativity constraint in the MCR-ALS calculations.

Experimental matrices were subjected to rank analysis using singular value decomposition (SVD) methodology; both singular values and the profile of the corresponding eigenvectors were considered in determining matrix ranks.

Use of MCR-ALS to Calculate Spectra

In this work, MCR-ALS methodology was used to obtain the estimated spectra for those analytes whose experimental spectra could not be recorded owing to the unavailability of the products in pure form. This entailed using the concentrations of the analytes involved in the reaction as determined with a reference method, as well as the NIR spectra for the analytes where available (e.g., those for glycerol, the mixture of caprylic and capric acids, and the triglyceride). This information was used as equality constraints[10] in every iteration by the methodology included in the software GUIPRO. The initial estimates,

prior to the iteration process by ALS, were constructed by the needle search methodology[10,14,15] implemented in the software GUIPRO.

MCR-ALS calculations were based on the concentrations for five sample components as obtained with reference methods and the recorded NIR spectra for glycerol, the acid mixture and the triglyceride. The estimated spectra were subsequently used to monitor changes in the reacting species in new esterification batches. The use of pure spectra as initial estimates or as equality constraints in calculating the concentration profiles by MCR-ALS suppresses rotational ambiguity, which in NIR spectroscopy is increased by the low spectral selectivity of the analytes present in the studied samples. The flowchart of Fig. 2 shows the different steps of the application of ALS in this study.

RESULTS AND DISCUSSION

Figure 3 shows the variation of the NIR spectra (subjected to math treatments commented in Section 3.5) during the course of the reaction. The largest spectral difference was the absorption decrease in the 2000–2200 nm region, which corresponded to the O–H bond in glycerol. This band did not disappear completely owing to the presence of excess glycerol and the absorption of O–H bonds in the mono- and diglycerides. The variation of the absorbance at 1200 nm is due to the SNV spectral pretreatment, which increases the small differences in this region.

Application of MCR-ALS to the Body of Spectral Data

The SVD calculated mathematical rank for the matrices representing the different studied batches was 2, as was that for the augmented matrix including the spectra for the three processes. The augmentation is done in a column-wise methodology,[7] which is the most appropriate method to follow the evolution of the reaction. This rank was confirmed by the eigenvectors of the SVD: while those for the first and second singular values evolved similarly in the three processes, that for the third value exhibited no clear-cut, similar trend in any process. The SVD calculations for the augmented matrix in a column-wise methodology exhibited an identical behavior.

Although the number of factors by rank analysis of the data matrix is calculated to be 2, the chemical system studied theoretically consists of 6 components, namely: glycerol, the mixture of caprylic and capric acids —which was taken to be a single component—the mono-, di-, and triglyceride, and water. Water is present in low proportions owing to the high reaction temperature used, the constant agitation and the continuous stream of nitrogen, which caused its distillation as it is produced during esterification. Also, the triglyceride is a minor component owing to the use of excess glycerol. Therefore, the chemical system is assumed to consist of four compounds only. Mono- and

