Investigation of Free Glycerin and Potassium Ion Adsorption by DudaLite DW-R10 Ion-Exchange Resin

A Major Qualifying Project Report submitted to the faculty of Worcester Polytechnic Institute in partial fulfillment of the requirements for the Bachelor of Science

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Abstract

Due to the current energy crisis, alternative fuels are becoming increasingly relevant. One of these fuels, biodiesel, can be produced relatively easily from common feedstocks such as vegetable or waste oil. Before use, biodiesel needs to be refined because byproducts not removed by separation or demethylization can cause corrosion of the engine. Dry-ion exchange resins are often used to refine biodiesel because the dry-wash process is relatively quick and simple.

The primary purpose of this project was to design and test a small-scale biodiesel refinery to accompany the biodiesel reactor in Goddard Hall at Worcester Polytechnic Institute. Tests were performed at varying levels of biodiesel contamination and flow rate to determine the efficiency and life-span of the dry wash resin, DudaLite DW-R10 Ion-Exchange Resin. Glycerol and potassium, the two primary impurities associated with unrefined biodiesel, were measured using a glycerol assay, potassium ion selective electrode, and titration. It was determined that the resin refined the biodiesel to industry standards.

From the results of this study, users of this dry-ion exchange resin and others will have a comprehensive understanding of its efficiencies. The execution of this project will enhance the utility of the refined product, be of use as a laboratory experiment for other chemical engineering students, and encourage other sustainable energy initiatives on campus.

Table of Contents

Abstract 2
Table of Figures 5
Table of Tables 8
Table of Equations 8
Introduction 9
Background 10
History of Biodiesel 10
Biodiesel Production Reaction 11
Refining Methods 12
Water Washing 13
Ion-Exchange for Dry-Washing Biodiesel 14
Membrane Separation 15
Project Overview 17
Process Flow Diagram 17
Method 18
Procedures 18
Safety 26
Determining Dilutions 27
Results & Discussion 29
High Velocity Dry Washing: 29
Extended Trial Testing 37
Equilibrium Determination 39
ISE Electrode Performance 41
Titration Performance 44
Conclusion 46
Large Column Design 46
Recommendations 47
Testing Velocity 47
Testing Glycerol Concentration 48
Testing Potassium Concentration 48
Methanol Mitigation 49
Works Cited 50
Appendix 52
ASTM Standards 52
Material Safety and Data Sheets 55
DudaLite DW-R10 DryWash Resin: 55
Raw Data 62

11/6/13 High Velocity Run Data *62*
11/18/13 High Velocity Run Data *66*
11/19/13 High Velocity Run Data *69*
12/4/13 High Velocity Run Data *73*
12/5/13 High Velocity Run Data *76*
12/6/13 High Velocity Run Data *78*
Optimizing Dilution *82*
Extended Run *85*
Adsorption Isotherm *97*

Table of Figures

Figure 1: Overall Transesterfication Reaction Producing Biodiesel and Glycerol 12
Figure 2: Process Flow Diagram 18
Figure 3: Optimizing Dilution 27
Figure 4: 11/6/13 Glycerol Optical Density 30
Figure 5: 11/18/13 Glycerol Optical Density 31
Figure 6: 11/19/13 Glycerol Optical Density 32
Figure 7: 12/4/13 Glycerol optical Density 33
Figure 8: 12/4/13 Glycerol Optical Density - Dilution 1 34
Figure 9: 12/5/13 Glycerol Optical Density - Dilution 2 34
Figure 10: 12/6/13 Unit Operations 1&2 Biodiesel Mixture Optical Density 35
Figure 11: Extended Run Glycerol Profile 38
Figure 12: Extended Run Average Gylcerol Profile 39
Figure 13: Equilibrium Adsorption Optical Density 40
Figure 14: Equilibrium Adsorption Isotherm 41
Figure 15: Potassium Concentration Profile 45
Figure 16: Optical Density - 11/6/13 - Minute 20 after adding Working Reagent 63
Figure 17: Optical Density - 11/6/13 - Minute 21 63
Figure 18: Optical Density - 11/6/13 - Minute 22 64
Figure 19: Optical Density - 11/6/13 - Minute 23 64
Figure 20: Optical Density - 11/6/13 - Minute 24 65
Figure 21: Optical Density - 11/6/13 - Minute 26 65
Figure 22: Optical Density - 11/7/13 - Minute 27 66
Figure 23: Optical Density - 11/6/13 - Minute 28 66
Figure 24: Optical Density - 11/18/13 - Minute 20 after adding Working Reagent 67
Figure 25: Optical Density - 11/18/13 - Minute 21 68
Figure 26: Optical Density - 11/18/13 - Minute 22 68
Figure 27: Optical Density - 11/18/13 - Minute 23 69
Figure 28: Optical Density - 11/18/13 - Minute 24 69

Figure 29: Optical Density - 11/19/13 - Minute 20 after adding Working Reagent 70
Figure 30: Optical Density - 11/19/13 - Minute 21 71
Figure 31: Optical Density - 11/19/13 - Minute 22 71
Figure 32: Optical Density - 11/19/13 - Minute 23 72
Figure 33: Optical Density - 11/19/13 - Minute 24 72
Figure 34: Optical Density - 11/19/13 - Minute 24 73
Figure 35: Optical Density - 12/4/13 - Minute 20 after adding Working Reagent 74
Figure 36: Optical Density - 12/4/13 - Minute 21 74
Figure 37: Optical Density - 12/4/13 - Minute 22 75
Figure 38: Optical Density - 12/4/13 - Minute 23 75
Figure 39: Optical Density - 12/4/13 - Minute 24 76
Figure 40: Optical Density - 12/6/13 - Minute 20 after adding Working Reagent 77
Figure 41: Optical Density - 12/5/13 - Minute 21 77
Figure 42: Optical Density - 12/5/13 - Minute 22 78
Figure 43: Optical Density - 12/5/13 - Minute 23 78
Figure 44: Optical Density - 12/9/13 - Minute 20 after adding Working Reagent 79
Figure 45: Optical Density - 12/9/13 - Minute 21 80
Figure 46: Optical Density - 12/9/13 - Minute 22 80
Figure 47: Optical Density - 12/5/13 - Minute 23 81
Figure 48: Optical Density - 12/5/13 - Minute 24 82
Figure 49: Optical Density - Optimizing Dilution - Minute 20 after adding Working Reagent 83
Figure 50: Optical Density - Optimizing Dilution - Minute 21 83
Figure 51: Optical Density - Optimizing Dilution - Minute 22 84
Figure 52: Optical Density - Optimizing Dilution - Minute 23 84
Figure 53: Optical Density - Optimizing Dilution - Minute 24 85
Figure 54: Optical Density Extended Run - 7pm 12/10/13 to 6am 12/11/13 - Minute 20 after adding Working Reagent 87
Figure 55: Optical Density Extended Run - 7pm 12/10/13 to 6am 12/11/13 -Minute 21 87
Figure 56: Optical Density Extended Run - 7pm 12/10/13 to 6am 12/11/13 - Minute 22 88

Figure 57: Optical Density Extended Run - 7pm 12/10/13 to 6am 12/11/13 -Minute 23 88

Figure 58: Optical Density Extended Run - 7pm 12/10/13 - 12pm 12/12/13 - Minute 20 after adding Working Reagent 89

Figure 59: Optical Density Extended Run - 7pm 12/10/13 - 12pm 12/12/13 - Minute 22 89

Figure 60: Optical Density Extended Run - 7pm 12/10/13 - 12pm 12/12/13 - Minute 23 90

Figure 61: Optical Density Extended Run - 7pm 12/10/13 - 12pm 12/12/13 - Minute 24 90

Figure 62: Optical Density Extended Run - 7pm 12/10/13 - 12pm 12/12/13 - Minute 25 91

Figure 63: Optical Density Extended Run – 1pm 12/12/13 – 12am 12/13/13 - Minute 20 91

Figure 64: Optical Density Extended Run – 1pm 12/12/13 – 12am 12/13/13 - Minute 21 92

Figure 65: Optical Density Extended Run – 1pm 12/12/13 – 12am 12/13/13 - Minute 22 92

Figure 66: Optical Density Extended Run – 1pm 12/12/13 – 12am 12/13/13 - Minutes 23 93

Figure 67: Optical Density Extended Run – 1pm 12/12/13 – 12am 12/13/13 - Minute 24 93

Figure 68: Optical Density Extended Run – 1am 12/13/13 – 1pm 12/13/13 - Minute 20 after adding Working Reagent 94

Figure 69: Optical Density Extended Run – 1am 12/13/13 – 1pm 12/13/13 - Minute 21 94

Figure 70: Optical Density Extended Run – 1am 12/13/13 – 1pm 12/13/13 - Minute 22 95

Figure 71: Optical Density Extended Run – 1am 12/13/13 – 1pm 12/13/13 - Minute 23 95

Figure 72: Optical Density Extended Run - 2pm 12/13/13 – 2pm 12/16/13 - Minute 22 after adding Working Reagent 96

Figure 73: Optical Density Extended Run - 2pm 12/13/13 – 2pm 12/16/13 - Minute 23 96

Figure 74: Optical Density Extended Run – 2pm 12/13/13 – 2pm 12/16/13 - Minute 24 97

Figure 75: Adsorption Isotherm Data - Minute 20 after Loading Working Reagent 98

Figure 76: Adsorption Isotherm Data - Minute 21 98

Figure 77: Adsorption Isotherm Data - Minute 22 99

Figure 78: Adsorption Isotherm Plate Data - Minute 23 99

Figure 79: Adsorption Isotherm Data - Minute 24 100

Figure 80: Adsorption Isotherm Data - Minute 25 100

Table of Tables

Table 1: Glycerol Assay Standards 20
Table 2: ISE Results 42
Table 3: Titration Results 44
Table 4: ASTM and EN Standards for B100 Biodiesel 52
Table 5: DudaLite Composition 56
Table 6: 11/6/13 Optical Density 62
Table 7: 11/18/13 Optical Density 66
Table 8: 11/19/13 Optical Density 69
Table 9: 12/4/13 Optical Density 73
Table 11: 12/5/13 Optical Density – Dilution 2 76
Table 12: 12/6/13 UO Mixture Optical Density 78
Table 13: Optimizing Dilution 82
Table 14: Extended Run 85
Table 15: Adsorption Isotherm 97

Table of Equations

Equation 1: Overall Biodiesel Production Reaction 11
Equation 2: Glycerol Concentration Determination 22
Equation 3: Soap Calculation 26
Equation 4: Nernst Equation 43
Equation 5: Electrode Slope 43

Introduction

Due to economic, environmental, and security reasons, new fuel sources are being sought out. Even though they are gaining popularity, biofuels still make up less than 4% of total fuels consumed worldwide. Biodiesel is an alternative fuel source that can be used as a replacement for traditional diesel. It is derived from vegetable oils or animal fats; some common feeds are soybean, rapeseed, canola, sunflower, corn, palm kernels, animal fats, and recycled oil. In the United States, soybean and rapeseed are the most commonly used oils (Cheng & Timilsina, 2011). Biodiesel is produced by chemical transesterification of triglycerides from oils and fats with alcohol. This process yields a fatty acid alkyl ester, commonly referred to as biodiesel (Knothe, Krahl, & Gerpen, 2010). Refining the biodiesel after production improves the quality of the product and allows for unreacted alcohol to be recycled. Standard diesel engines that burn biodiesel instead of traditional diesel emit lower amounts of carbon monoxide, particulate matter, and unburned hydrocarbons (Van Gerpen, 2005).

Currently, the Chemical Engineering Department of Worcester Polytechnic Institute operates a biodiesel production lab through the traditional transesterification process. However, the lab lacks the infrastructure to refine the biodiesel so the finished product is not utilized. Recently, the Chemical Engineering Department purchased a heater capable of running on biodiesel. Using the unrefined biodiesel as fuel would cause premature corrosion and degradation of the unit. The biodiesel produced in the biodiesel production lab could be used to fuel the heater if a refining process was also introduced.

The goal of this project was to design and assess the efficiency of a refining process employing an ion-exchange resin. Creation of a refining process would enhance the biodiesel's utility for the Chemical Engineering Department of Worcester Polytechnic Institute and also provide students with another unit operation laboratory experiment. This research would also provide further insight into the efficacy of ion-exchange resins since there is currently not much research in this area.

Background

History of Biodiesel

The development of diesel technology began in 1893 when Rudolph Diesel published a paper containing a design for an engine that compressed vegetable oils to the point of combustion (The History of Biodiesel, 2010). Diesel engines were introduced to replace less stable steam powered engines in agriculture because petroleum wasn't readily available. At the 1911 World's Fair in Paris, Rudolph Diesel put the first diesel engine on display. Rudolph Diesel performed a significant amount of work in the development of the diesel engine for the combustion of vegetable oil, but after his death in 1913, petroleum became more widely available. With the increase in the availability of petroleum, the diesel fuel that we are familiar with today became a primary fuel source. Diesel engines were designed around the specifications of petroleum-based fuel rather than vegetable oils (A Clean Burning Alternative Fuel from Renewable Resources, 2012).

Considerable attention was not given to biodiesel until the fuel crisis of the 1970's spurred by the Organization of Arab Petroleum Exporting Countries (OAPEC) oil embargo of 1973. Consequently, the 1970's were a time of uncertainty in the energy industry marked by fuel shortages and high fuel prices (Staff, 2010). The process of transesterification is as old as diesel technology itself, but interest in process improvement for diesel technology spiked during the Oil Crisis. Improvements were needed because diesel engine specifications of the 1970's had changed since the inception of the technology. Initially, diesel engines were designed to compress the more viscous vegetable oils but the newer engines were built to burn less viscous petroleum-based diesel. Biodiesel from vegetable oils today have a viscosity that is compatible with current diesel engines (A Clean Burning Alternative Fuel from Renewable Resources, 2012).

As new energy sources are explored to slow global climate change and sustain the world's economy, biodiesel, and biofuels in general, will likely gain importance (Cheng & Timilsina, 2011). The Environmental Protection Agency (EPA) has set mandates for biofuel usage in the future, requiring increased utilization of these fuels. For 2014, about 10% of all fuel will be from

renewable sources, with 1.16% from biomass-based diesel (Office of Transportation and Air Quality, 2013). As biodiesel and other biofuels gain importance in the energy industry, research regarding the refining process for these fuels will be increasing important.

