

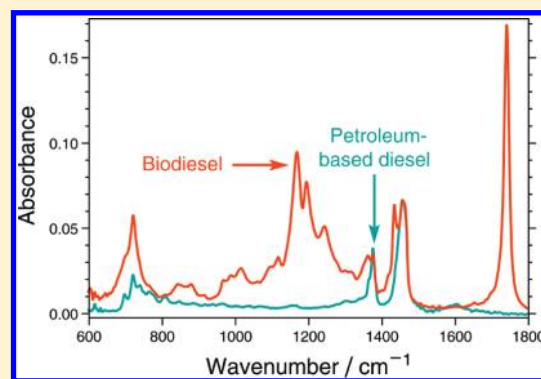
# Quantitative Investigations of Biodiesel Fuel Using Infrared Spectroscopy: An Instrumental Analysis Experiment for Undergraduate Chemistry Students

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## S Supporting Information

**ABSTRACT:** Biodiesel has gained attention in recent years as a renewable fuel source due to its reduced greenhouse gas and particulate emissions, and it can be produced within the United States. A laboratory experiment designed for students in an upper-division undergraduate laboratory is described to study biodiesel production and biodiesel mixing with conventional petroleum-based diesel fuel using Fourier transform infrared (FTIR) spectroscopy. The production of biodiesel from oil via a transesterification reaction is monitored by the intensity of the C–O ester peak ( $1098\text{ cm}^{-1}$ ), whereas the mixing of biodiesel and petroleum-based diesel is monitored by the C=O ester stretch ( $1746\text{ cm}^{-1}$ ). The impact of water on the precision of the biodiesel mixture is determined using a Karl Fischer titration to monitor water content. Students also gain experience with method validation using multiple sampling platforms (liquid cell, ATR-cell, and IR card). At the conclusion of the experiment, students are able to use FTIR to quantitatively monitor reactions and determine mixtures, determine the impact of water content on quantitation, and evaluate the strengths and weaknesses of different sampling platforms for various applications.



**KEYWORDS:** Upper-Division Undergraduate, Analytical Chemistry, Environmental Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Applications of Chemistry, IR Spectroscopy, Instrumental Methods, Laboratory Equipment/Apparatus, Quantitative Analysis