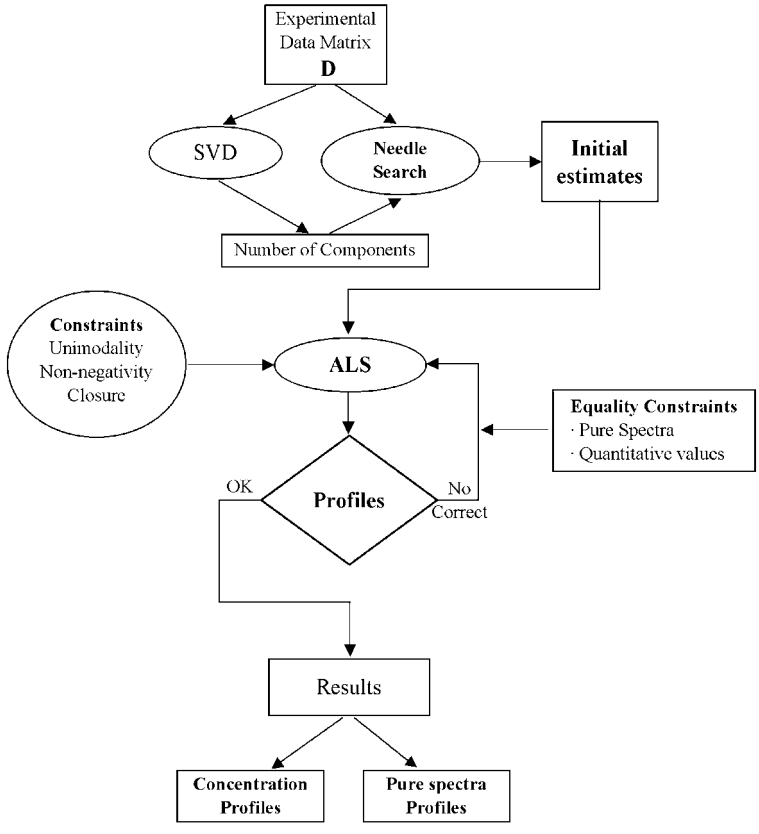

Figure 2. The flowchart shows the different steps of the application of ALS in this study.

diglycerides are chemically highly similar and exhibit very similar NIR spectra as a result. This is probably why the rank of the data matrix is much lower than the number of compounds involved. Although the esterification process involves 6 different compounds, the actual number of spectral components is 3 (viz. glycerol, the acid mixture and the glycerides), but the experimental data are consistent with a rank of only 2.

The augmentation matrix methodology did not improve the rank analysis of the data set. Therefore, we worked with a single matrix **D** corresponding to process A. Application of the MCR-ALS algorithm in combination with non-negativity and unimodality constraints for two components (coinciding with the mathematical rank of the matrix), provided the spectral and concentration profiles which afford no clear-cut conclusions: a comparison with the data matrix reveals that spectrum of component 1 coincides with the last spectrum in matrix **D** and the opposite to component 2. This analysis of

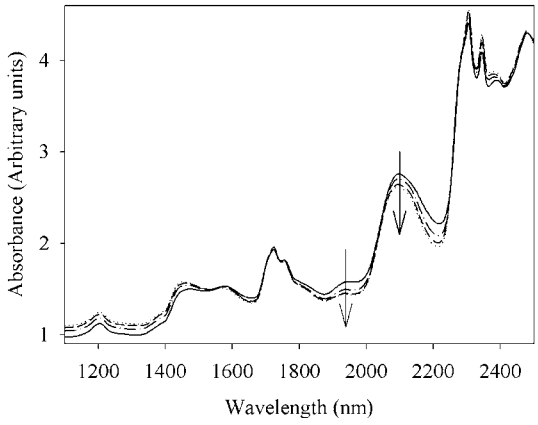

Figure 3. NIR spectra for the reaction products. The arrowheads indicate the temporal evolution of the spectra at 40 (—), 80 (- · -), 120 (--), and 340 min (· · ·).

matrix **D** gives poor results due to the complexity of the system studied. To obtain good results the number of components needs to be near to the number of absorbing species involved in the reaction.

The analysis of matrix **D** under the assumption of 3 components resolves the second component into two; this is reflected in the fact that two of the estimated components exhibited highly similar spectral profiles. This precluded the use of a number of components exceeding the rank of the data matrix and hence resolving rotational ambiguity.