Biodiesel Production Reaction

The current method of producing biodiesel in the Chemical Engineering Department of Worcester Polytechnic Institute utilizes a transesterification reaction. The methanol is added to the feedstock oil and is heated and stirred. A potassium hydroxide catalyst is added to the mixture to facilitate the reaction and reduce the overall reaction time. The primary product is biodiesel but glycerol is also produced as a byproduct. Since glycerol has a density much greater than that of biodiesel, the two products naturally separate and the glycerol falls to the bottom of the vessel. The glycerol can be drained off the bottom until only the desired product, biodiesel, remains.

When using soybeans as the feedstock for soybean oil and biodiesel, which is the feedstock used in the laboratory setup in Goddard Hall at Worcester Polytechnic Institute, there are two main byproducts: the expeller and glycerol. The expeller is a solid residue created when the soybeans are processed to obtain the oil. Instead of waste, the expeller is a valuable product used for cattle meal. Glycerol is a byproduct of the transesterification of the triglyceride fraction of the oil (Mazzieri, Vera, & Yori, 2008).

Biodiesel from soybean oil, the feedstock used in the laboratory setup at Worcester Polytechnic Institute, is typically produced by base-catalyzed transesterification using a catalyst such as sodium or potassium hydroxide. In order to drive the reaction to completion and achieve the industry purity standards, it is carried out in two steps. In the first step of the reaction, the potassium hydroxide reacts with methanol to produce a methoxide ion catalyst, as shown in Equation 1. Byproducts of this step are small amounts of potassium soap and water.

Equation 1: Overall Biodiesel Production Reaction

$$KOH + H_3COH \rightleftharpoons H_3CO^- + K^+ + H_2O$$

Next, the triglycerides (the feedstock oil) undergo nucleophilic attack by the methoxide catalyst. This results in the mono-alykl esters (the biodiesel) and the glycerol byproduct. This reaction is summed up in Figure 1.

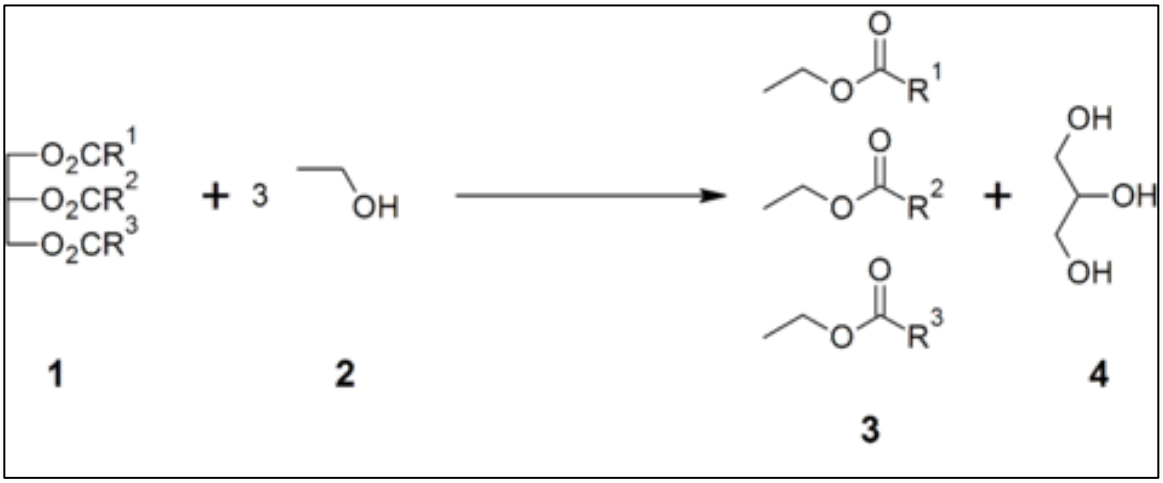

Figure 1: Overall Transesterfication Reaction Producing Biodiesel and Glycerol

Refining Methods

After the reaction is complete, glycerol is separated based on density by gravity settling or centrifugation. There are still impurities that remain in the biodiesel. These include remaining glycerol, soaps, the catalyst, water, methanol, and mono-, di-, and triglycerides (Mazzieri, Vera, & Yori, 2008). It is important to refine biodiesel in order to improve fuel combustion performance, maximize power delivered to the motor, increase the life of the diesel engine, and reduce emissions of noxious chemicals. For example, glycerol that remains in the biodiesel polymerizes when heated causing coke and tarnish to form on engine injectors and cylinders (Biodiesel, 2013) At low temperatures, glycerides form small crystals which increases the cloud point of the biodiesel (Vera, et al.). For these reasons, it is important to make sure that impurity levels do not exceed the ASTM standards after refining. The ASTM D6751 and EN 14214 standards for free glycerin and total glycerol are 0.02% and 0.25%, respectively (Jaaskelainen, 2009). The ASTM standards for other impurities can be found in the

Biodiesel feedstocks may need to be treated with several refining steps, as follows:

1. Degumming: This is necessary if there are large amounts of phosphatides present (Vera, et al.).
2. Deodorization: This is defined as the removal of aldehydes, ketones, pesticides, fungicides, and herbicides by distillation (Vera, et al.).
3. FFA reduction: This can be done by steam stripping, caustic stripping, solvent extraction, glycerolysis, or acid esterification (Vera, et al.).
4. Bleaching: This is the removal of remaining pigments, soaps, insoluble, peroxides, phospholipids, and metals from the solution (Vera, et al.).

Hydrophilic adsorbents are used in adsorption for the refining of biodiesel because most of the impurities contained within it are polar compounds. Silicas have a high saturation capacity for glycerol and glycerides and also have an affinity for soaps, FFA, metals, and salts. Utilizing adsorption units instead of water washing reduces wastewater effluents and does not require washing, oil-water separation, and wastewater treatment units.

Water Washing

Water washing is the most common method used for purifying biodiesel. In this process, water is used to dissolve methanol, glycerin, soap and other impurities in the fuel. This method is effective because methanol has a higher affinity for water than oil and the other contaminants, like soap, also dissolve readily (Mazzieri, Vera, & Yori, 2008). Since the methanol is dissolved in the water and not reacted, there is no methanol gas produced, which is both flammable and toxic (Tech, 2012).

While conservative methods can process biodiesel at a ratio of 1 part water to 5 parts biodiesel, typically, the ratio is 1 to 1, or even greater. Therefore, large volumes of effluent water are produced (Vera, et al.). Since water that remains in the fuel can corrode an engine and cause side reactions with the glycerides to form soaps and glycerol, it is important that the fuel is dried before use. Water, soaps and free fatty acids can deteriorate parts of the engine. Sodium and potassium, both used as catalysts, can form soaps deposited on the engine and cause catalytic polymerization reactions (Faccini). Because of the washing and drying processes, additional structures may have to be added to the refinery such as washing, oil-water, and

wastewater treatment units (Vera, et al.). Untreated effluent water has a negative environmental impact. After minimal treatment, the effluent water can be used for irrigation. (Tech, 2012)

This process can be automated and is flexible. An aggressive method can take just a few hours to reach completion, while a less aggressive method could take as long as a week. Since it takes several washes to purify the biodiesel, typically less aggressive methods are used first and followed by more aggressive ones. Proceeding in this order reduces the likelihood of emulsions, which are mixtures of water, biodiesel, and other byproducts.

Ion-Exchange for Dry-Washing Biodiesel

Dry-ion exchange resins are used during the refining stage of biodiesel production in order to remove any excess byproducts that were not removed prior by separation or demethylation. This particular method of using resin to extract any contaminates out of the unrefined biodiesel is called dry-washing.

Dry-washing, compared to wet-washing, has several large advantages and is becoming a more popular alternative process. In wet-washing, the inclusion of additional water to the process creates many of the disadvantages, including increased cost and production time. The first main advantage of dry-washing is that it decreases production time down to just a few hours. Another critical factor to consider is that the dry-wash process can also severely lower laboratory costs. One of the largest expenses when wet-washing biodiesel is the supply of water and the process of removing and disposing of said water. Furthermore, the dry-wash process yields biodiesel of excellent quality. Without the addition of water, the biodiesel produced through dry-washing can attain less than 500 parts per million (ppm) water content, which currently meets ASTM standards (Dugan, 2007). In wet washing, the resulting water content tends to exceed 1,000 ppm. Therefore, the wet-washing process would need to incur additional expenses to match the quality of the dry-washing process. Finally, the factor that makes dry-washing so much more efficient and effective than wet-washing is the resin's ability in the dry-washing process to be reused. In order to maintain the quality performance of the resin, flushing the resin with methanol can restore some of its absorbent capabilities. The

introduction of methanol to the resin releases the contaminants caught in the resin, and carries them out of the system. Once the methanol begins to run clear after flushing out the contaminants, the methanol can be left to drain out of the column, and the resin should be ready for reuse.

What makes the dry-washing process so effective relies entirely upon the resin that is used. Some of the more popular resin brands taken into account for this project are Amberlite and Purolite. Both have near similar prices, are both based on a gel-type resin, and function almost exactly the same. The main difference between the two products is in the amount of clean biodiesel that they can produce. Typically, Amberlite tends to be able to produce more pure biodiesel per pound of resin than its competitor Purolite. Purolite is a dry wash ion exchange resin used to refine biodiesel. It has similar properties and functionality compared to Amberlite, but it is more cost effective. The resin is placed in a column forming a bed and the biodiesel is pumped through. It can be used until spent and disposed of or it can be regenerated using a methanol wash (Purolite PD206 Dry Wash Resin, 2013). Purolite is estimated to treat approximately 100 gallons of biodiesel for every pound of resin used. Amberlite on the other hand, claims to be able to produce somewhere in the range of 900-1600 pounds of biodiesel for every pound of resin used (Amberlite BD10DRY - Frequently Asked Questions). If the density of biodiesel was assumed to be approximately 0.9 g/cm^3 or 7.51 lb/gal, then Amberlite would claim to treat about 120-215 gallons of biodiesel for every pound of resin used.

Membrane Separation

In recent years, membrane separation has begun to receive increasing attention as a viable method of refining biodiesel. Traditionally, biodiesel has been processed by methods such as gravity separation, water washing, and acid washing. Membrane technology has been shown to exhibit several advantages over these conventional separation methods such as improved cost efficiency and increased specific mass transfer area (Atadashi, Aroua, & Abdul Aziz, 2011). Despite these and other advantages, the efficacy of membrane separation has not been studied extensively.

Currently, membrane technology is commonly used for water purification and protein and gas separation (Gomes, Pererira, & de Barros, 2010). While it has not been studied extensively with regards to biodiesel, several studies have shown that it is a promising separation technique. Dube et al. compared a membrane separation process to the standard water washing process. The study found that membrane separation not only succeeded in purifying biodiesel to ASTM standards, but that it also drastically reduced the amount of water required to remove free glycerol. The membrane separation process used merely 2.0g of water per liter of treated fatty acid methyl ester (FAME) while the water washing process used 10 liters of water per liter of treated FAME. This reduction in the water necessary to complete the separation both simplifies the process and reduces the overall cost (Saleh, Tremblay, & Dube, 2010).

Yong Wang et al. studied a ceramic membrane separation process for the purification of biodiesel. Utilizing a membrane of pore size 0.1 μm, Yong Wang et al. pumped the unrefined biodiesel at a flux of, which reduced the free glycerol content to 0.0108%, which meets the ASTM standard. Following the filtration through the membrane, the biodiesel solution was mixed with methanol at a flux of . This step further purified the biodiesel due to the excellent solubility of free glycerol and soap in the methanol. Like the previously mentioned study, this study yielded virtually no waste water (Wang, Wang, Liu, Ou, Tan, & Tang, 2009).

Project Overview

The goal of this Major Qualifying Project was to investigate the effectiveness of Dudalite DW – R10 ion exchange resin for refining biodiesel produced in the Worcester Polytechnic Institute (WPI) Unit Operations Lab. Before this project, the chemical engineering department had a lab capable of producing biodiesel, but no method to refine the final product. This resulted in the biodiesel not being utilized. This project involved research of different refining processes, the design of a dry washing column, execution of the design, and testing of the refined biodiesel. The design was created based on research into refining methods and necessary parts were ordered. Once construction of the small testing column was complete, the team designed and ran experiments.

Process Flow Diagram

Figure 2 is a process flow diagram of the dry washing system used for testing and unit operations production dry washing. The smaller column was used for determining breakthrough and exhaustion of the resin. The smaller column was use for the majority of testing before implementing the main column. The main column was setup to run Unit Operations diesel, but life of the column has not been verified experimentally. Selection of which column will perform the washing is done using the 3 way valve at the top of the columns. Raw diesel product is pumped using a high precision liquid chromatography pump. All biodiesel product is fed into a final product tank. The columns are always ran with fuel being fed top down.