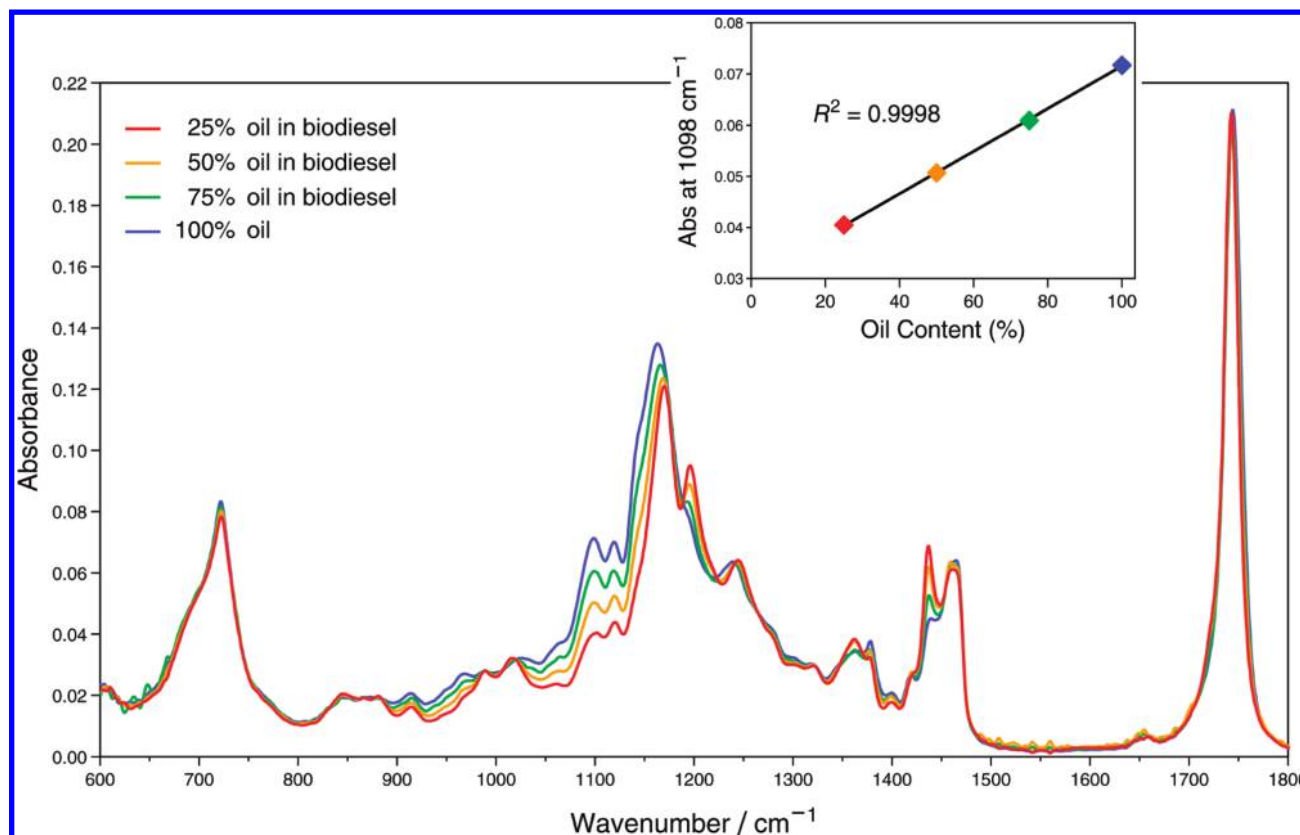
Biofuels, and biodiesel in particular, have recently become a significant research focus<sup>1–3</sup> because they are renewable,<sup>4</sup> their production is energy efficient,<sup>5,6</sup> and they emit less greenhouse gases (i.e.,  $\text{CO}_2$ ) over their life cycle<sup>7</sup> and less particulate matter during combustion than standard fuels.<sup>8,9</sup> Further, biodiesel is nontoxic,<sup>10</sup> biodegradable,<sup>10</sup> and can be used as a 20% blend in most diesel equipment with little-to-no modification.<sup>11</sup> Because the United States has the fourth greatest biodiesel production potential,<sup>12</sup> increasing production to match that potential would significantly reduce the dependence on imported oil. Disadvantages of biodiesel include higher viscosities, densities, and flash points.<sup>13</sup> In colder environments, the higher cold-filter plugging point of biodiesel can be problematic,<sup>14–16</sup> as the diesel fuel flow is restricted due to gel formation at higher temperatures<sup>17</sup> than petroleum-based diesel.<sup>18,19</sup> Unlike petroleum-based diesel, biodiesel has long-term storage issues related to thermal and oxidative stability, as esters in the fuel can undergo numerous reactions changing their fuel properties;<sup>20–22</sup> in addition, it tends to take-up water and support microbial growth.<sup>22,23</sup>

From a chemical perspective, biodiesel is composed of fatty acid methyl esters (FAME) that are produced via transesterification of the ester-containing triglycerides in oils as they react with alcohols (e.g., methanol) in the presence of a catalyst (e.g., sodium hydroxide); reaction schemes are included in

Clarke et al., 2006.<sup>24</sup> The initial oil can come from plants, animal fats, and algae.<sup>25–28</sup> Petroleum-based diesel consists of a mixture of aliphatic alkanes (paraffins) and cycloalkanes (naphthenes) (80–90%), as well as aromatic hydrocarbons (10–20%). Most hydrocarbons for petroleum-based diesel are in the  $\text{C}_9$ – $\text{C}_{20}$  size range.<sup>29</sup> The presence of FAME in biodiesel is a key chemical difference from petroleum-based diesel that can be probed with infrared spectroscopy.<sup>30</sup>

