Summer Scholar Report

Applying $_1$H NMR spectroscopy to develop a kinetic model for the transesterification of glycerol fatty acid triesters

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Introduction

Biodiesel (Fatty Acid Methyl Esters, FAME) is an alternative fuel made from renewable vegetable oils that can be used in diesel vehicles without engine modifications. Biodiesel is a possible replacement for petroleum diesel due to reduced greenhouse gas emissions, unburned hydrocarbons, carbon monoxide, particulate manner and nitrogen oxides[1]. A diesel engine can run on an 80/20 (B20) mix of petroleum diesel to biodiesel, and with adjustments (to avoid possible clogging) can run 100% (B100) biodiesel[2]. Over the past 10 years our research group has been studying the chemistry of small-scale (500mL-4L) biodiesel synthesis by base-catalyzed transesterification of vegetable oils, and determined that this process is highly inefficient and wasteful[3]. A detailed model of the kinetics and mechanism for glyceryl fatty acid transesterification has not been developed. A better understanding of this chemical process could be important in solving these issues through the application of green chemistry principles in modifying reaction parameters, and in the design of specific catalysts for transesterification.

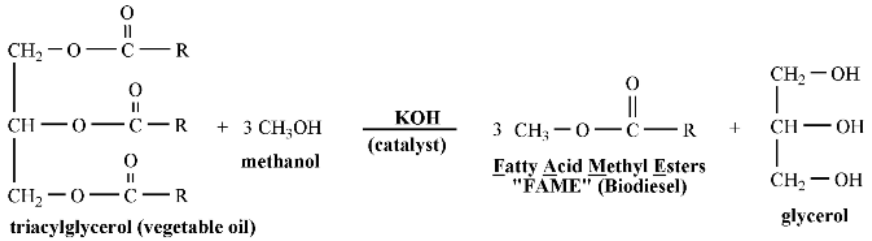

Figure 1: *Chemical reaction for the transesterification of vegetable oil to biodiesel.*

The transesterification of simple esters is a traditional chemical reaction taught in college-level organic chemistry, Figure 1[4]. However, understanding the transesterification of fatty acid triglycerides is more challenging due to complex substrate conformations, reaction solubility challenges, identification of intermediates, and uncertainty as to the sequence of tri-ester exchange, Figure 2. It is not clear, for example, if the transesterification is a progression of three, sequential reactions at each of the three tri-acyl glycerides (C1-C2-C3), or if there is rate limiting exchange of the methylene ester groups (C1 vs C3) versus the methine ester group (C2), Figure 2.

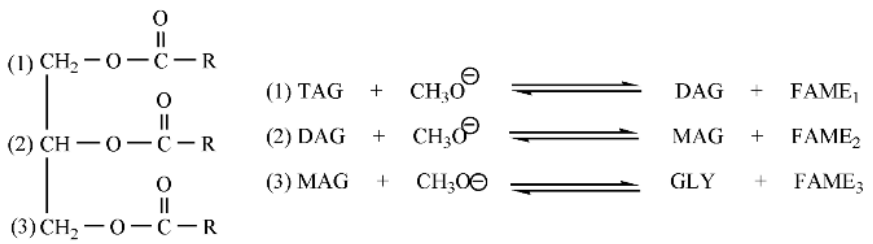

Figure 2. *Triacyl glyceride transesterification with methoxide: TAG = triacylglyceride, DAG = diacylglyceride, MAG = monoacylglyceride, GLY = glycerol, FAME1,2,3 = Fatty Acid Methyl Esters (biodiesel).*

^{1}H NMR spectrometry is a rapid, quantitative instrumental method for time-based monitoring of transesterification reactions based on the integration of select proton signals. Although vegetable oil transesterification into methyl esters has been studied by ^{1}H NMR[5-7], very little work has been done studying the reaction progress and kinetics by this method. The goal of this work was to develop a ^{1}H NMR experimental method to study the time course of vegetable oil transesterification, that may eventually lead to a detailed kinetic model that would identify whether rate limiting transesterification occurs at C1-C3 or C2 as the glyceryl triester is converted into the di- and mono- ester, and finally free glycerol.

Methodology

General. All reagents were purchased from Sigma-Aldrich or Fisher Scientific and used without further purification. Transesterification reactions were conducted using store bought brand name soybean oil. All glassware was washed with Micro-90 cleaner, and then rinsed with deionized water and acetone before oven drying at 80oC.

Transesterification Procedures. Transesterification reactions were run at constant temperature (60oC or 25oC) using a standard 25 mL batch method with 3:1 mole ratios of methanol:ester (25.1 mmole triglyceride), and 2.51 mmole potassium hydroxide as catalyst. Aliquots were removed at timed intervals and quenched by dilution in acetone-d6 containing 0.05% TMS. The molecular mass of the triglyceride (vegetable oil) and biodiesel product were estimated based on the molecular mass of oleic acid as a model fatty acid.