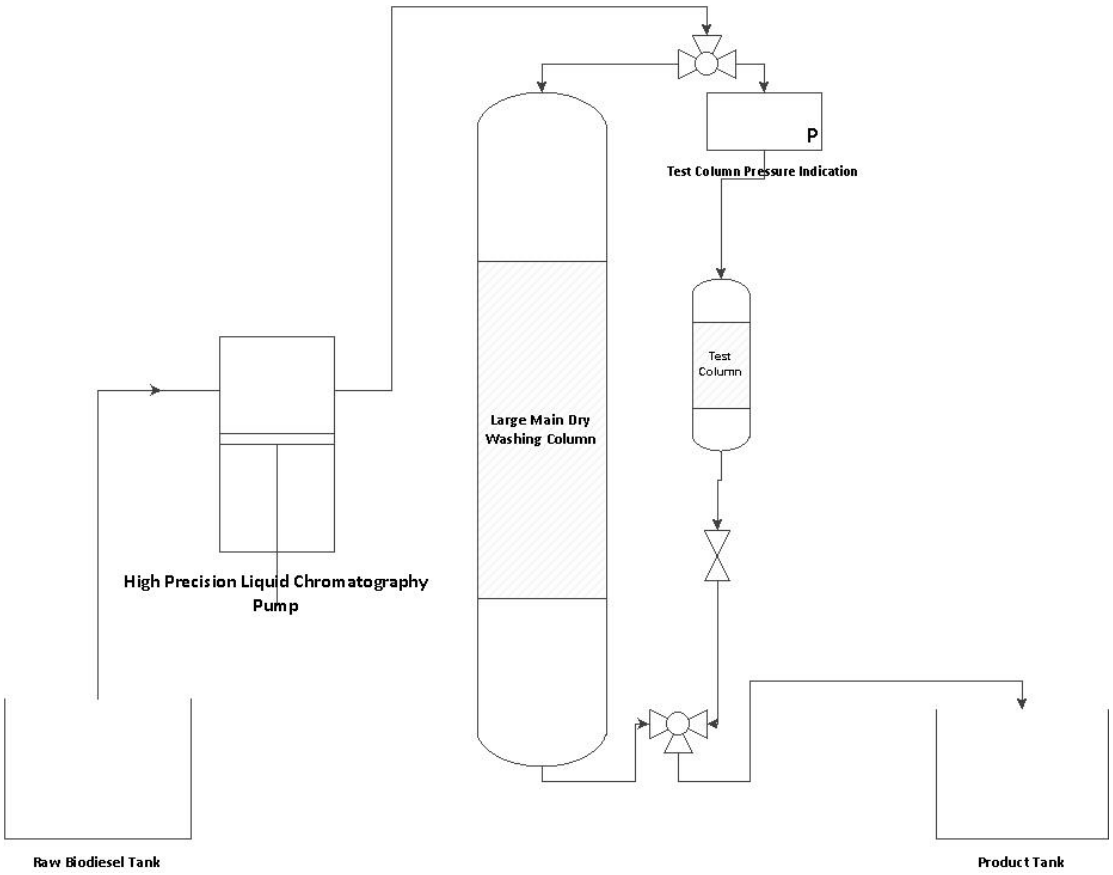

Figure 2: Process Flow Diagram

Method

Procedures

Setup

- All parts were acquired:
    - 2 – 3way connectors
    - 2 glass columns
    - 14 fittings and ferrules
    - 1 High Precision Liquid Chromatography Pump
    - Finishing tank
    - Feed Tank
    - Raw diesel
    - Teflon tubing

- Pressure Gauge

1. Fittings and Tefzel cones were installed on the tubing sections
2. The pressure gauge was installed after the 3 way column selection valve.
3. The sections of piping and fittings were installed per the diagram
4. It was ensured that column selector valve was directing flow to the top of the small testing column.
5. The valves leaving the columns were checked to ensure flow to the finishing tank
6. After double checking that all connections were secure, water was pumped through the system to check for leaks.
7. The valves were rotated to check for proper operation.
8. The columns were drained of all water and allowed to dry.
9. The columns were loaded with resin per loading instructions and resealed.

Loading Column

There is an explosion risk due to potential static discharge when loading a previously used column with fresh resin, especially if the relative humidity is less than 60%. In order to minimize risk, the column was rinsed with biodiesel containing less than 2.5% methanol by weight before beginning to load the column (Amberlite BD10DRY - Frequently Asked Questions).

1. Once a column was dried with pressurized air and Kim wipes, the desired amount of resin was weighed out.
2. Enough Steel wool is placed in the bottom of the test column (Kim wipe for the large column) to stop the resin from falling out, but not restrict flow severely. The bottom fixture is then threaded into place and secured.
3. The resin is poured into the top of the column. Due to swelling of the resin, the amount of dry resin added should never fill the column more than 1/3 full.
4. The top of the column was sealed.
5. Enough biodiesel was fed from the bottom of the column to cover the resin. The resin was allowed to swell for one hour before beginning to run biodiesel through the column.
6. The biodiesel was run from the top of the column at the desired flow rate.

Unloading Column

1. Stop the flow of biodiesel.
2. The valve at the bottom of the column was opened so that the remaining biodiesel in the column could drain out.
3. Compressed air was applied to the resin bed for one hour to further dry the resin.
4. The bottom of the column was opened and the resin was emptied out and put into containers suitable for transporting.
5. Residual resin was blown out using compressed air.
6. Since the resin is non-hazardous, the spent resin is incinerated. When disposing of resin, local regulations regarding disposal should be consulted.

Cleaning Column

1. After unloading the column, the column was flushed with methanol. The volume of methanol used was five times the bed volume.
2. The methanol was drained from the column.
3. The column was allowed to dry. If time was a constraint, compressed air was run through the column.
4. Column was returned to service.

Glycerol Assay Procedure

The procedure for measuring glycerol concentrations via the EnzyChrom Glycerol Assay Kit was as follows:

1. Before testing the biodiesel for glycerol, five of each of the standards shown inTable 1 were created:

Table 1: Glycerol Assay Standards

No	STD + H_2O	Vol (µL)	Glycerol (mM)
1	10 µL + 990 µL	1000	1.0
2	6 µL + 994 µL	1000	0.6
3	3 µL + 997 µL	1000	0.3
4	0 µL + 1000 µL	1000	0

- Each standard was created by diluting 10 µL of the provided 100 mM glycerol (100 µL total) with 990 µL distilled water. Each of the ten standards was placed into separate wells of a clear 96-well plate and stored in a refrigerator for future use. These standards acted as a comparison point for the actual samples that were tested.

2. The purchased (clean) biodiesel was tested to validate whether or not it was actually free of glycerol. Much like the 10 µL standards, the samples were prepared by diluting 10 µL of biodiesel with 990 µL of distilled water. These samples were placed in separate wells of a different 96-well plate.
3. The standards and actual samples were then tested for glycerol concentration:
    - For each reaction well (both the standard and the actual sample), the Working Reagent was created with the following (provided) materials:
        - 100 µL Assay Buffer
        - 2 µL Enzyme Mix
        - 1 µL ATP
        - 1 µL Dye Reagent
    - 100 µL of the Working Reagent was added to each reaction well. The plates were tapped to mix the solutions.
    - All samples were then incubated at room temperature for 20 minutes.
    - The optical density of each sample was determined via a colorimeter. Each plate was read at an optical density of 570 nm (average of 550-585 nm).
4. The glycerol concentration for each sample was then calculated via the following steps:
    - The difference between the optical density of the standards (first three rows of Table 1and that of the pure distilled water (last row in Table 1) was calculated. These differences were then plotted against the standard glycerol concentrations (shown in the fourth column of Table 1).
    - The glycerol concentration of the sample was then calculated via Equation 2: Glycerol Concentration Determination

Equation 2: Glycerol Concentration Determination

$$[Glycerol] = \frac{OD_{sample} - OD_{H_2O}}{Slope}$$

OD_{sample} is the optical density of the sample. OD_{H2O} is the optical density of pure distilled water (last row in Table 1). The slope is the slope from the aforementioned plot.

5. Steps 2-4 were executed in order to validate whether or not the purchased biodiesel was actually free of glycerol or not. Once a certain batch of biodiesel was confirmed to be clean, it was ready to be properly tested.
    - Several samples were created where glycerol was added to the clean biodiesel. This mixture was then passed through the column.
    - Once the biodiesel was purified, samples were prepared just as they were in step 2.
    - The glycerol concentration was determined just as it was in steps 3-4.

Potassium Ion Specific Electrode Procedure

Equipment:

- Thermo Scientific Orion ISE meter
- Cole-Parmer ISE electrode, potassium, double-junction, BNC
- Magnetic stirrer
- DI Water
- Potassium electrode filling solution
- Sample containers
- 1mL pipet
- Volumetric flask
- Graduated cylinder
- Beaker
- 0.1M KCl potassium calibration standard
- Potassium ionic strength adjuster

Procedure:

1. Remove the module from the storage vial and attach it to the electrode handle.

2. Ensure that the electrode has enough filling solution. The level of filling solution should always be above the level of the sample in the beaker.

3. Prepare a 10-2K+ standard into a 100mL flask, and fill the flask to the 100mL line with DI water.

4. Connect the potassium electrode to the BNC port on top of the ISE meter and turn on the meter by holding down the power button.

5. Press the accept key located on the middle-right of the meter and align the ISE icon on the top line. Press the calibration key on the top left of the meter to begin calibration.

6. Rinse the electrode with DI water and soak it in the 10-2

7. Once the ISE icon stops flashing, press the digits key located on the middle-left of the meter. Then change the value with the arrow keys until the meter displays the correct concentration value of the prepared standard.

8. Repeat steps 5-7 for each concentration of standard solution prepared to ensure the desired range of potassium is within calibration. Then press the measure key to save the calibration.

9. For measuring, press the settings mode key located on the bottom left of the meter. Scroll through the available options until ISE is displayed on the top line and press the accept key.

10. Then check the ISE measurement units and calibration range on the following screen. Press the accept key again to lock in any changes.

11. Once the settings are placed, press the measure key located on the top right of the meter to return back to measurement mode.

12. Prepare the sample to be analyzed by diluting 1mL of the biodiesel product into 50mL of DI water.

13. Rinse the electrode with DI water and blot dry with a lint free tissue. M potassium standard solution. Pipette 10mL of known 0.1 M.

14. Place the electrode in the sample and wait for the ISE icon to stop flashing, which indicates that the reading is stable. Press the measure key to log the measurement.

15. Remove the electrode from the sample and rinse with DI water. Blot the electrode dry and place it in the next sample and repeat step 13.

16. Once all samples have been measured, rinse the electrode with DI water and return the module to the original storage vial.

Potassium Titration Procedure

Titration was performed in order to determine the potassium content in various samples of biodiesel. The specific procedure that was used is detailed below.

Equipment:

- Biodiesel samples
- Isopropyl alcohol
- 0.01 M HCl solution
- Phenolphthalein
- Bromophenol blue
- Burette
- Magnetic stirring plate
- Various beakers (50, 100, and 250 mL were common)
- 5 and 10 mL micropipettes
- Micropipette tips
- Eyedroppers (for phenolphthalein and bromophenol blue)
- Weighing scale

Procedure:

1. A specific amount of biodiesel (in grams) was measured via the scale in a 50 ml beaker. This was dependent on the availability of biodiesel. For example, 10 grams was used for certain samples whereas only 3 grams was used for others.

2. A specific amount of isopropyl alcohol was measured in a separate beaker. As in the previous step, the amount varied based on availability of biodiesel. In all cases, the amount of isopropyl alcohol (in mL) required was ten times the amount of biodiesel (in grams). For example, if 10 grams of biodiesel was used, then 100 mL of isopropyl alcohol was necessary.

3. The biodiesel from the first step and the isopropyl alcohol from the previous step were combined in a suitable beaker. This was done to dilute the biodiesel in a consistent fashion every time.

4. With eyedroppers, ~5 drops of phenolphthalein and ~10-20 drops of bromophenol blue were added to the solution from the previous step. The specific amount of bromophenol blue depended on the volume of the previous solution. In all cases, the solution turned from a murky yellow (the color of biodiesel) to dark blue.

5. A 50 mL burette was completely filled up with the 0.01 M HCl solution.

6. Underneath the burette, a magnetic stirring plate was set up and the biodiesel solution was placed on it.

7. With the stirring plate turned on, the 0.01 M HCl solution was slowly dispensed into the biodiesel solution. Once the color of the solution in the beaker turned from dark blue to yellow, the titration was ceased.

8. From the burette, the amount of 0.01 M HCl added to the solution was measured and recorded.

9. With the result from the previous step, the concentration of potassium soap in the biodiesel was calculated according to the following equation:

Equation 3: Soap Calculation

$$PPM\ Soap = \frac{(mL\ HCL) * .01 * 320.56}{g\ Biodiesel} * 1000$$

10. The first term in the numerator is the result from the previous step. The second term (0.01) is the concentration of the HCl solution, which is constant. The third term is the catalyst factor for potassium hydroxide, which was used for the production of the biodiesel [1]. The term in the denominator is simply the amount of biodiesel used, in grams.

11. The calculated result from the previous step was recorded.

Safety

Equipment:

- Non-Latex Protective Gloves
- Goggles/ Safety Glasses
- Experimental Hood

Determining Dilutions

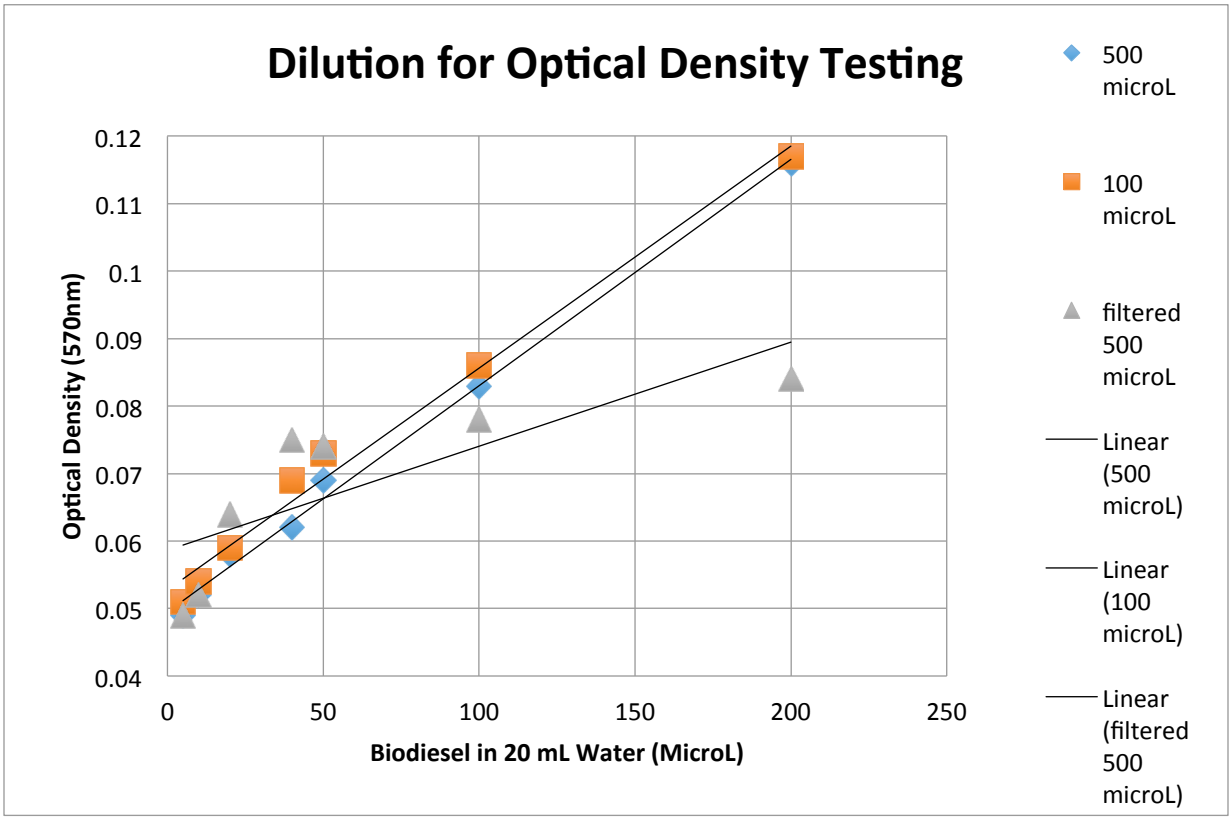

Figure 3: Optimizing Dilution

The glycerol assay used in the project is only valid and accurate for optical density values between 0 and 1. To determine an optimal dilution scheme for the glycerol assay, relative concentrations were determined by spiking clean biodiesel in three different ways: 503 µL of glycerin in 1600mL of clean purchased diesel, 103 µL of glycerin in 1500mL of clean purchased diesel, and 503 µL of glycerin in 1600mL of clean purchased diesel followed by vacuum filtering. Figure 3 was established by diluting these three biodiesel solutions in 20 ml of distilled water. Through this dilution method, one can see the relative amounts of dissolved glycerol in the solution. It was found that after biodiesel solutions spiked with 503 µL of glycerin and 103 µL of glycerin were processed, they exhibited similar dilution behavior. The vacuum filtered solution, however, showed lower relative glycerol concentrations at higher dilutions.