Biofuels are incorporated into petroleum-based diesel via splash blending to produce federally approved B20 mixtures; the index B\*\*, represents the percent biodiesel in petroleum diesel. To splash blend biodiesel, petroleum-based diesel is loaded into a vessel followed by biodiesel and the subsequent mixing is sufficient for use in vehicles.<sup>31</sup> However, blend levels are frequently inconsistent, ranging from 1% to over 80% biodiesel across the United States in samples labeled B20.<sup>10</sup> This is a serious concern as diesel with greater than 20% biodiesel has the potential to damage fuel system parts.<sup>32</sup> The fuel's water content following storage is of additional concern; thus, various methods have been developed to quantify the amount of water in fuel and remove it. These biodiesel methods frequently use the Karl Fischer titration,<sup>20,21,33,34</sup> a coulometry

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**Figure 1.** Infrared spectra for different percentages of biodiesel in starting oil. Inset is the absorbance at 1098 cm<sup>-1</sup> for different mixtures showing the linearity of the response.

technique with high accuracy, high precision, and extended dynamic range for determining trace water concentrations.<sup>35–37</sup>

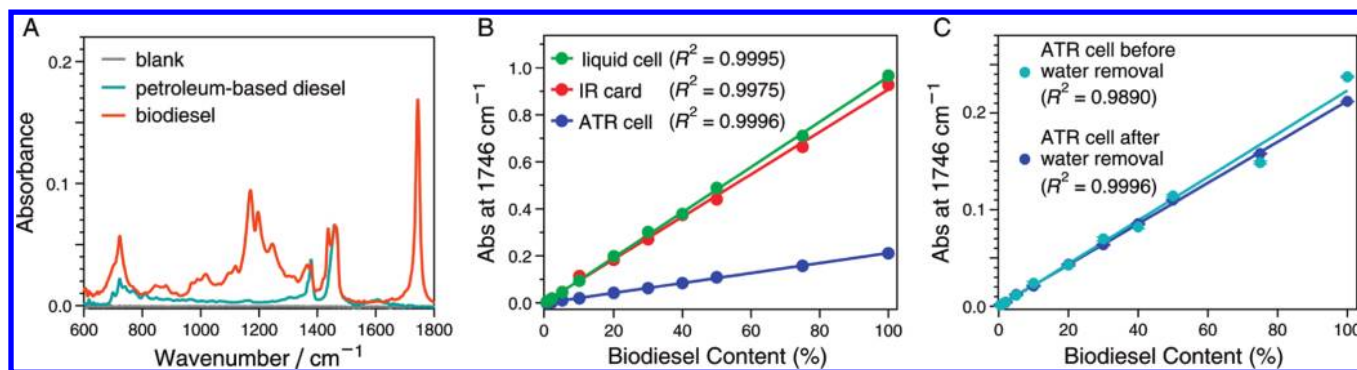
The interest in biofuels has led educators to incorporate biodiesel into the teaching laboratory by instructing students to make biodiesel<sup>24,38</sup> and test properties such as viscosity<sup>24</sup> and heat of combustion.<sup>39</sup> The laboratory experiment described herein allows students in an upper-division course to study the production and mixing of biodiesel quantitatively using Fourier transform infrared spectroscopy (FTIR), a technique readily available at most institutions. Since 1966, over 100 articles have used or discussed infrared spectroscopy (IR) in this *Journal* (selected references: 40–64); however, most of these articles have focused on using IR for the qualitative identification of vibrational bands in structural determinations.<sup>41,56,58–61</sup> Less than a dozen articles have focused on using IR quantitatively.<sup>40,46,48,50–54,62–64</sup> The limited number of quantitative infrared experiments is likely due, in part, to the use of expensive attenuated total reflectance (ATR) cells<sup>54</sup> or cumbersome liquid cells with fragile salt plates.<sup>50</sup> Herein, a third option, inexpensive IR cards<sup>65</sup> are shown to be an adequate substitute for quantitative analysis of environmental samples.<sup>63</sup> Further, students will apply these quantitative analysis tools to samples with important environmental and real-world ramifications.

