A Comparison of Attenuated Total Reflectance-FTIR Spectroscopy and GPC for Monitoring Biodiesel Production

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ABSTRACT: Gel permeation chromatography (GPC) and attenuated total reflectance (ATR)-FTIR spectroscopy were used to monitor the products of transesterification of waste frying oil in methanol to FAME or biodiesel. To evaluate the reliability and reproducibility of each method, quantitative analyses of mixtures of standards (TG, DG, MG, FAME, and glycerol) as well as lipid products of transesterification were carried out. The reproducibility of each method was found to be within ±1–5%. The differences between the results of the two methods were less than ±2%. The GPC method showed good separation of the intermediate products MG and glycerol from the TG starting material and FAME, but DG were not completely separated from TG. GPC gave good quantitative results for MG and FAME, but the TG and DG analyses required correction, depending on the mole ratio of TG/DG. In contrast, ATR-FTIR spectroscopy could only give quantitative data for the sum of TG + DG + MG.

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KEY WORDS: Acid-catalyzed transesterification, ATR-FTIR spectroscopy, biodiesel production monitoring, FAME, GPC, waste frying oil.

Measurement of the conversion of TG to FAME (biodiesel) is required for monitoring and control of biodiesel production as well as for fundamental studies of the reaction kinetics. The products of transesterification may contain DG and MG as intermediates as well as glycerol and FAME as final products. Thus, it is important to be able to measure the yield of biodiesel accurately and to identify and quantify the various reaction components and by-products during the course of the reaction and to analyze the final FAME product for the presence of TG, DG, MG, and glycerol. It is also desirable that the analytical method be quick and easy to use.

Many analytical methods have been used for the study of biodiesel transesterification (1). Among these, GC and LC-GC have been most widely used because of their accuracy in quantifying not only TG and FAME but also the intermediate reaction products, DG and MG (2,3). However, GC requires sample derivatization, which is time-consuming. TLC and NMR spectroscopy also have been used but have tended to be less reliable for quantitative purposes and require a somewhat lengthy analysis time (4,5). HPLC provides excellent quantitative results but also requires lengthy sample preparation and analysis time (6,7). GPC, however, provides excellent quantitative data but requires less analysis time (8). NIR spectroscopy has been used to measure conversion of TG to FAME, but the reaction intermediates could not be monitored (5).

Recent advances in IR spectroscopy and signal transmission have resulted in improved monitoring devices. Attenuated total reflectance (ATR)-FTIR spectroscopy is considered to be an attractive analytical method primarily because of its broad applicability to monitoring chemical reactions and identifying a variety of organic compounds, robustness to industrial environments, ease of use, and rapid data acquisition capabilities. Absorption bands in the mid-IR range of 4000 to 1500 cm^{-1} are typically due to the presence of functional groups (e.g., –OH, C=O, N–H, CH_3). Although ATR-FTIR has been used for the determination of FFA in vegetable oils, it has not been used to analyze TG, DG, and MG individually because of the similarity of their spectra (9). However, GPC is well able to analyze each of these intermediates, as well as FAME, individually (8).

The objective of this study was to compare and assess the suitability of ATR-FTIR spectroscopy and GPC for accurate and reproducible analysis of the intermediates and products of the transesterification of TG to FAME from waste frying oil. These methods are applicable to both the acid- and base-catalyzed transesterification products of waste and virgin vegetable oils.

EXPERIMENTAL PROCEDURES

Reaction conditions. Methanol (reagent grade; ACP Chemicals Inc., St-Léonard, Québec, Canada), sulfuric acid (ACS grade; BDH Chemicals Inc., Toronto, Ontario, Canada), and waste canola oil (Sam's University Tavern, Ottawa, Canada) were used in the experiments. The FFA content of the waste oil was determined by GPC to be ~6 wt%. The oil was used directly in the transesterification. Nine different feed compositions [methanol/sulfuric acid/oil mole ratios: 98.1:1.5:0.4; 95.6:3.5:0.9; 94.6:3.5:1.9; 96.5:1.5:2.0; 96.9:2.5:0.6; 95.1:1.5:3.4; 95.6:2.5:1.9; 97.3:1.5:1.2; 96.2:2.5:1.3 (replicated)] were carried out at 70 and 80°C for a total of 20 runs. All of the experiments and the sample analyses were carried out in random order to minimize any potential bias. Experiments were conducted in a jacketed 5-L stainless steel reactor equipped with a two-plate turbine impeller set to a mixing

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speed of 400 rpm. The reactor was equipped with a reflux condenser, sampling port, thermocouple, and temperature controller. Pressure was allowed to vary for the 70°C runs from 170 to 308 kPa and for the 80°C runs from 267 to 294 kPa. Each experiment was conducted for 4 h.