These results suggest that by vacuum filtering the biodiesel, one may be able to improve the performance of the column. With less glycerol content to process, the column would have a

longer usable life. This test provided enough information to determine a dilution of 200 µL of biodiesel product in 10mL of Deionized water for the extended run testing. Considering the results of this dilution test, vacuum filtering before dry washing the fuel should be further investigated.

Results & Discussion

The overall goal of this Major Qualifying Project was to study the performance of DudaDiesel DW-R10 dry wash resin, for the application of washing biodiesel produced in the Unit Operations lab. The primary basis for performance was how well the resin removed glycerol and free potassium ion. Three major test types were used to fully investigate the performance of this dry wash resin. The major testing types were: high velocity washing, extended run washing, and equilibrium testing. Using the data collected from these various types of experiments, the group was able to predict the breakthrough of the resin in a large scale column depending on mass of resin in the column.

High Velocity Dry Washing:

The Unit Operations biodiesel produced typically has a glycerol concentration greater than 1000ppm directly after production. Per the literature provided by DudaDiesel, the maximum flow rate for processing biodiesel with glycerol concentration greater than 1000ppm is 1.75 times the bed volume. It was decided to investigate this claim made by the supplier. To test the flow rate suggestion by DudaDiesel the biodiesel was pumped at flow rates higher than the suggested maximum. The data for the various runs can be seen below.

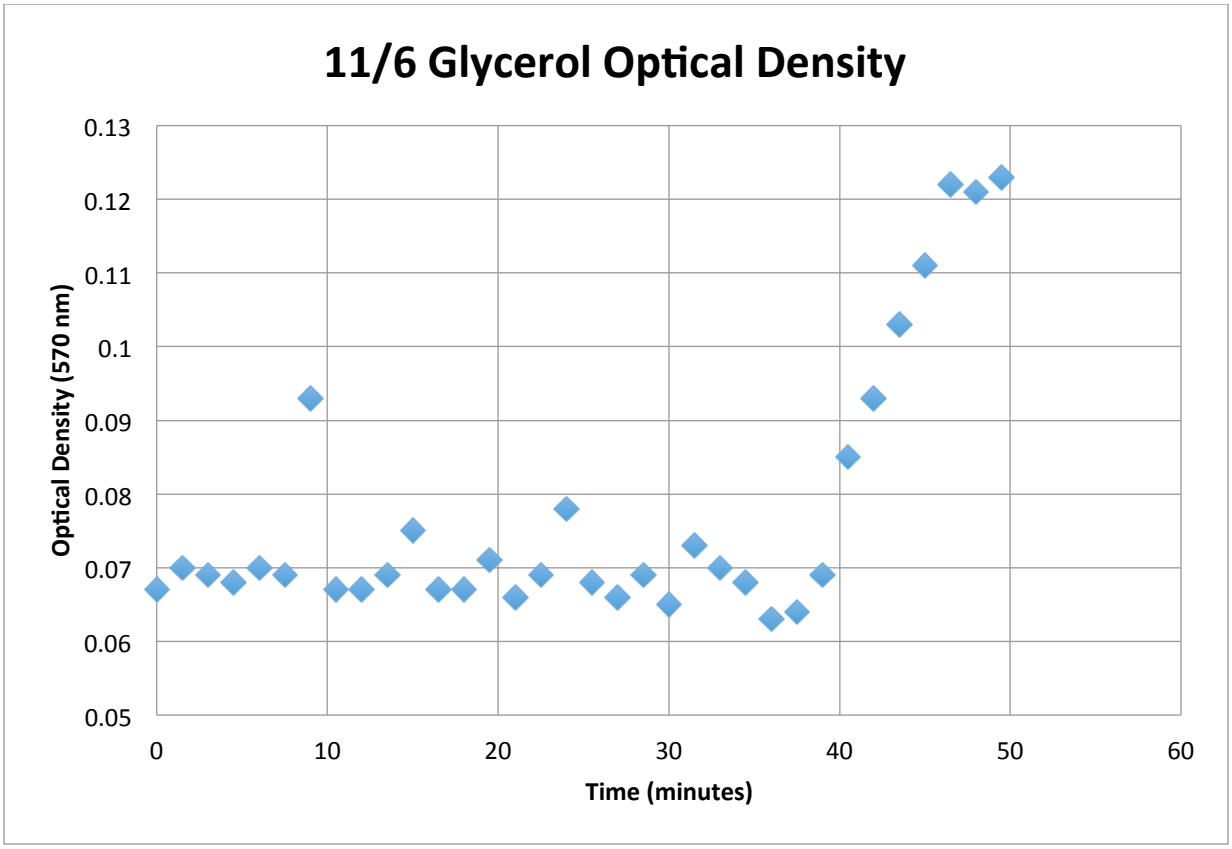

Figure 4: 11/6/13 Glycerol Optical Density

100 µL of pure glycerin was added to 1500 mL of clean biodiesel to simulate biodiesel with a glycerol concentration of 1000 ppm. This diesel was passed through the small test column at a rate of 22.7 ml/min through 0.75 grams of dry wash resin. The suggested flowrate for this amount of resin is roughly 5 ml/h. The product diesel was sampled off the column for 15 seconds every 1.5 minutes. Each sample was diluted and from Figure 4 it can be seen that the simulated 1000 ppm biodiesel performance. For roughly 40 minutes the column performed well. Complete exhaustion occurred at about 45 minutes. The data was taken every 1.5 minutes to ensure that a sufficient number of data points was obtained. From this point forward, the goal was to replicate this result to ensure reproducibility.

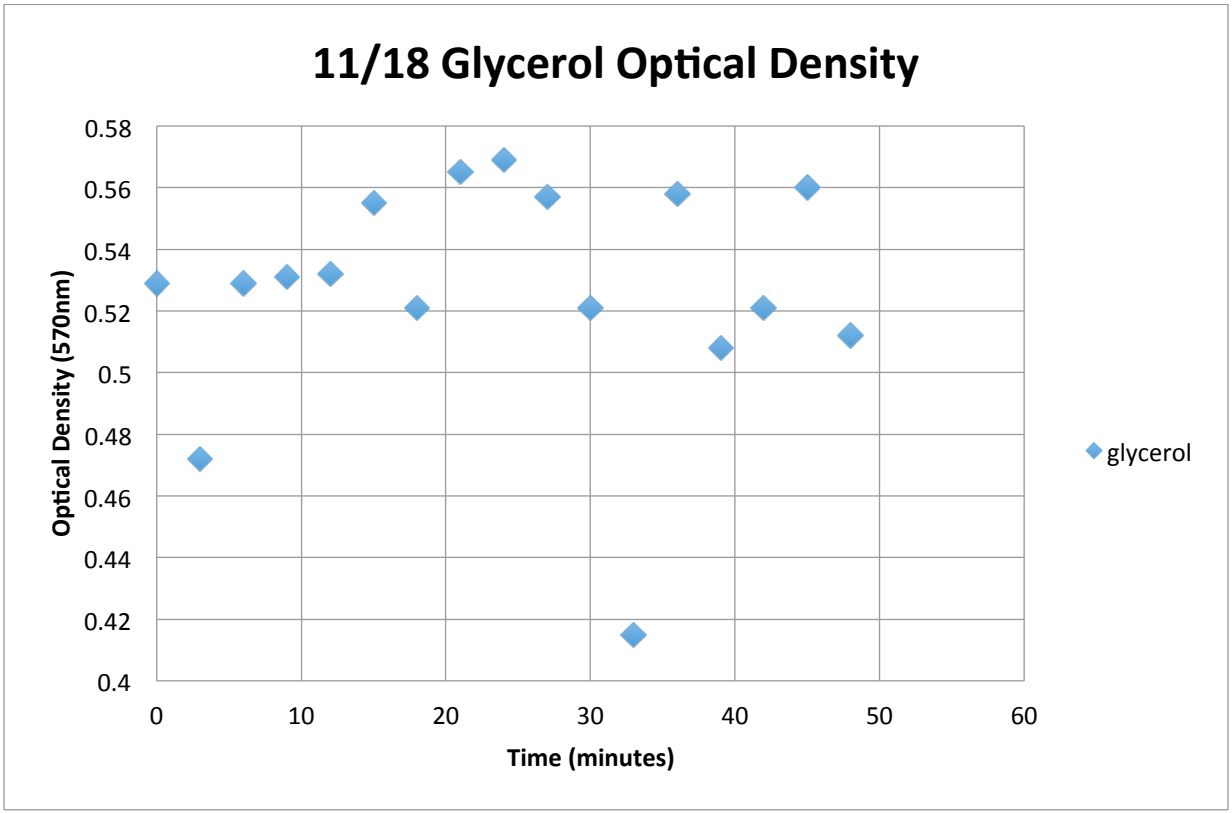

Figure 5: 11/18/13 Glycerol Optical Density

After observing the column exhaustion and breakthrough as shown Figure 4, it was decided to test a biodiesel mixture with a higher glycerol concentration. It was expected that column exhaustion would occur more rapidly with a higher glycerol concentration. The solution consisted of 500 µL of glycerin in 1600mL of B100 purchased biodiesel. The results of this test can be seen in Figure 5. These results do not exhibit the distinct breakthrough trends as seen in Figure 5. These results suggest that the column may have been overwhelmed by the glycerol concentration and the high flow rate. By overwhelming the column, the resin was not able to effectively remove the glycerol from the solution. The optical densities in Figure 5 are clearly higher than those shown in Figure 4 due to a different dilution ratio.

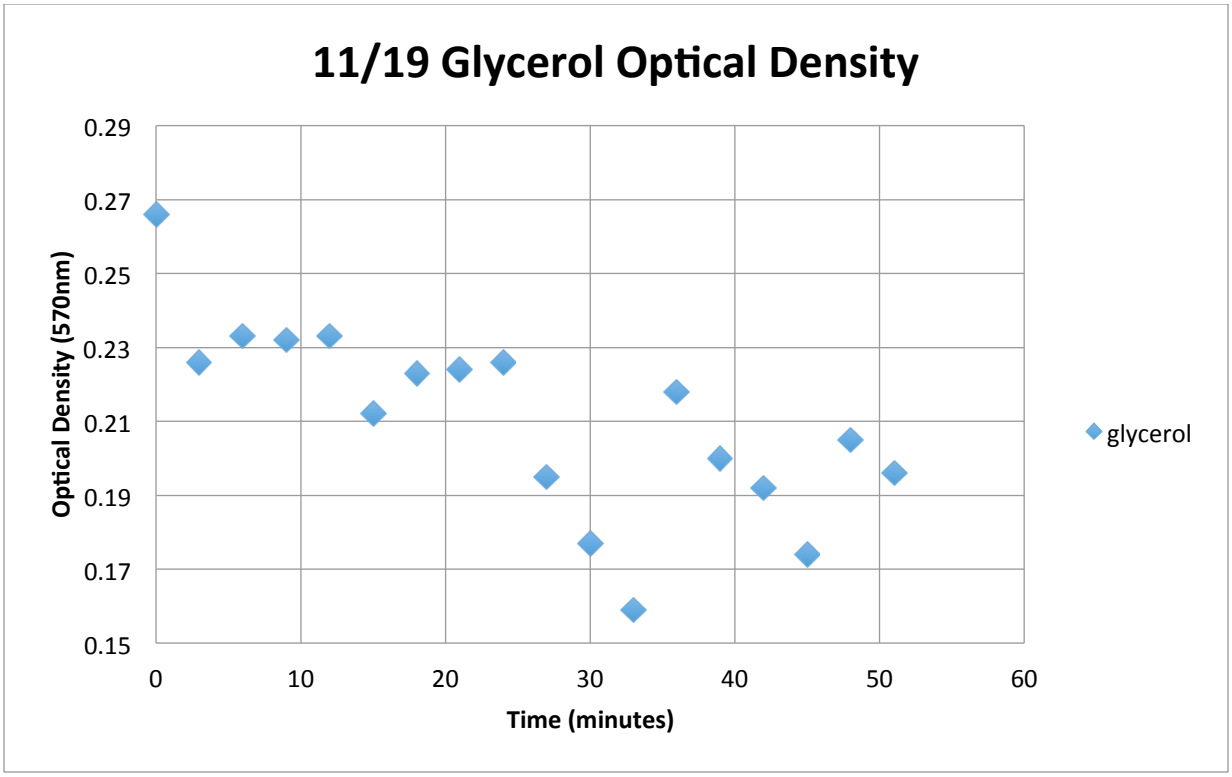

Figure 6: 11/19/13 Glycerol Optical Density

Since Figure 5 suggested that the column was overwhelmed, it was decided that the test shown in Figure 4 should be reproduced to ensure the accuracy of the results. Figure 6 highlights the result of a solution of 103 µL of glycerol in 1550 mL of clean B100 biodiesel which was passed through 0.747 grams of resin at a rate of 22.7 mL/min. The data seen here is inconsistent with both the literature from DudaDiesel and Figure 4. The data shows a general downward trend of glycerol concentration. This suggests that the resin became more effective as time proceeded. Traditionally, packed bed adsorption performance is best at the very beginning of a run. This is due to the amount of unoccupied space on the material surface diminishing; therefore a reduction in glycerol uptake would be exhibited. The data shown in Figure 6 therefore contradicts the literature and lab tests.