Sampling and Sample Preparation. Good signal-to-noise ratios were obtained using 1.0 mL of acetone-d6 to quench 25 μL reaction aliquots. Aliquot volumes were measured with gas-tight syringes, flushed thoroughly between each aliquot to avoid contamination. ^{1}H NMR spectra were obtained immediately after being quenched, and then scanned

again twenty-four hours later. No changes in NMR spectra were observed over a 24-hour period.

Instrumentation and Analysis. Nuclear Magnetic Resonance (NMR) spectra were obtained on a JEOL ECX-400 MHz instrument. Quantitative NMR (qNMR)[8] was used to determine reaction progress based on theoretical percent yield. In qNMR the quantity of a particular analyte (biodiesel) is determined by comparing the integrated value of an analyte signal of known number of protons to the integrated value of a known internal standard (Figure 3, equations 1 and 2). Maleic acid was used as the reference standard (vinyl protons at 6.3 ppm, 2H)[9], to quantitate the biodiesel formed based on the appearance of the -OCH3 methyl ester signal at 3.6 ppm (3H).

$$1)\quad \left(\frac{\text{x mmol Biodiesel}}{\text{y mmol Maleic Acid}}\right) * 3 = \text{Expected Integration FAME}$$

$$2)\quad \left(\frac{\text{Integration FAME}}{3}\right) * \text{y mmol Maleic Acid} = \text{x mmol Biodiesel}$$

Figure 3. *Equations used to determine reaction progress by Quantitative NMR.*

Calibration Curves and Data Handling. Maleic acid was kept constant at 25 mL of a 0.689 M stock solution (0.0172 mmole), and an aliquot of pure biodiesel was added (5, 10, 15, 20, and 25 μL) to create five standards. qNMR analysis with normalization of the maleic acid signal for each sample resulted in integrations of the $–OCH_3$ methyl ester signal (3.6 ppm) for comparison to theoretical integration. A straight line with good correlation RÇ = 0.9749 was obtained (data not shown).

Results and Discussion

Acetone-d6 was found to effectively and completely quench the transesterification reaction, with no evidence of side reactions over a 24 hour period. Also, maleic acid functions as a suitable internal reference for qNMR quantification due to a clear signal downfield from all other signals of interest, resulting in easy and accurate calculations of methyl ester concentration.

The rates of triacylglyceride transesterification were examined at 60oC and 25oC. We initially attempted to study the transesterification kinetics at 60oC as this is the typical temperature for biodiesel production. However, it was found that the initial rate of transesterification at 60oC was too fast to measure by our sampling method, and that

equilibrium was reached within one minute (data not shown). At 25oC a rapid initial rate was clearly observed that was linear for approximately 25-30% of the reaction as shown by the concentration-time data graphed in Figure 4. The rapid initial rate was followed by a slower reaction phase which reaches equilibrium in approximately 3 hours.

To our knowledge, this is the first time a rapid initial phase has been reported for vegetable oil transesterification. As our data suggest that this rapid phase accounts for approximately 25-30% of maximum yield, it is tempting to speculate that this implies a rapid exchange of a C1 glyceryl methylene ester group, followed by slower (rate limiting) exchange of the remaining two glyceryl esters, Figure 2. At this time we were not able to determine the equilibrium product composition based on the signals of the C1-C3 glyceryl methylene protons (4.1-4.4 ppm). Furthermore, the C2 methine proton signal (5.25 ppm) overlaps with the fatty acyl olefinic proton signal (5.35 ppm), Figure 5, making it difficult to accurately integrate the two signals. The equilibrium concentration of biodiesel produced at 25oC is approximately 60% of completion, and underlies the importance for using an excess of methanol and removing the glycerol product to "force" the reaction to completion.