However, if the experimental data matrix is expanded in the iteration process with the spectral information available (viz. the recorded spectra for the reagents) as equality constraints, the resulting profiles exhibit a better-defined, more consistent pattern than the previous calculations. Thus, the use of MCR-ALS methodology with three components, unimodality and concentration and spectral non-negativity constraints, and the recorded NIR spectra for glycerol and the acid mixture as initial estimates in the ALS iterations, provided the profiles shown in Fig. 4. These profiles are more consistent with our knowledge [16] of the system. Thus, compound 1 clearly coincides with the acid as its curve falls to virtually zero—the reactant is in a substoichiometric proportion. Also, component 2 coincides with glycerol as its curve initially falls and then levels off at about 30%—which is typical of an excess reactant. Finally, the third component coincides with the glycerides, which are used as a whole in the MCR-ALS calculations on account of their spectral similarity. As can be seen, the inclusion of external information (viz. the spectra for the pure reactants) allows a system with an inadequate rank to be resolved and concentration profiles consistent with available knowledge about the system to be obtained. In fact, the incorporation of the spectra for the pure reactants resolves rotational ambiguity between them,

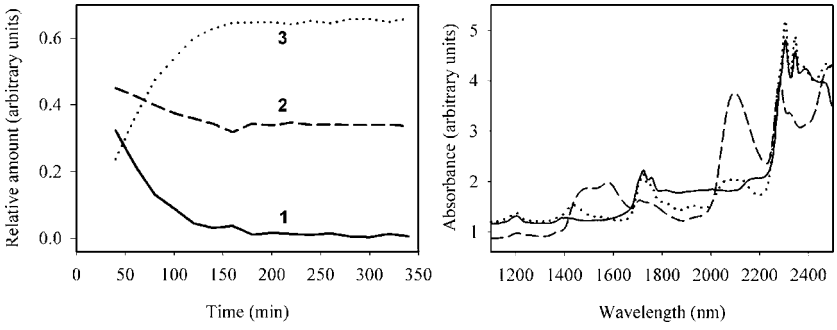

Figure 4. Concentration (left) and spectral profiles (right) obtained by using MCR-ALS with three components and the spectra for the pure components. The solid line corresponds to the acid mixture, the dashed line to glycerol, and the dotted line to glycerides. Variance captured: 99.99%.

which was one of the problems posed by the studied system. This fact reduces the correlations that decreased the rank of the experimental matrix and allowed glycerol to be accurately discriminated from the acid mixture. The captured variance for this calculation is 99.99% and the correlation coefficient between the estimated and original spectra is greater than 0.999 for both glycerol and the acid mixture.

However, the spectral information included as equality constraints in the iterative process was insufficient to solve a system of **4** components; in fact, the concentration profiles were inconsistent with available knowledge (16) about the reaction. Intensity ambiguity was poorly resolved, so, we considered the use of external quantitative values in order to obtain accurately scaled concentration profiles and hence the best approximation to the actual spectral profiles. Thus, the MCR-ALS model was expanded with the concentration values for batch samples that were previously analysed using reference methods (viz. gas chromatography and titration). Five different components were considered, namely glycerol, the acid mixture, and the three types of glyceride (mono-, di-, and tri-). Also, the recorded spectra for glycerol, the acid mixture and the triglyceride, as well as the corresponding concentrations were used as equality constraints. The only parameters not included were the spectra for the mono- and diglyceride, which must be estimated precisely by MCR-ALS. The concentration and spectral profiles thus obtained are shown in Fig. 5.

The estimated spectra profiles for the mono- and diglyceride are very similar; the strongest difference is in the 2000–2200 nm region, where the monoglyceride exhibited a higher absorbance on account of its greater number of O–H groups per molecule. The coefficients of correlation between the estimated and recorded spectra are higher than 0.999 for glycerol, the acid mixture, and the triglyceride. The estimated concentration