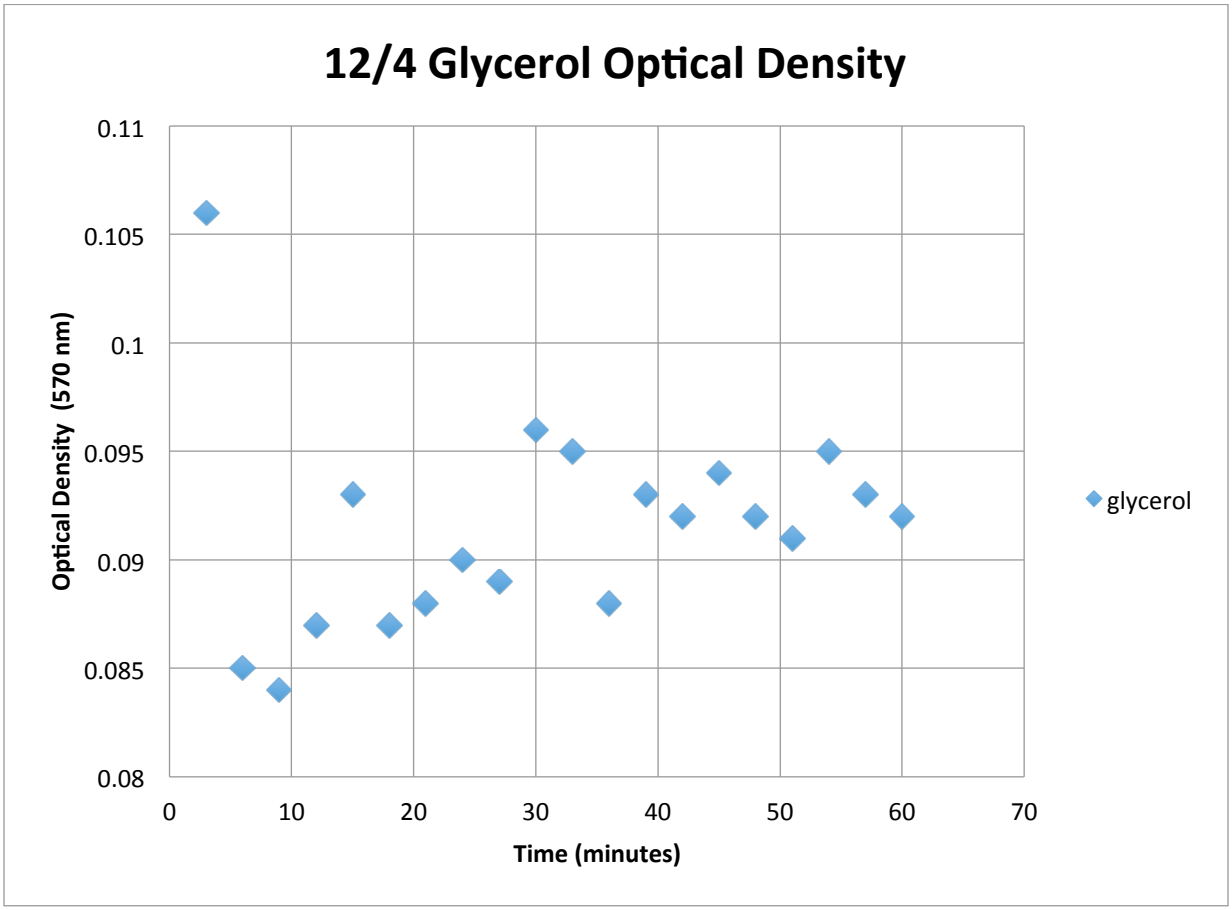

Figure 7: 12/4/13 Glycerol optical Density

In an effort to produce results consistent with the literature, the processed diesel from the test associated with Figure 5 was processed in 0.75 grams of resin at the maximum flow rate of 22.7 mL/min. The results shown in Figure 7 were not ideal but showed the performance of the column trending appropriately. Initially, the concentration of glycerol was high but it then decreased rapidly. After this drop, the data shows a slight upward trend which signified the degradation of the column as unoccupied sites were filled up.

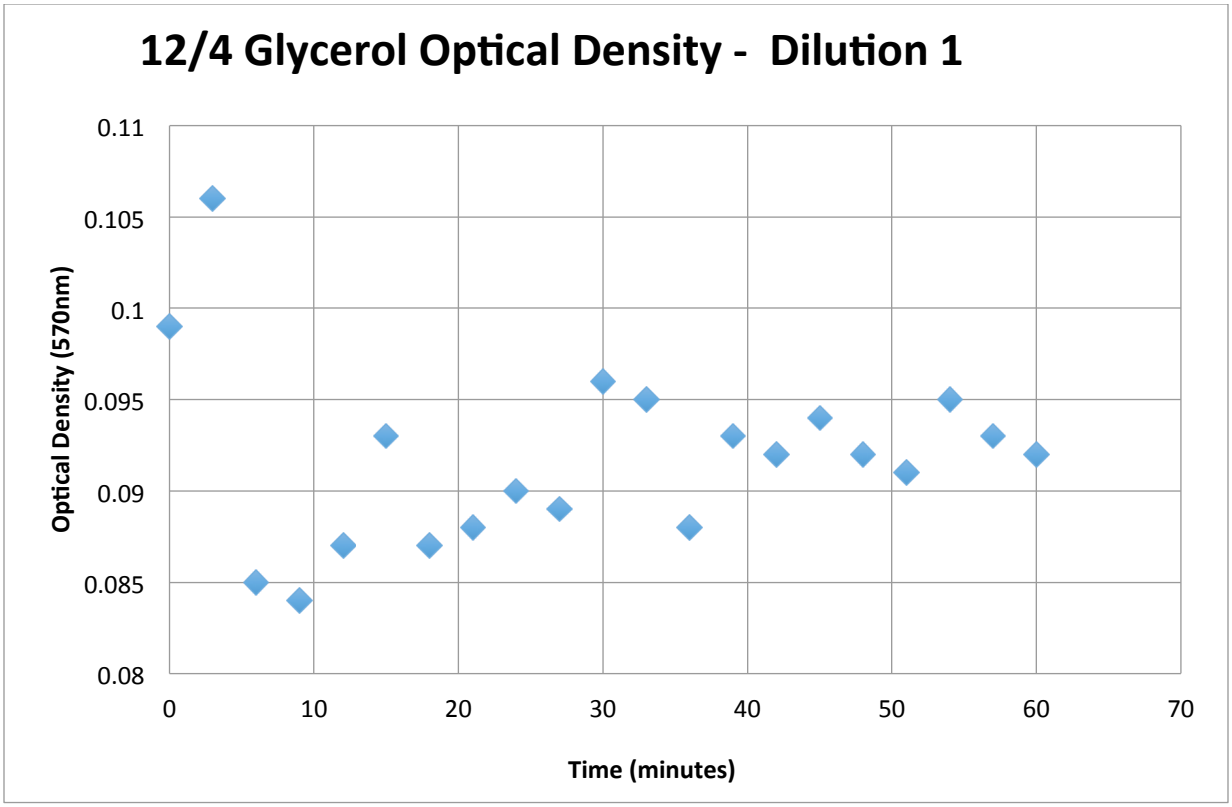

Figure 8: 12/4/13 Glycerol Optical Density - Dilution 1

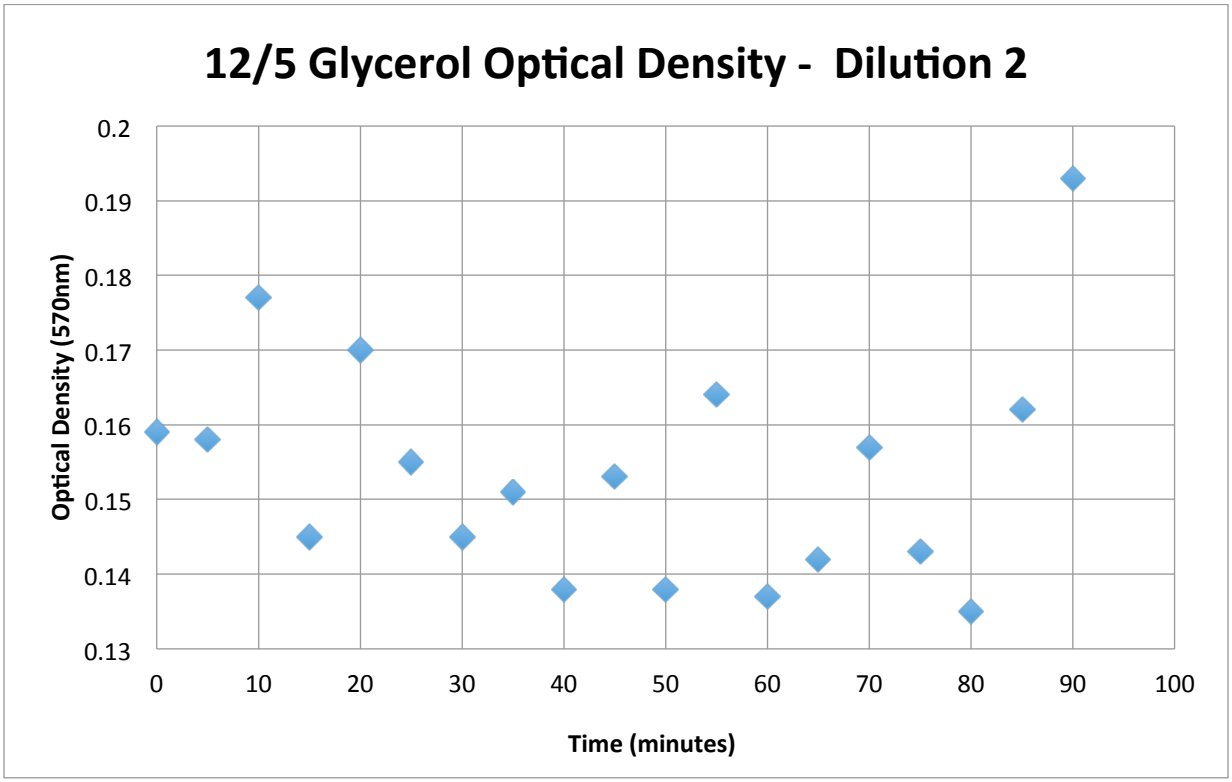

Figure 9: 12/5/13 Glycerol Optical Density - Dilution 2

Figure 8 and Figure 9 represent the test results from processing a solution of 500 µL glycerin in 1600 mL of B100 purchased biodiesel after vacuum filtering. This was a small investigation into the effectiveness of vacuum filtering for the removal of glycerol. Considering the assay results from Figure 5, it was found that filtering was somewhat effective. Figure 8 shows that the glycerol level was initially high followed by a rapid decrease. Following this decrease, the data began to show breakthrough. As seen in Figure 9 there was an increase in glycerol concentration starting around 80 minutes. The data does not represent a typical breakthrough curve. The starting glycerol concentration was lower than the final concentration, which should not be possible because glycerol was not being added to the solution. Additionally, the shapes of the two curves are different. This suggests that the data could be erroneous because the same diesel was tested in both data sets. There could have been an issue with glycerol dissolving in the solution.

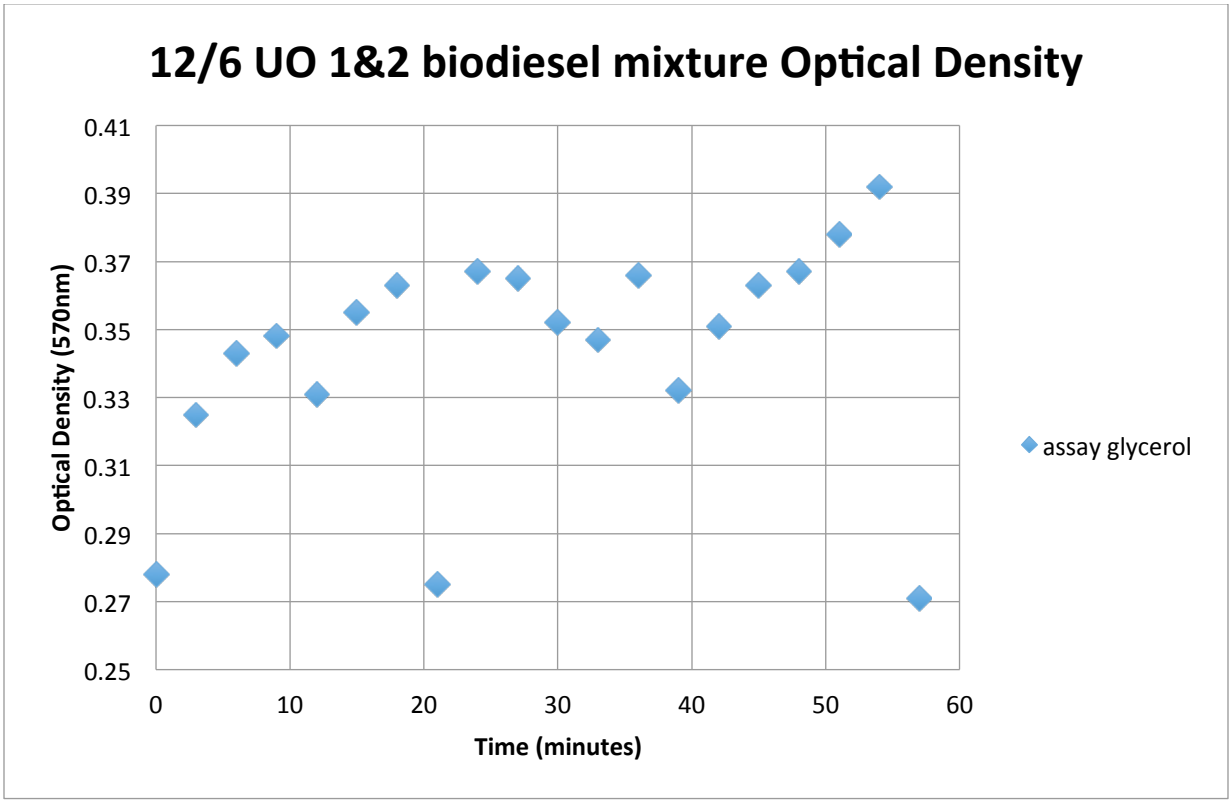

Figure 10: 12/6/13 Unit Operations 1&2 Biodiesel Mixture Optical Density

After trying to replicate the results from Figure 4 and finding no success, it was decided to attempt to run the biodiesel produced by the Unit Operations reactor system. This data can be

seen in Figure 10. Like the data of Figure 6, the data seen here is not typical for column adsorption. Once again, it can be seen that using high velocity through the packed bed is not a viable method of washing biodiesel. The glycerol concentration continued to rise with time. This rise could be due to the perturbation of undissolved glycerol on the bottom of the tank. If this happened, the concentration of glycerol would increase as the remaining biodiesel was diminished.