For each experiment, ten 5-mL samples were taken over the course of the reaction and to each was added 1 mL of distilled water, then 5 mL of petroleum ether. After gentle mixing and settling, the upper petroleum ether layer was removed and washed with 1–2 mL of a methanol/distilled water mixture (90:10, vol/vol). The upper layer of the subsequent mixture was removed, the solvents were evaporated, and the samples, which were free of glycerol and acid, were finally dried in a desiccator *in vacuo*. All samples were analyzed by both GPC and ATR-FTIR spectroscopy.

GPC analysis. The GPC method of Darnoko *et al.* (8) was used, with some modifications, for simultaneous analysis of the transesterification products. A Waters Corp. (Mississauga, Ontario, Canada) GPC system consisting of an HPLC pump, a controller, a differential refractive index detector, and Waters Millennium 32^{TM} software was used for the analyses. Two 300 × 7.5 mm columns of 3 µm diameter and 100-Å pore size Phenogel (Phenomenex, Torrance, CA) connected in series were used. The mobile phase was HPLC-grade THF at a flow rate of 1 mL/min at 38°C. The sample injection loop was 200 µL, and the running time was 25 min. Each sample was diluted with THF to a concentration of 20 mg/mL, of which 10 µL was injected into the 200-µL loop. Prior to injection, the solutions were filtered through a 0.2-µm polytetrafluoroethylene syringe filter.

Calibration curves were generated for six standards (Sigma-Aldrich, Oakville, Ontario, Canada): triolein (TG), diolein (DG), monoolein (MG), methyl oleate (FAME), oleic acid (FFA), and glycerol. The injection masses were plotted against the peak areas. Each standard was injected three times at five different concentrations. The calibration curves of the standard solutions showed good linearity ($R^2 > 0.99$). The retention times of the standards varied slightly from injection to injection, but the relative retention (rel. ret.) times remained constant (rel. ret.: TG, 1.00; DG, 1.04; MG, 1.10; FFA, 1.16; FAME, 1.17; glycerol, 1.26). A typical chromatogram of a mixture of standards (Fig. 1A) shows good separation of all components except TG and DG. In other experiments (not shown here), separation of FFA and FAME was also found to be incomplete.

For the transesterification samples, the fractional molar conversion of oil to FAME at time t was calculated using

$$X = \frac{N_{\mathrm{FAME}\,(t=t)}}{N_{\mathrm{oil}\,(t=0)}} \qquad [1]$$

where X is the fractional molar conversion, $N_{\mathrm{FAME}\,(t=t)}$ is the number of moles of FAME at time t, $N_{\mathrm{oil}(t=0)}$ is the original number of moles of oil in the sample calculated from the sum of the amount of TG plus the equivalent amounts of DG, MG, and FFA present at t=0, using the calibration curves:

$$N_{\mathrm{oil}(t=0)} = N_{\mathrm{TG}(t=0)} + 2/3N_{\mathrm{DG}(t=0)} + 1/3N_{\mathrm{MG}\,(t=0)} + 1/3N_{\mathrm{FFA}(t=0)} \qquad [2]$$