## ■ EXPERIMENT

The experiment consists of four sections: (i) monitoring the production of biodiesel by the progress of the transesterification reaction, (ii) quantification of mixtures of biodiesel and petroleum-based diesel, (iii) determination of the impact of water on quantification using the Karl Fischer

titration to determine water content, and (iv) sample platform performance. The student work in pairs and the entire experiment took two, 3-h lab periods. It is important for students to have familiarity with FTIR,<sup>66</sup> different sampling cells,<sup>66</sup> figures of merit,<sup>66</sup> and Karl Fischer titrations<sup>36,37,67</sup> prior to the laboratory to maximize student gain from the experiments. This can be accomplished through a prelaboratory assignment or quiz.

Biodiesel can be made following the procedure set forth in Clarke et al.,<sup>24</sup> which is based on the industrial scale reaction in Wenzel et al.<sup>68</sup> Different starting oils may be used (e.g., olive oil or canola oil), which students may bring or be provided; filtering may be required for samples from food fryers. The starting oil is reacted with methanol and sodium hydroxide or sodium methoxide as the catalyst. The students titrate the sample oil to determine the concentration of free acid and the appropriate amount of catalyst to use.<sup>24</sup> Students then collect an initial IR spectrum and further IR spectra as they systematically add catalyst and approach the equivalence point. It is important to avoid adding too much catalyst, which can lead to soap production. Samples were analyzed by students on a PerkinElmer Spectrum RX FTIR spectrometer. Three IR platforms were used in the different experiments. The first platform was a Real Crystal IR Card (Model: 0000-7096, International Crystal Laboratory) with a 9.5 mm KBr window.<sup>65</sup> Coverslips with a clip mount were used to minimize evaporation of the samples. The cards can be reused by cleaning with methylene chloride and the efficiency of the cleaning was confirmed with FTIR. The fuel samples, 10  $\mu$ L, were applied to the cards with a micropipet. Also, a sealed liquid IR cell with KBr windows was used with a 0.05 mm path



**Figure 2.** (A) Infrared spectra showing the absorbance of biodiesel versus diesel. (B) Comparison of absorbance versus biodiesel content for three sampling platforms (liquid cell, IR card, and ATR cell) with dried samples. (C) Comparison of absorbance versus biodiesel content for the ATR cell for samples with and without water (error bars are standard deviation of 16 runs).

length and 16 mm aperture (Model: S-3, International Crystal Laboratory). Finally, fuel samples were analyzed with an attenuated total reflectance (ATR) cell (PIKE MIRacle ATR cell). In all cases, 4 spectral scans at 2 cm<sup>-1</sup> resolution were averaged; peak absorbances and areas were recorded at 1098 cm<sup>-1</sup>.

For quantification of biodiesel and petroleum-based diesel mixtures, biodiesel produced in the first portion of the laboratory may be splash mixed with commercially available petroleum-based diesel or commercial biodiesel mixtures may be used, if available. For the data shown herein, samples were obtained from Pearson Ford, San Diego, CA (B20 and B100). The protocol used here called for the application of 10  $\mu$ L delivered by micropipet onto the 9.5 mm aperture of the IR card. Peak absorbance and areas were recorded for the ester carbonyl stretch at 1746 cm<sup>-1</sup> and integration was performed from 1720 to 1780 cm<sup>-1</sup>. Water content was determined by a Karl Fischer titrator (DL39 titrator Mettler Toledo GmbH). Karl Fischer analysis was performed with Aquastar CombiCoulomat fritless reagent, a mixture of methanol, sulfur dioxide, and two proprietary components. Water-in-oil standards were also obtained from Aquastar in a range of 15 to 30 ppm (EMD Chemicals, Inc., an affiliate of Merck KGaA, Darmstadt Germany). Biodiesel samples were soaked in molecular sieves (Fluka UOP Type 3A) for 2 days to remove trapped water. Fuel samples were prepared by mass on an analytical balance ( $\pm 0.0001$  g) using capped glass vials and diluted with ultra-low sulfur diesel fuel (ULSD). Ordinary or linear least-squares analysis of the data is appropriate for this application.