Overall, high velocity washing was found to be ineffective. The results presented in this section show an inconsistent performance at higher velocities through the packed bed for this particular DudaDiesel dry wash resin. These inconsistencies could have arisen from a multitude of sources. The primary source for the poor data is thought to be wall effects of the diesel flow. As the diesel entered the column, there could have been adhesion to the wall. This in combination with the high velocity could have forced the liquid to flow around the packed resin bed.

The high velocity run performed on November 6, 2013 resulted in data that was consistent with the literature for exhaustion of a resin. However, this data may not be as consistent as it could have been. Data like this could also be seen if a slug of undissolved glycerol was fed through the column. When this concentrated amount of glycerol passed through the column, it would show results similar to column exhaustion.

With regards to the atypical data from the tests discussed above, it can be seen that the initial data point (time zero) was occasionally lower than the final data point. This could have been due to the sampling position of the feed line relative to the point where the time zero was taken.

The replication of the original November 6, 2013 test results was never successful. It was determined that high velocity washing was an ineffective way to wash biodiesel and remove glycerol. Of the seven sets of data presented, only two of the sets present compelling evidence that glycerol can be removed effectively until exhaustion of the resin beads occurs. These results led to the investigation of washing biodiesel at the DudaDiesel's specifications.

Extended Trial Testing

After running several high-velocity tests and examining the results, it was determined that the data was unsatisfactory. The rate at which biodiesel was being processed by the column was too fast to properly extract glycerol from the solution. In an effort to perform the tests in a reasonable time frame, the effectiveness of the test may have been compromised. Operating at a slower flow rate, however, would likely allow the resin to function as it would in an actual biodiesel production process.

With this in mind, it was decided to process unrefined unit operations' biodiesel at a much slower rate. In consideration of DudaDiesel's literature, the volumetric flow rate was set to 5 ml/hr. To achieve this much smaller flowrate, a high precision liquid chromatography (HPLC) pump was used. Calculations based on estimated contamination levels and the literature predicted that breakthrough would occur at approximately 40 hours at the selected flow rate. For the first few hours, samples were collected and tested every hour to make sure that contamination levels had dropped, showing that the resin was working and absorbing the contaminants. Once it was clear that the resin was performing appropriately, sample collection was performed every four hours. As the time approached 40 hours, sample collection was performed at one hour intervals again to ensure an adequate number of data points during this critical period.

A sudden increase in glycerol concentration was expected shortly after breakthrough, which would have shown that the resin was exhausted and no longer absorbing contaminants. Breakthrough was observed at the expected time, but not exhaustion. Instead, the test was continued for much longer and a slight increase in glycerol concentration in the product was observed.

The results of this test can be observed in Figure 11 and Figure 12. During the first few hours, concentration levels were low. This shows that the resin was performing well and that it was absorbing most of the glycerol from the biodiesel. While there was deviation from sample to sample, at around hour 40, the glycerol concentration levels became noticeably higher. By hour 80, the glycerol concentration became more constant. It appears that the last six data points

were out of range of the previous data points. The average concentration of the final six points was 1.84 mmol/L while the average of all points after the 80th hour was 1.95 mmol/L. The trend observed in Figure 11 and Figure 12 is that of a typical breakthrough curve for the glycerol assay. The results can be split into three sections. The initial section, roughly between hours 0 and 40, is when the resin was performing optimally. Between hours 40 and 80, the effectiveness of the resin began to decrease. By hour 80, the resin was performing at a consistently lower effectiveness. Based on these results, breakthrough seems to have occurred between 40 and 80 hours. (Note: 5% performance loss, breakthrough, is shown by the red line on the following figures.)

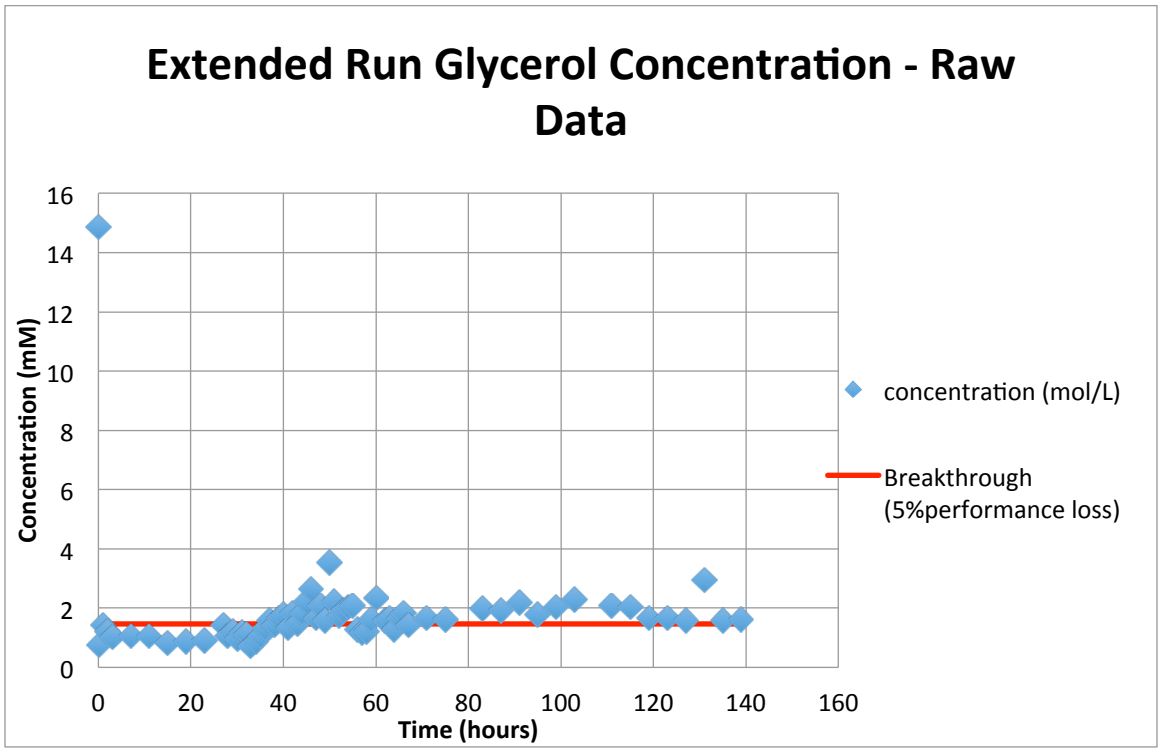

Figure 11: Extended Run Glycerol Profile

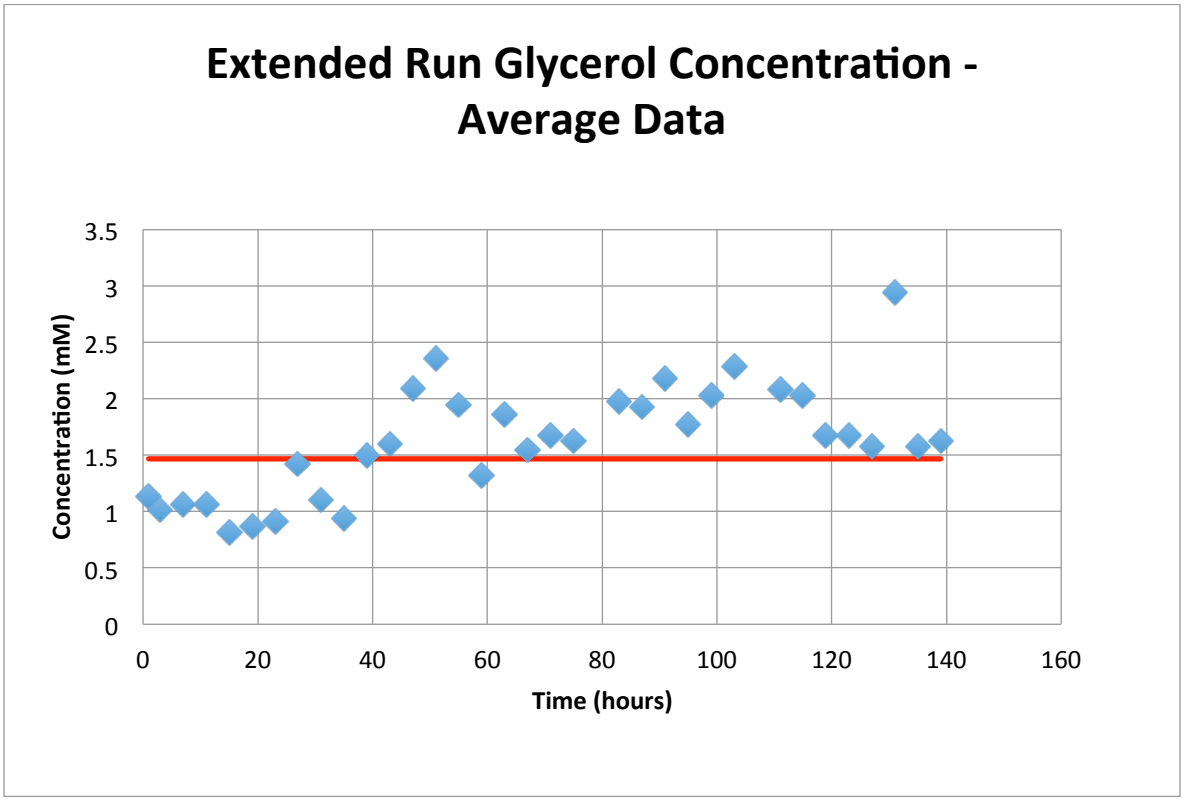

Figure 12: Extended Run Average Gylcerol Profile

With this in mind, what had been calculated to be the time it would take to reach breakthrough was actually the time that it took the resin to begin to lose functionality. The calculations performed prior to beginning the experiment were based off of information found on the resin website, not based on a scientific journal or a similar experiment. The definition of breakthrough was different for different sources and it may be that the understanding of what the resin website believed breakthrough to be was different than the definition used for this major qualifying project.

Equilibrium Determination

In effort to determine the equilibrium performance of the resin material an experiment was develop to generate an adsorption isotherm. The test was conducted at room temperature in a series of test tubes. 20ml of biodiesel was used for each test tube and the amount of resin placed in each tube was varied. The resin was allowed to sit in the solution for approximately 2 months. It was decided that 2 months was sufficient to reach equilibrium. The results of this test can be seen in the table below.

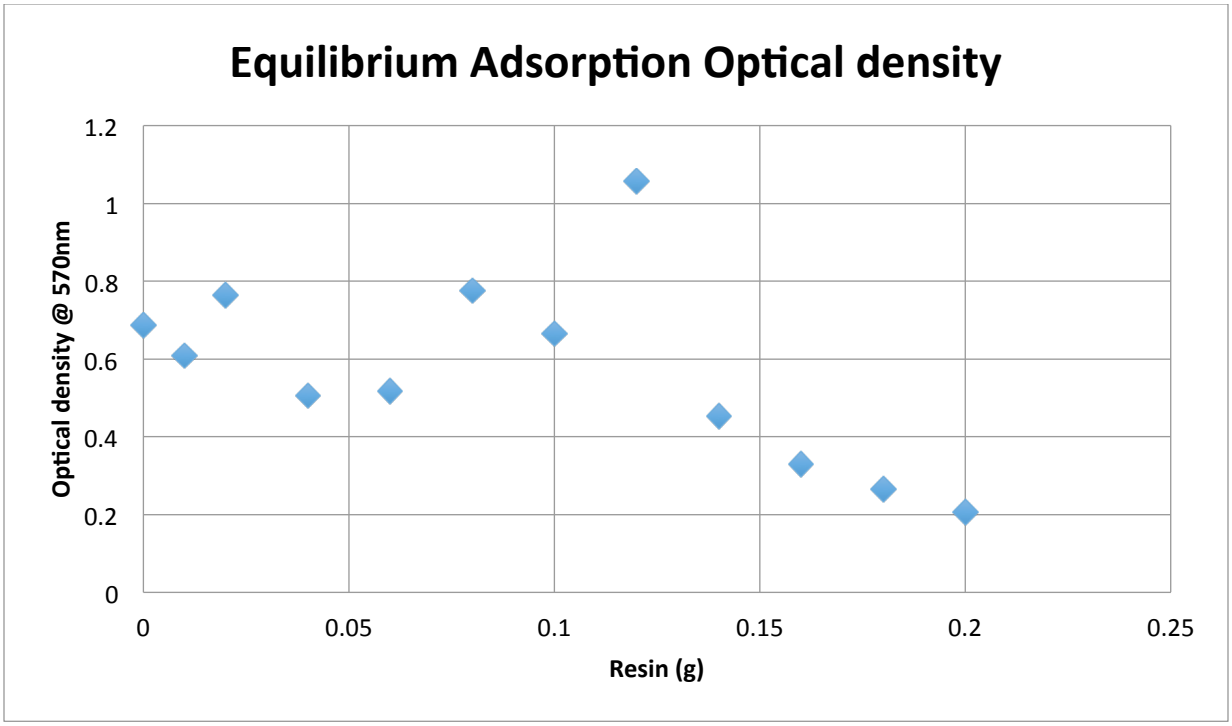

Figure 13: Equilibrium Adsorption Optical Density

It is found that additional resin would generally result in a lower optical density. Therefore more resin would lead to a larger amount of the glycerol being removed. It can be seen that there are certain data points that are above the starting concentration. These data points and the data point for 0.1 grams or resin appear to be outliers. These outliers could have arose from sampling biodiesel too close to the resin bed. More glycerol could have been sampled off the resin bead surface and not just the biodiesel. This would lead to the spike in the glycerol concentration seen. After removing the outliers from the data set the isotherm adsorption graph was generated.