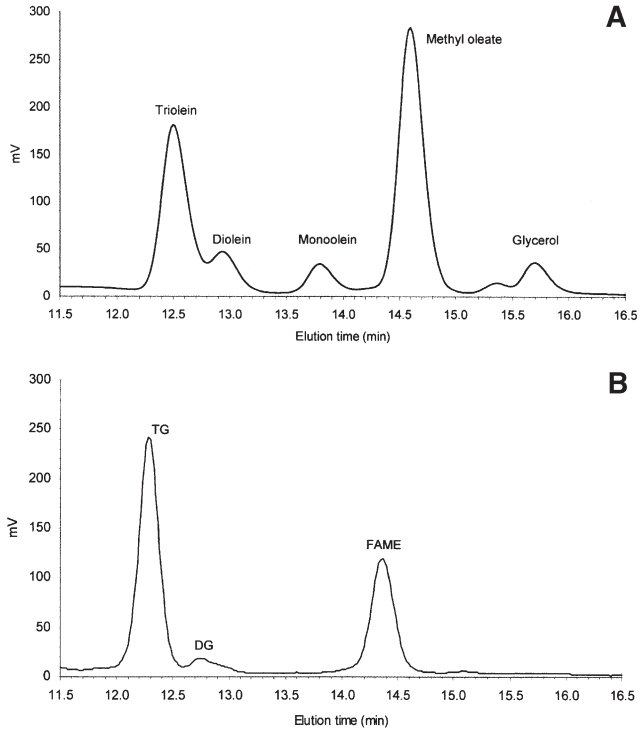

FIG. 1. Gel permeation chromatograms of (A) a mixture of standards. The sample concentration was 0.39 mg/mL TG, 0.12 mg/mL DG, 0.072 mg/mL MG, 1.00 mg/mL FAME, and 0.085 mg/mL glycerol, and the injection volume was 200 µL. (B) Sample taken from a transesterification reaction at 180 min (feed composition: 96.2 mol% MeOH, 2.5% acid, and 1.3% oil, at 70°C).

where $N_{\mathrm{TG}(t=0)}$, $N_{\mathrm{DG}(t=0)}$, $N_{\mathrm{MG}(t=0)}$, and $N_{\mathrm{FFA}(t=0)}$ represent the number of moles of TG, DG, MG, and FFA, respectively, at time 0. Analysis of a sample of lipid products of transesterification is shown in Figure 1B.

ATR-FTIR analysis. The reaction was monitored using a ReactIR 1000^{TM} (ASI Applied Systems Inc., Millersville, MD) ATR-FTIR spectrometer. A detailed description of this apparatus is found elsewhere (10). Neat sample (0.1 mL) was placed on the probe tip, and spectra were recorded at a resolution of 8 cm^{-1} using 64 scans under 1 min against air as the background. An ATR-FTIR spectrum of waste frying oil is shown elsewhere (11).

ATR-FTIR monitoring of the transesterification reaction is based on the absorbance of characteristic functional groups in the lipid products, the glycerol by-product having been removed prior to analysis. The changes in absorbance at 1378 cm^{-1}, which are attributed to the terminal CH_3 groups in TG, DG, MG, FFA, and FAME, and to the OCH_2 groups in the glycerol moiety of TG, DG, and MG (12), were monitored during the course of the transesterification reaction (Fig. 2). The conversion of TG to FAME involves the loss of the glycerol moiety, resulting in a decrease in peak height at 1378 cm^{-1}. The conversion of oil to FAME at time t can thus be defined by this decrease in peak height during the reaction. However, the concentration of terminal CH_3 groups remains constant and identical

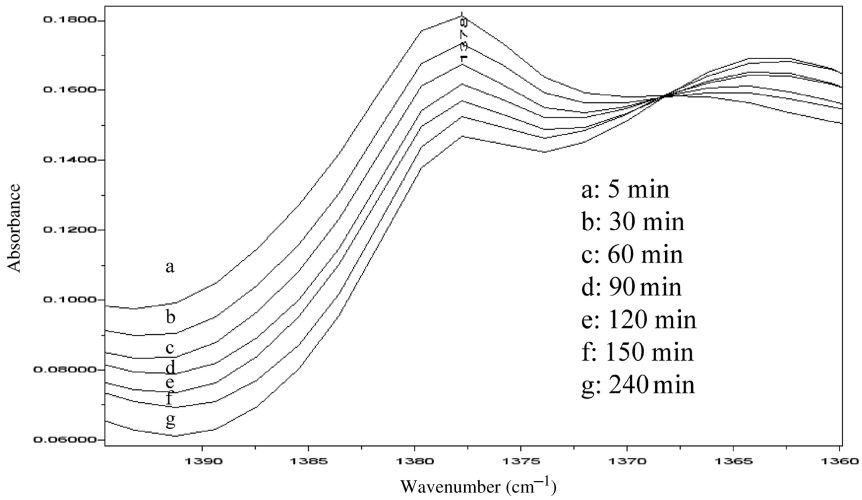

FIG. 2. Changes in absorbance at 1378 cm^{-1} of waste frying oil during the course of the transesterification reaction.