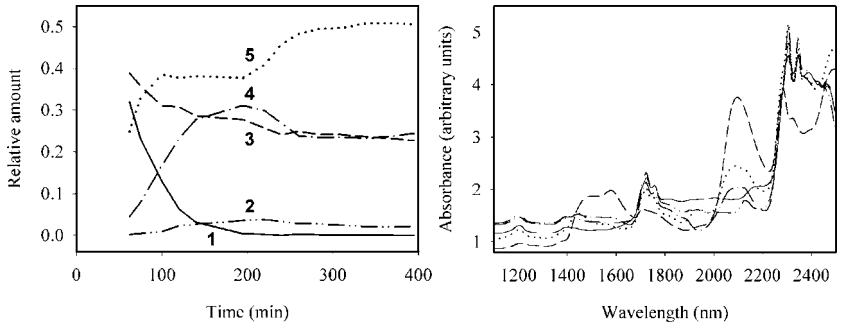

Figure 5. Concentration (left) and spectral profiles (right) obtained by using MCR-ALS with 5 components and the spectra for the pure reactants. (—) Acid mixture, (--) glycerol, (· · ·) monoglyceride, (- · -) diglyceride, and (- · · ·) triglyceride. Variance captured: 99.98%.

profiles have the same as the profiles from the reference methods. After 200 min, there is equilibrium between glycerides where transesterification reactions may occur. There is easy to see that after 200 min, the concentration profiles of diglyceride, triglyceride, and glycerol fall and the profile of monoglyceride increases. At that period of time, the fatty acid has been totally consumed, so, in principle, reaction could be finished at 200 minutes. Actually, glycerol reacts with diglyceride and triglyceride to form monoglyceride. This is an important step in this industrial process.

The spectra for the mono- and diglyceride in Fig. 5 were used to estimate the corresponding concentration profiles by applying MCR-ALS to new esterification batches (B and C); only the recorded spectra for glycerol and fatty acids, and the estimated spectra for the mono- and diglyceride, in conjunction with the usual non-negativity and unimodality constraints, were used. The profiles thus obtained are shown in Fig. 6.

The concentration profiles are consistent with the expected result for both the reactants and products. Thus, the acid concentration decreases to a residual level after about 150–200 min. The glycerol content levels off at 25% over the same period. The monoglyceride content is high from the start in both processes because the sampling was done 40 min after the beginning of the reaction for the process B and approximately 80 min for C. On the other hand, the diglyceride content increases in a rapid manner similarly as in Fig. 5. The concentration profiles of the two processes are similar because they have been carried out in similar experimental conditions. Not only are the concentration profiles consistent; the concentration scale agrees with the results obtained by titration and gas chromatography.

Therefore, the use of estimated and recorded spectra as initial estimates in the ALS iterative process allows one to quantify the target analytes in the esterification reaction between glycerol and a mixture of caprylic and capric acids.

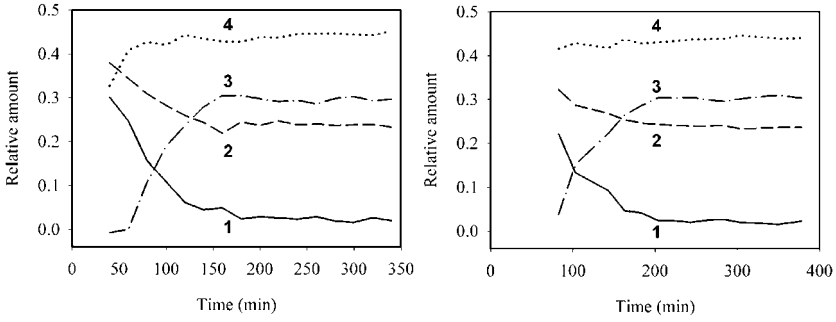

Figure 6. Concentration profiles for batches B (left) and C (right) as obtained by using MCR-ALS methodology with four components and spectral information only. (—) Acid mixture, (--) glycerol, (· · ·) monoglyceride, and (- · -) diglyceride.

CONCLUSIONS

The application of MCR-ALS methodology to a complex chemical system provides information about the course of the reaction; however, the high correlation between the spectra for the products and the inadequate rank of the experimental data matrix considerably restrict the quality of the spectral and quantitative information thus obtained. The incorporation of spectral information (viz. the spectra for the pure reactants) helps resolve rotational ambiguity, but not intensity ambiguity. The inclusion of quantitative information (concentrations) for the components of the chemical system completely resolves both. The spectral profiles thus obtained allow the concentration profiles for components in new esterification batches to be accurately estimated.

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