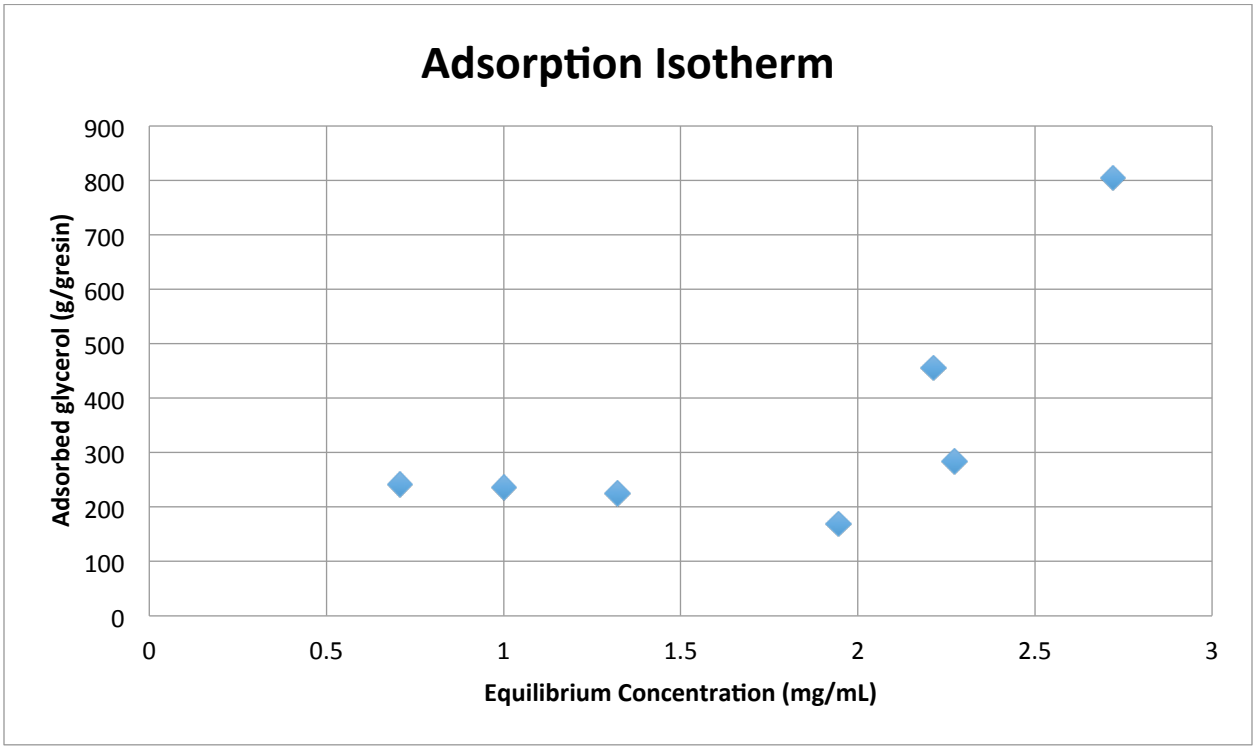

Figure 14: Equilibrium Adsorption Isotherm

Figure 14 shows that adsorption of the material is very low at lower resin concentrations. This is unfavorable for packed bed performance. This data suggests that higher resin performance occurs at higher solute concentrations, but well at low concentrations (Price, 2003). These adsorption results are correct, because the resin swells as more hydrophobic materials attach to the resin surface. With a smaller concentration of glycerol in the solution, the resin didn't swell as much as it could, which limited the availability of active space. As seen from isotherms of this shape, there could have been a weak interaction between the resin and glycerol, but a strong interaction between glycerol bonding to glycerol currently on the resin surface (Fletcher, 2008). This would have led to better resin performance at higher concentrations. Since the resin tends to sit at the bottom of the test tube, the test tube should be rotated periodically in further testing.

ISE Electrode Performance

The purpose of using the ion selective electrode (ISE) in tandem with the Thermo Scientific Orion 4-Star pH/ISE meter was to determine the potassium catalyst content left over in all biodiesel samples. More specifically, the electrode used is called a 'Double Junction Reference

Electrode' (Thermo Fisher Scientific Inc, 2008). Additionally, testing of the biodiesel samples before and after refining was conducted in order to evaluate the performance of the refining process.

The initial issue that was encountered was that the Orion 4-Star meter was designed for smaller concentrations of potassium content, which could explain why the results varied so much day to day. Although the ISE user guide states that the Orion 4-Star meter is acceptable to use with double junction ISE, the meter could only read up to a maximum of 10ppm (10,000ppb) potassium (Thermo Fisher Scientific, 2008). However, the ISE concentration range is stated to be 0.4ppm (400ppb) to 39,000ppm ($3.9*10^7$ppb) (Thermo Fisher Scientific Inc, 2008). The ISE also required that all samples be in aqueous solution in order to attain a stable reading. Since biodiesel is viscous, which causes biodiesel to stick to itself; an aqueous solution may not have been attainable even when heavily diluted. In addition, the prepared standards did not produce consistent results, which eventually led to the decision to utilize titration as an alternative method. Finally, because this electrode was explicitly for potassium content, testing of store bought biodiesel was unreliable considering the catalyst for that biodiesel was unknown.

Table 2: ISE Results

Averages						
Date:	12-Nov	18-Nov	19-Nov	20-Nov	4-Dec	4-Dec
*in ppb						
Purchased Oil		**10.5**	**16.7**	**0.55**	**1.8**	**0.08**
UO S1	**160**	**28**	**32**	**1.6333**	**7.2**	**1.7**
UO S2	**38**	**19**	**13**	**0.18**	**3**	**1.2**
UO S3		**0.006**	**0.8167**	**0.0019**	**6.2**	**0.5**
UO S4		**0.0185**	**0.6667**	**0.0011**	**7.2**	**0.2**
Refined Oil			**0.425**	**0.01**	**6.3**	**0.15**

All physical conditions were kept stable throughout testing. Cleaning, set-up, and storage of all equipment were consistent each day. Preparation and mixing of all standards and samples was kept constant. In addition, the batteries of the Orion 4-Star meter were changed multiple times to ensure no discrepancies in the results as well. However, room temperature may have been a factor on the electrode's performance since it varied tremendously each day. According to the ISE user guide, "Standards and samples should be at the same temperature. A 1°C difference in temperature for a 10^{-3} M potassium solution will give rise to about a 2.5% error." In addition, the user guide also claims that, "the electrode can be used at temperatures from 0°C to 40°C."

Further, the ISE user guide states that "reproducibility is independent of concentration," and that the limiting factors are usually, "temperature fluctuations, drift and noise." While the exact temperature of the lab was unknown on a daily basis, there is a possibility that the electrode was submitted to temperatures below that range. Some discrepancies in the results could also be attributed to the fact that samples were also produced on different dates and with different feedstocks (canola oil, soybean oil).

Overall, the variance in the lab room temperature could be the biggest contributing factor to unpleasant results with the ISE, especially considering how the electrode measures potassium content. When the ISE is dipped into a sample solution, an electrode potential develops based on the amount of free potassium present. This potential is defined by the Nernst equation:

Equation 4: Nernst Equation

$$E = E_o + S * \log(A)$$

Where E is the electrode potential, E_o is a constant reference potential, A is the potassium ion activity level, and S is the electrode slope. Furthermore, the slope is dependent on the temperature, and is defined by the equation:

Equation 5: Electrode Slope

$$S = \frac{2.3 * R * T}{n * F}$$

Where R and F are constants, n is the ionic charge, and T is temperature in degrees K. This demonstrates that even if the electrode is able to accurately measure the potassium ion activity level in a given sample, the temperature can still ultimately alter the overall electrode potential. Therefore, when the temperature changes between days of testing, the result displayed through the Orion 4-Star meter can be dramatically distorted.

Titration Performance

Titration, like the ISE Probe, was used to determine the potassium content remaining in various biodiesel samples. The decision to use titration arose from the fact that the ISE Probe yielded inconsistent results. Additionally, titration is a reliable method and all of the necessary materials and equipment was available. For these reasons, titration made sense as a viable alternative to the ISE Probe.

The procedure and specific materials and equipment used are discussed in the Titration Procedure section. Everything regarding titration, from material ratios to equipment cleaning, was kept consistent and done according to this procedure.

Compared to the ISE Probe, titration yielded remarkably more consistent results. Before titrating samples that were critical to this project's results, several trial runs were conducted with various spare samples of biodiesel. The results are tabulated below in Table 3.

Table 3: Titration Results

Potassium Concentration (PPM)			
	UO#1	UO#3	UO#4
1	577	962	641
2	577	962	577
3	577	962	545
4	561	-	577
5	534	-	-
6	513	-	-
AVG	557	962	585

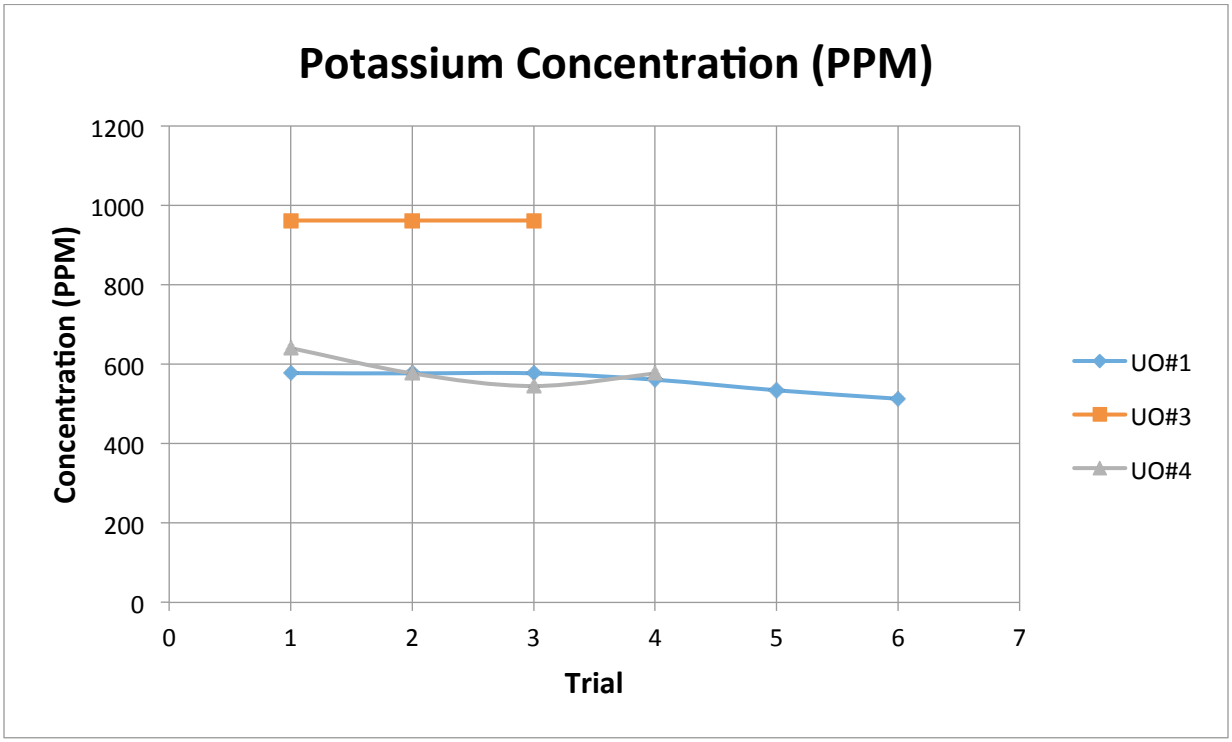

Figure 15: Potassium Concentration Profile

As shown in Figure 15, the trials were consistent. These trials were done primarily as a test to determine how viable titration would be. They served a second purpose, however, in that they were used to gain an understanding of how much potassium content was present in the various biodiesel samples. This purpose was important because it was necessary to obtain an initial concentration of potassium in a biodiesel sample prior to processing it. As the results show, the potassium content varied slightly between different samples, but it was, overall, in a similar range. Any differences resulted from the fact that the biodiesel samples were produced at different times and sometimes with different feedstocks (canola oil, soybean oil, etc.).

Once these trials were executed, titrations were performed in order to determine the potassium content in the biodiesel that was processed via the extended run. As it turns out, there is little to discuss regarding the extended run because the resin effectively removed all potassium for the entire duration of the test. Several titrations were performed across the 155 hours; every single one yielded no potassium content. This shows that the resin was extremely efficient at removing potassium. This result makes sense because the potassium content was actually quite low prior to being processed.

Inconsistencies in the titration results, although few and far between, were present. Three possible reasons for these inconsistencies are discussed below. The first reason was that the temperature of the lab varied, sometimes substantially, based on the outside temperature. The lab was actually known to have poor insulation and ventilation and, as a result, was sometimes considerably drafty. These temperature fluctuations could have had an impact on the biodiesel samples and the various materials used for titration. A second possible reason was that some biodiesel samples were sometimes added to others in order to conserve containers. Care was always taken to mix samples that were made with the same feedstock; as the samples were made on different occasions, however, conditions may have been inconsistent and the resulting biodiesel could have been different. A third possible reason was that some titrations on the same biodiesel sample were performed on different days. As a result, the potassium content could have settled to the bottom of the container during that time.

Conclusion

Overall it was determined that high velocity washing is not effective for dry washing biodiesel. Biodiesel should not be processed at a flow rate greater than 1.75 times the dry bed volume of the packed resin bed. The optimum dilution for raw unit operations biodiesel is roughly 200 microliters in 10 milliliters of deionized water. Resin exhaustion was never determined during extended testing and could not be verified for high velocity washing. Resin breakthrough was seen clearly in the extended run. At around 40 – 80 hours, the resin degraded to an overall lower performance state. Using the data gathered from the extended run testing of the smaller column, a larger column for Unit Operations was developed and is ready for service.