## HAZARDS

Proper safety equipment should be worn at all times (laboratory coat, safety goggles, and gloves appropriate to the chemicals being handled). All solvents and samples should be handled with care and disposed of following guidelines for organic waste disposal. Methylene chloride is a particularly toxic solvent and needs to be stored in a halogenated organic waste container and not commingled with other organic waste. The AquaStar CombiCoulomat fritless reagent is toxic and flammable and care should be taken when handling it. Open flames should be avoided with all samples and solvents and samples should be prepared in a well-ventilated facility. If the precautions described above are followed, the laboratory represents minimal hazard.

## RESULTS AND DISCUSSION

### Monitoring the Progress of a Transesterification Reaction

The FTIR spectra collected during the transesterification process can be used to quantitatively monitor the reaction. The spectra of blends from 25 to 100% (w/w) are overlaid and displayed in Figure 1. The most intense peak in the spectrum, the ester carbonyl stretch at 1746 cm<sup>-1</sup>, cannot be used to monitor the reaction as the ester C=O stretch is of the same intensity for both the starting oil (triglycerides) and the biodiesel (fatty acid methyl esters). However, the peak at 1098 cm<sup>-1</sup> (methyl-ester C–C–O stretch<sup>69</sup>) can be used to monitor the biodiesel increase during the transesterification (Figure 1 inset) and displays a high correlation coefficient ( $R^2 = 0.9998$ ).

### Quantification of Mixtures of Biodiesel in Diesel Fuel and Motor Oil

For the preparation of vehicular-grade B20, it is necessary to monitor the concentration of biodiesel in the sample. This analysis can be accomplished using FTIR. Figure 2A shows IR spectra from a blank, 100% petroleum-based diesel, and 100% biodiesel. The C=O stretch at 1746 cm<sup>-1</sup> is ideal for this application as there is no absorbance at that wavenumber by petroleum-based diesel. The absorbance at 1746 cm<sup>-1</sup> is shown in Figure 2B versus % biodiesel in diesel for the three sampling platforms (liquid cell, ATR cell, and IR card). Figure 2C compares % biodiesel in diesel samples for which water was and was not removed with the ATR cell, with error bars representing the standard deviation of 16 runs. Removal of water reduces the variation between sources and improves the precision of the calibration curves used to determine the amount of biodiesel in a biodiesel/diesel blend. The value of  $R^2$  improved from 0.9890 to 0.9996 with water removal on the ATR cell; results were typical to those observed by students. The lower correlation coefficient for samples with water contamination is due to hydrogen bonding of the water with the ester carbonyl group, which changes the line shape and subsequently leads to changes in the peak height or integrated area. The variation in water between samples leads to the variation in absorbance and lower precision, discussed below and shown in Table 1.

### Determining the Impact of Water in Biodiesel

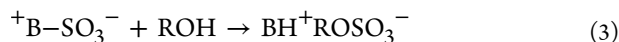
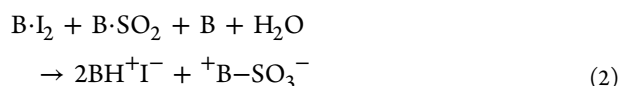
To determine the amount of water in a sample, the students are encouraged to perform a Karl Fischer titration.<sup>35,67</sup> This coulometric technique uses an anode solution consisting of an alcohol (typically methanol), a base (B, typically imidazole), SO<sub>2</sub>, I<sup>-</sup>, and a mixture of organic solvents to prevent moisture