to the final amount of the FAME product, and therefore all peak heights are adjusted by subtracting the absorbance of FAME (Fig. 2). Thus, the fractional molar conversion of oil to FAME is

$$X = \frac{\text{adjusted peak height at } 1378\ \text{cm}^{-1}{}_{(t=0)} - \text{adjusted peak height at } 1378\ \text{cm}^{-1}\ (t=t)}{\text{adjusted peak height at } 1378\ \text{cm}^{-1}\ (t=0)} \quad [3]$$

RESULTS AND DISCUSSION

GPC analysis. Three mixtures of known amounts of triolein, diolein, monoolein, and methyl oleate were injected between three and five times each into the GPC system (Table 1). The reproducibility of the method was found to be very good, the SE of the injections being very low. Comparison of the measured moles with the actual values showed that the differences for both FAME and MG were on average <6%, but for TG and DG the differences were as high as 11 and 28%, respectively (Table 1). This was likely due to the slight overlap of the TG and DG peaks. Because there was always significantly more TG than DG present in the samples, the amount of TG was often underestimated and that of DG was overestimated (Table 1). However, the fractional recoveries (i.e., mean mole measurement divided by actual moles injected) of TG and DG were

TABLE 1
Gel Permeation Chromatography Analysis of Mixtures of Standards (all units in moles $\times 10^4$ unless otherwise indicated)

	Triolein	Diolein	Monoolein	Methyl oleate
Mixture 1				
Actual	8.00	1.73	1.30	4.60
Mean measurement	7.52	2.21	1.24	5.09
SE ($\times 10^7$)	8.7	8.7	15.0	23.0
Recovery (%)	94.0	127.7	95.9	110.7
Revised mean measurement	8.21	1.81	1.24	5.09
Revised recovery (%)	103.2	104.4	95.9	110.7
Mixture 2				
Actual	2.65	0.85	0	24.0
Mean measurement	2.36	1.03	0	24.5
SE ($\times 10^7$)	26.0	4.5	—	79.0
Recovery (%)	89.2	120.1	—	102.1
Revised mean measurement	2.64	8.68	—	24.5
Revised recovery (%)	99.9	101.6	—	102.1
Mixture 3				
Actual	2.19	2.38	6.84	13.8
Mean measurement	1.99	2.52	6.75	14.2
SE ($\times 10^7$)	1.3	2.5	2.0	6.4
Recovery (%)	90.7	106.0	98.7	103.0
Revised mean measurement	2.29	2.24	6.75	14.2
Revised recovery (%)	104.6	94.2	98.74	103.0

TABLE 2
Attenuated Total Reflectance-FTIR Spectroscopy Analysis of Known Mixtures of Oil and FAME (all units in mole fractions unless otherwise indicated)

Mixture	Actual FAME	Mean measured FAME	Difference between mean and actual (%)	SE
1	0.100	0.112	11.7	0.00088
2	0.250	0.246	–1.7	0.00067
3	0.500	0.526	5.2	0.00265
4	0.750	0.760	1.4	0.00219
5	0.900	0.893	–0.8	0.00153

found to be a linear function of the TG/DG mole ratio. The recoveries of TG and DG were therefore corrected using linear equations relating the TG/DG mole ratio to the actual TG and DG amounts injected (revised fractional recovery of TG = 0.0119 TG/DG + 0.8536; revised fractional recovery of DG = 0.0266 TG/DG + 1.0947). The revised recovery data were adequate (Table 1) and were used to calculate a revised mean measurement.

With an increase in the TG/DG molar ratio, the recovery of TG approached 100%, but that of DG became less reliable (Table 1). However, in this study, DG was not of major concern, since, at reaction times greater than 2 h, the amount of DG was always found to be negligible (see Fig. 1B). Thus, the analysis of samples taken toward the end of a reaction could be considered highly reliable. The presence of any MG was below the detection limit of the GPC. The original oil was composed of about 82 wt% TG, 12% DG, and 6% FFA. The low amounts of DG and MG in the reaction samples were a result of the high methanol/oil ratios (e.g., 50:1 to 250:1) used, which pushed the equilibrium of the reaction to completion.

Comparison of our GPC results with those of Darnoko *et al.* (8) showed general agreement in spite of the differences in apparatus and sample preparation. However, they did not report any recovery problems for TG and DG.