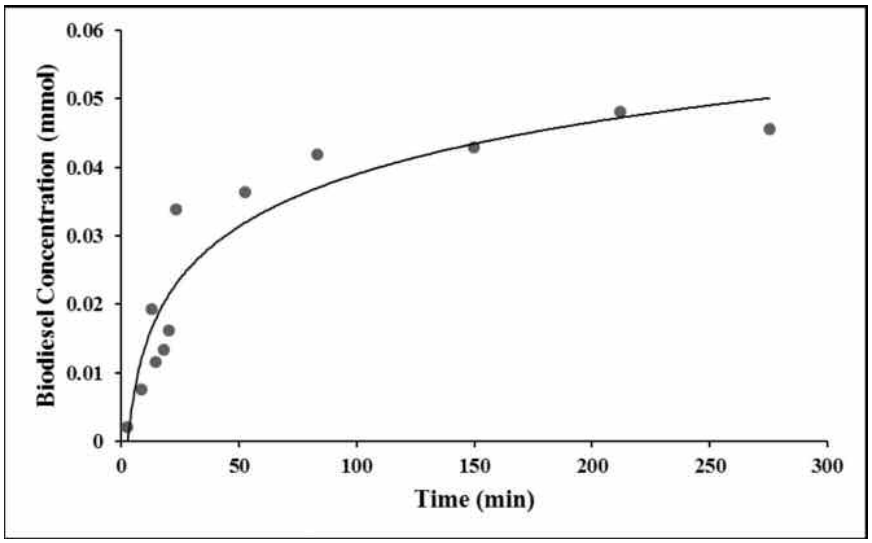

Figure 4. *Time course of increasing biodiesel concentration vs time at 25oC from the transesterification of 25.1 mmol of vegetable oil. Each data point represents a 25μL aliquot of the reaction mixture, where the maximum observed yield of biodiesel product in this plot would be 0.0753 mmoles, Figure 1.*

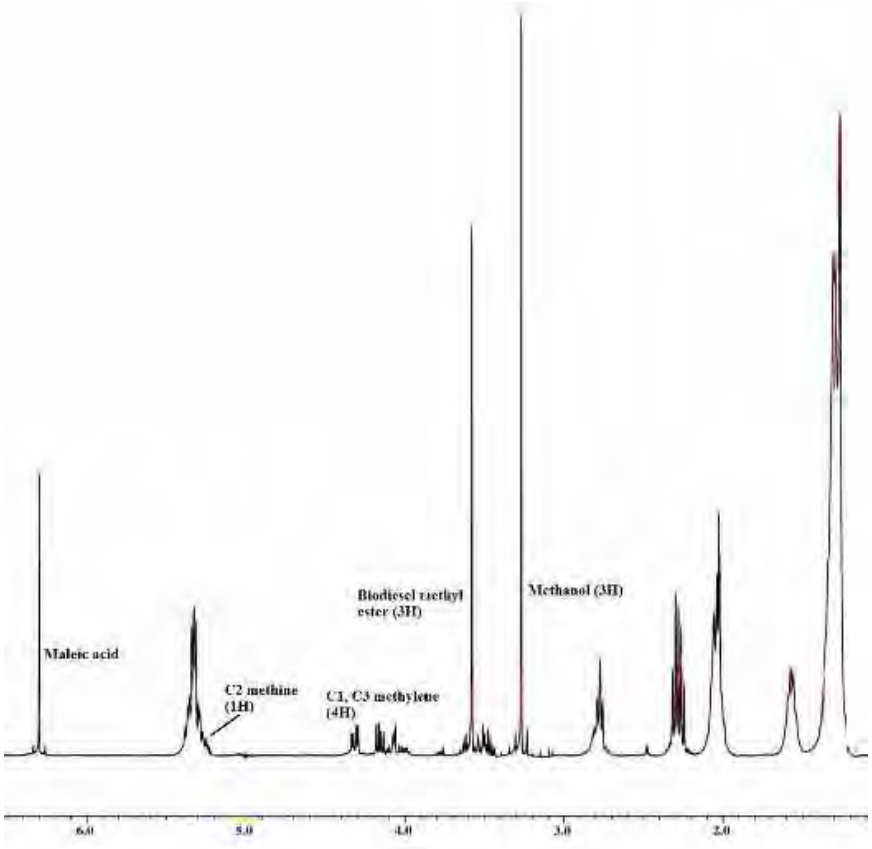

Figure 5. *^{1}H NMR spectrum of transesterification reaction taken at equilibrium. Chemical shifts: maleic acid vinyl protons (2H, 6.4 ppm), C2 glyceryl methine (1H, 5.25 ppm), C1-C3 glyceryl methylenes (4H, 4.1-4.4 ppm), biodiesel –OCH_3 methyl ester (3H, 3.6 ppm), methanol (3H, 3.25 ppm). Signal at 5.35 ppm is attributed to fatty acyl olefinic protons.*

Conclusions and Future Work

We have developed a simple, efficient and reproducible qNMR analytical method to monitor the transesterification of fatty acyl triglycerides. This method will be used to continue our work to better understand the kinetics and mechanism of small-scale biodiesel synthesis, and facilitate the optimization of reaction parameters and screening of new catalysts. Our results also suggest that room temperature (25oC) is preferred for rate studies on fatty acyl transesterification reactions, but more must be done in order to finalize rate analysis and determining reaction order. Our ongoing work is focused on determining the product distribution over the reaction time course. The complex mixture may contain tri-, di-, and mono-glycerides, free glycerol, biodiesel (methyl ester) and unreacted methanol. Furthermore, we need to conclusively determine the sequence of transesterification for the methylene vs methine carbons to conclusively identify reaction intermediates. Due to the complexity of the C1-C3 methylene signals, and overlap of C2 methine signal with fatty acyl olefinic protons (Figure 5), we will investigate whether 2D NMR can be employed to resolve these signals.

Acknowledgements
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