Table 1. Comparison of the Figures of Merit for the Sampling Platforms

Sampling Platforms	Sensitivity (Abs units per % biodiesel)	Avg. Precision at 1746 cm <sup>-1</sup>	Std. Dev. Conc. (%)	Limit of Quant. (% biodiesel in diesel)	Limit of Linearity (% biodiesel in diesel)	Cost/ \$ (approx)	Ease of use and cleaning
Liquid cell	0.0097		3.4	1.9	100	300.00	Hard
IR card	0.0092		4.0	5	100	3.00	Simple
ATR cell	0.0021	0.0004	0.7	1.3	100	3000.00	Medium
ATR cell (without water removed)	0.0022	0.0012	3.9	1.3	100	3000.00	Medium

trapping in micelles or emulsions.<sup>67</sup> The cathode solution contains reagents that can be reduced at the cathode and is separated from the anode solution by an ion-permeable membrane. A precise electrolysis current is applied between large platinum electrodes immersed in the cathode and anode solutions. This current yields a constant source of I<sub>2</sub> at the anode (eq 1) and subsequent consumption of water in the solution (eqs 2 and 3):<sup>67</sup>



From these reactions, it is clear that 2 mol of electrons consumes 1 mol of water. To signal the complete consumption of water and the end point of the titration, a small, independent pair of platinum electrodes is also immersed in the anode solution for bipotentiometric detection.<sup>67</sup> The total amount of water consumed during the titration is then determined from the amount of charge passed during the electrolysis (current × time) and Faraday's law.

The improvement in the correlation coefficient for the fit between percent biodiesel in diesel and absorbance at 1746 cm<sup>-1</sup> can be monitored by making measurements before and after drying with molecular sieve. If the initial sample contains negligible water, it can be added stepwise to determine the effect on quantitation.

### Sample Platform Performance

Method validation is a very common and necessary process for chemists in industrial settings.<sup>70,71</sup> Figures of merit for three sampling platforms, ATR cell, liquid cell, and IR card, are compared in Table 1 (sensitivity, precision, limit of quantitation, limit of linearity, ease of use, and cost). As can be seen from Table 1, the ATR cell has lower sensitivity than the liquid cell or IR cards; the lower sensitivity can be observed as lower slope in Figure 2B. Further, it is clear that higher precision is achieved after water is removed. The linearity of the fit over the range of B05 to B100 shows the dynamic range (limit of quantification to limit of linearity) of the method, which matches industrial results.<sup>72</sup>

Students should be prompted to discuss the strengths and weaknesses of each method, both in the context of this experiment and other experiments where these methods might be used. For example, IR cards are useful in this application because biodiesel forms a uniform film due to its viscosity, is compatible with salt plates, and does not rapidly evaporate. If spectra are collected immediately after application, the loss of sample from the surface has a negligible effect in the range B5 to B100. Below B5, the variability increases compared to the liquid cell and the ATR cell due to faster evaporation of

petroleum-based diesel. Students should be challenged to think of samples that will not form a uniform layer, are not compatible with salt plate, or evaporate quickly, thus, rendering IR cards ineffective. For more volatile samples, the fixed path length of the liquid cell and ATR cell can be weighed against the greater time to clean and fill the liquid cell and higher cost of the ATR cell. Students should describe scenarios where each of the three sampling methods would be preferable based on compromises in performance, throughput (analyses per unit time), and price.

## CONCLUSIONS

Herein, we describe a laboratory experiment designed for students in an upper-division undergraduate laboratory to study the biodiesel production and biodiesel mixing with conventional diesel fuel using infrared spectroscopy. At the conclusion of this experiment, students will understand the steps to produce biodiesel mixtures that can be used in vehicles from starting oils. Students will learn to use FTIR as a quantitative analytical technique based on structural changes during the transesterification reaction and splash mixing with petroleum-based diesel. From this laboratory, students will be able to use figures of merit to evaluate the strengths and weaknesses of different sampling platforms and determine the appropriate choices for different applications. Students will also understand how the presence of water can lower the precision of the FTIR measurements and be able to use Karl Fischer analysis to monitor the water content as the sample is dried. To link the experiment to ongoing discussions regarding the positive and negative environmental impacts of biodiesel, instructors are encouraged to have students discuss the relative merits of biodiesel in a laboratory report or classroom presentation or discussion. Overall, during this experiment data are collected on an environmentally relevant system and combined with quantitative analysis to create a challenging experiment for the upper-level undergraduate laboratory.

## ASSOCIATED CONTENT

### Supporting Information

Instructions for students and instructors. This material is available via the Internet at <http://pubs.acs.org>.

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