The retention times of FFA and FAME were very close to each other; thus, the presence of FFA in the sample could have been masked by the FAME peak. Early samples (as early as 0.5 min) taken from the reaction were examined specifically for the presence of FFA. It appeared that there was no FFA in any of our samples except for the initial oil and early samples, and it is assumed that FFA were totally converted to FAME because of the large excess of methanol used in our study (mole ratio, 75:1 methanol/oil). Since most of the glycerol was removed in the washing step of our sample preparation, little or no glycerol was found in the GPC chromatograms.

In summary, the GPC method provided adequate sample recovery, excellent reproducibility, straightforward sample preparation, and relatively short analysis times (<25 min).

ATR-FTIR spectroscopy. Five mixtures of waste oil and FAME produced from waste oil of differing compositions (Table 2) were examined by ATR-FTIR spectroscopy. Each mixture was analyzed three times. The measurement-to-measurement variation was found to be very small: The SE of the analyses was less than 0.00265, showing very good reproducibility. The measured mole fractions of FAME of the five mixtures were within ±12% of the known compositions (Table 2). As a result, a calibration curve was not required for the ATR-FTIR analysis, and Equation 3 was used to calculate conversion in the transesterification samples. The analysis time was short (1–2 min per sample). The disadvantage of the technique is that DG and MG cannot be quantified individually because of their similar structure to TG. This did not affect our measurements significantly since the contents of DG and MG were very low (<1%) in all samples except in the initial oil and in the early stages of the reaction. Application of ATR-FTIR spectroscopy to samples containing large amounts of DG and MG would not be feasible, but chemometric methods may circumvent this problem. Nonetheless, ATR-FTIR spectroscopy provided a reliable conversion of oil to FAME data in a very short time.

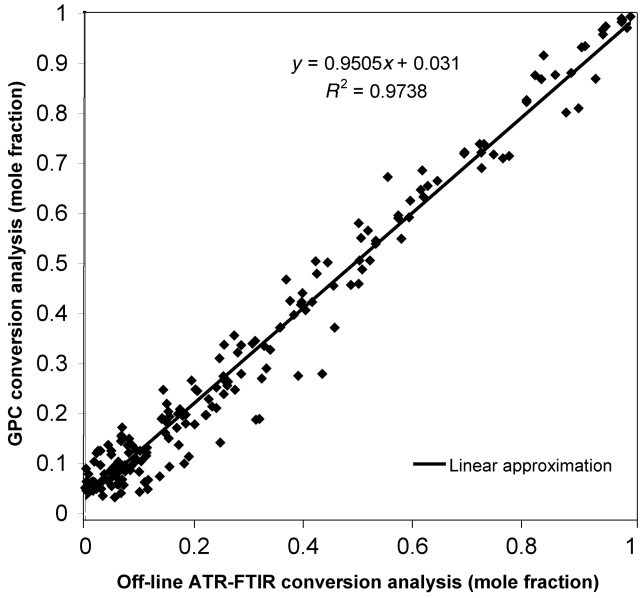

FIG. 3. Gel permeation chromatography (GPC) vs. attenuated total reflectance (ATR)-FTIR spectroscopic analyses for all production experiments (i.e., waste oil to FAME transesterification).

Comparison of ATR-FTIR spectroscopy to GPC analyses. A paired comparison was carried out between the results of the composition analyses from the two methods. Five mixtures of waste oil and FAME from waste oil (mole ratio oil/FAME 89:11, 76:24, 45:55, 25:75, 10:90) were analyzed by the two methods nine times each for a total of 45 measurements. The 95% confidence interval for the difference between the measured contents of FAME obtained by the two methods in the mixtures (calculated using either Equation 1 or 3) was found to be –0.0114 to 0.0001 mol%, indicating no significant difference.

All of the samples (total of 100) from the waste oil transesterification experiments were analyzed by both ATR-FTIR spectroscopy and GPC. An overall comparison plot of all the analyses showed good agreement between the two methods,

the R^2 value being 0.9738 (Fig. 3). A paired comparison of the two methods was carried out for all of the data, giving a 95% confidence interval of (0.0088 to 0.0222) mol% for the difference in the values of the mole fraction between analytical methods. Although this interval did not include 0, paired comparisons for individual experiments resulted in 12 out of 20 95% confidence intervals that did include 0. Furthermore, most of the samples with high FAME content (>80%) showed excellent agreement between the methods. Because the differences were actually very small, the results of these two analytical methods can be considered equivalent.

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