Large Column Design

The primary testing for this MQP was done using the small chromatography column. This column has a volume of approximately 10ml. The large column has a usable volume of 150 ml. The primary use of this large column will be for the continual processing of the unit operations biodiesel production lab. This glass column has been filled with 55ml of dry resin to process

biodiesel. In addition to resin, there is one sheet of KIM Wipe at the bottom to act as a surface to hold the resin beads inside the column.

The resin is designed to expand to 2.25 times its original size under normal conditions. With a volume of 55ml, for example, it would be expected to increase to roughly 125ml. This will give roughly 25ml of free space for liquid above the packed bed of resin. 55ml of resin in the tube weighs approximately 47.4 grams. Using the average data presented from the extended run trial, it can be seen that 47.4 grams should be able perform at no less than 95% of maximum performance for 13.7L of biodiesel. Using this same data and extrapolating a linear regression, it was found that the column would perform at no less than 85% for roughly 40L.

The volumes presented can only be supported at a maximum flow rate of 1.75 times the dry bed volume. In the case of 55ml, the maximum flow rate is 92mL/h. This means that the column will only perform above 95% for 149 hours, and 85% for 435 hours (18.1 days). Due to the length of time required, these numbers were not verified over the course of experimentation. It is suggested that as UO diesel is produced, the biodiesel should be dry washed in the column. The amount of biodiesel that is processed should be noted in order to verify predictions.

Under initial performance testing, the large column was able to remove 99.7% of all glycerol present. In the test, the biodiesel had an initial glycerol concentration of 1100 ppm. The processed biodiesel had 4 ppm glycerol. Over time, the resin will naturally lose its ability to hold glycerol. The resin will lose 20% of performance over two years time. With this in mind, it is recommended that the resin is replaced yearly even if significant performance degradation is not seen. It is also recommended that a second column should be purchased to improve overall system efficiency. This setup is recommended by the resin manufacturer.

Recommendations

Testing Velocity

The majority of the high velocity testing was performed at maximum pump speed of 22.7 ml/min. As discussed earlier, high velocity testing proved to be ineffective at consistently

removing glycerol. Moving forward, further research should look at varying speeds above resin maximum speed. By varying the speed more effectively, the effects of flow rate on resin performance can be studied closer.

Testing Glycerol Concentration

During this MQP, glycerol was determined through a method of light absorbance after an aqueous glycerol solution was reacted with a working reagent. The working reagent was composed of enzymes, proprietary reagents, and a die. Depending on the color of solution a colorimeter could give a light absorbance number of 570nm light, which can then be related back to a glycerol content. This method is inaccurate, because the biodiesel solution must be diluted in water. By diluting in water it must be assumed that all glycerol is dissolved into the water phase and that none remains in the biodiesel phase.

It is recommended that further research should utilize a different method of glycerol determination. A method that would eliminate the dilution of biodiesel in water would be preferable. The use of raw wash diesel would improve the accurate of glycerol determination. It is also recommended that methods utilizing light absorbance for determination should be avoided. These methods should be avoided due to biodiesel being a colored solution. It is suggested that glycerol determination methods for gas chromatography or high precision liquid chromatography should be established. Using such methods of glycerol determination would provide a higher resolution of the overall composition of the biodiesel. (eg. amount of glycerol and tri, di, and mono glycerides)

Testing Potassium Concentration

Comparing the results of the Thermo Scientific Orion ISE meter and titration, it was clear that titration yielded much more consistent results. This was due to several different reasons. One reason was that the ISE probe has to rely on various electromechanical components in order for it to operate. Titration, on the other hand, is a reliable method that proved to be consistent on a daily basis. As titration is a much less complex method, there was a much smaller chance for errors and inconsistencies.

Another reason titration was more consistent was that the ISE Probe is a highly-specialized potassium concentration measuring tool designed for specific concentration levels. As discussed, the ISE Probe is meant for somewhat low concentrations of potassium, with a maximum possible reading of 10 ppm. Titration consistently yielded potassium concentrations within the range of 500-1000 ppm, so it was no surprise that the results of the ISE Probe were inaccurate and inconsistent. The probe is also designed to determine free potassium in aqueous solutions, not solutions like biodiesel.

Methanol Mitigation

Methanol is used in excess to drive the transesterification reaction to completion. Unreacted methanol can cause contaminants to stay suspended in the biodiesel instead of settling out. Increased contamination can cause the resin to exhaust prematurely. Methanol also poses a health risk during handling of biodiesel and glycerol, and should be removed. Furthermore recovery of methanol for reuse improves the process economics. Methanol can be removed from the product by three different methods; the 5% water pre-wash method, the GL 1 day process, and centrifuge or settling. The 5% water pre-wash method is the easiest to perform and is effective at removing some glycerin, excess methanol, soaps, catalyst, and other impurities. The GL 1 day process removes methanol and then allows the impurities to settle out over the period of a day since without methanol, the other impurities are immiscible in biodiesel. Settling or centrifugation allows the glycerin to settle out. The resin has an immense capacity for removing soap relative to glycerin; removing as much as possible glycerin first ensures the economical use of the resin (Purolite PD206 Dry Wash Resin, 2013).

The boiling point of methanol is 64.7 degrees Celsius and the boiling point of biodiesel is in the range of 315 to 350 degrees Celsius (National Renewable Energy Labratory, 2009). Excess methanol can be removed from the biodiesel by heating the product to just over the boiling point of methanol. A proposed appropriate temperature is 75 degrees Celsius. Adding a collecting condenser on the reactor would allow the methanol vapor to be collected and recovered. The methanol could then be recycled and used in future reactions which would help to reduce production costs. Heating of biodiesel can be dangerous since biodiesel is flammable so caution should be exercised.

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Appendix

ASTM Standards

Table 4: ASTM and EN Standards for B100 Biodiesel

Property	ASTM D975-08a		ASTM D6751-12 2-B 1-B		EN 590:2004		EN 14214:2012	
Flash point, min	No 1D 38°C No 2D 52°C	D93	**93°C**	D93	55°C	EN 22719	**101°C**	EN ISO 2719
Water & sediment, max	0.05% vol	D2709	**0.050% vol**	D2709				
Water, max					200 mg/kg	EN ISO 12937	**500 mg/kg**	EN ISO 12937
Total contamination, max					24 mg/kg	EN 12662	**24 mg/kg**	EN 12662
Distillation temperature (% vol recovered)	90%: 1D 288°C max 2D 282-338°C	D86	**90%: 360°C max**	D1160	65%: 250°C min 85%: 350°C max	EN ISO 3405		
Kinematic viscosity	1D 1.3-2.4 mm^2/s 2D 1.9-4.1 mm^2/s	D445	**1.9-6.0 mm^2/s**	D445	2.0-4.5 mm^2/s	EN ISO 3104	**3.5-5.0 mm^2/s**	EN ISO 3104
Density					820-845 kg/m^3	EN ISO 3675 EN ISO 12185	**860-900 kg/m^3**	EN ISO 3675 EN ISO 12185
Ester content	5% vol. max	EN 14078			5% vol. max FAME	EN 14078	**96.5% min**	EN 14103
Ash, max	0.01% wt	D482			0.01% wt	EN ISO 6245		
Sulfated Ash, max			**0.020% mass**	D874			**0.02% mass**	ISO 3987
Sulfur, max (by mass)	1D and 2D: S15 15 mg/kg S500 0.05% S5000 0.50%	D5453 D2622 D129[2]	**Two grades: S15 15 ppm S500 0.05%**	D5453	Two grades: 50 mg/kg 10 mg/kg	EN ISO 14596 EN ISO 8754 EN ISO 24269	**10.0 mg/kg**	EN ISO 20846 EN ISO 20884 EN ISO 13032
Copper strip corrosion, max	No 3	D130	**No 3**	D130	class 1	EN ISO 2160	**class 1**	EN ISO 2160
Cetane number, min	40	D613	**47**	D613	51.0	EN ISO 5165	**51.0**	EN ISO 5165

Cetane index, min					46.0	EN ISO 4264		
One of[3]: - cetane index - aromaticity	40 min 35% vol max	D976-80 D1319						
PAH, max					11% wt	IP 391 EN 12916		
Operability, one of: - cloud point - LTFT/CFPP	Report	D2500 D4539 D6371						
Cloud point			**Report**	D2500	Location & season dependant	EN 23015	**Location & season dependant**	EN 23015
CFPP					Location & season dependant	EN 116	**Location & season dependant**	EN 116
Carbon residue on 10% distillation residue, max	1D: 0.15% wt 2D: 0.35% wt	D524	**0.050% wt[5]**	D4530	0.30% wt	EN ISO 10370		
Acid number, max			**0.50 mg KOH/g**	D664			**0.50 mg KOH/g**	EN 14104
Oxidation stability			**3 hrs min**	EN 14112	25 g/m^3 max	EN ISO 12205	**8 hrs min**	EN 14112
Iodine value, max							**120[1] g Iod/100g**	EN 14111 EN 16300
Linolenic acid methyl ester, max							**12.0% wt**	EN 14103
Polyunstatured methyl esters, max							**1.00% wt**	EN 15779
Alcohol control			**0.2% wt methanol max, or 130°C flash point min**	EN14110 D93			**0.20% wt methanol max**	EN 14110
Monoglycerides, diglycerides & triglycerides, max			**MG 0.40% wt**	D6584			**MG 0.70% wt DG 0.20% wt TG 0.20% wt**	EN 14105
Group I metals (Na + K), max			**5 mg/kg**	EN 14538			**5.0 mg/kg**	EN 14108 EN 14109 EN 14538
Group II metals (Ca + Mg), max			**5 mg/kg**	EN 14538			**5.0 mg/kg**	EN 14538
Free glycerin, max			**0.020% wt**	D6584			**0.02% wt**	EN 14105 EN

Total glycerin, max			**0.240% wt**	D6584			**0.25% wt**	14106 EN 14105
Phosphorous, max			**0.001% wt**	D4951			**4.0 mg/kg**	EN 14107 prEN 16294
Lubricity, max	520 μm	D6079			460 μm	ISO 12156-1		
Conductivity, min	25 pS/m	D2624 D4308						
Cold soak filtration time (CSFT), max			**360 s**[4]	**200 s**	D7501			

(1) Spain's Royal Decree 1700/2003 sets the maximum iodine value at 140 to facilitate the use of soybean oil as a feedstock.
(2) D129 is only applicable to S5000 grades.
(3) Limits only apply to S15 and S500 grades.
(4) 200 s if fuel temperature ≤ -12°C.
(5) Tested on 100% sample but reported using 10% residual calculation.

Material Safety and Data Sheets

DudaLite DW-R10 DryWash Resin:

Identification of substance and of the company

Identification of the substance : DudaLite DW-R10 DryWash Resin

Use of substance: Purification of biodiesel

Name of manufacturer:

Duda Diesel

7055 Greenbrier Road Bldg A

Madison, AL 35756

Tel: 256-340-4866

Fax: 866-568-3412

Responsible person: Brian Duda

Email: support@dudadiesel.com

Emergency telephone: 256-340-4866

Hazards identification

Emergency overview

Physical state: amber, light brown, dark brown, gold, black solid bead

Odor: Not applicable

Contact with eyes: may cause temporary eye irritation

Contact with skin: may be slightly irritating to skin

Low hazard for usual industrial or commercial handling by trained personnel

OSHA regulatory status: This product is not hazardous according to OSHA 29CFR 1910.1200

Potential health effects

Inhalation: limited inhalation hazard at normal work temperatures

Eye contact: may cause temporary eye irritation

Skin contact: may be slightly irritating to skin

Ingestion: under normal conditions of intended use, this material does not pose a risk to health. However, ingestion may cause irritation and malaise

Chronic health effects: no other specific acute or chronic health impact noted.

Target organs: eye/skin

Potential physical/chemical effects: This product is a combustible per NFPA

Environmental effects: The environmental hazard of this product is considered limited

Composition/information on ingredients

Table 5: DudaLite Composition

Ingredient	Concentrations	CAS Number	R Phrases	Symbol
Symbol Sodium polystyrene sulphonate	98 – 99%	69011-22-9	-	-
Water	1-2%	7732-18-5	-	-

First aid measures

Inhalation: no specific first aid measures noted

Eye contact: any material that contacts the eye should be washed out immediately with

water. If possible, remove any contact lenses. Get medical attention if discomfort continues.

Skin contact: Wash skin with soap and water.

Ingestion: immediately rinse mouth and drink plenty of water (200-300ml). Get medical attention if irritation persists.

Fire-fighting measures

Flammable properties - NFPA rating fire=1

Extinguishing media: extinguish with foam, carbon dioxide, dry powder or water fog.

Unsuitable extinguishing media: not applicable

Special fire fighting procedures: self contained breathing apparatus and full protective clothing must be worn in case of fire

Unusual fire and explosion hazards: Not available

Hazardous combustion products: Monomers, residual organics, carbon and sulfur oxides

Protective measures: Selection of respiratory protection for fire-fighting: follow the general fire precautions indicated in the workplace.

Accidental release measures

Personal precautions: Keep people away

Spillage causes slippery surfaces

Environmental precautions: Do not allow to enter public sewers and water courses.

Methods of cleaning up: Sweep up as much as possible and transfer to plastic containers for recovery and disposal.

Handling and storage

Handling: Avoid contact with eyes and prolonged skin contact.

Storage:

Store at temperatures above zero degrees C

Store at temperatures below forty degrees C

Keep in original container

Keep container tightly closed to prevent the absorption of water

Store away from incompatible materials

Exposure controls/personal protection

Exposure limits: no exposure limits noted for substances

Exposure Controls: provide adequate ventilation

Occupational exposure controls

Respiratory protection: if engineering controls do not maintain airborne concentrations below recommended exposure limits (where applicable) or to an acceptable level (in countries where exposure limits have not been established), an approved respirator must be worn. In the United States of America, if respirators are used, a program must be instituted to assure compliance with OSHA standard 63 FR 1152, January 8, 1998. Respirator type: High efficiency particulate respirator

Eye protection: Risk of contact wear approved safety goggles

Hand protection: Risk of contact wear protective gloves. Suitable gloves can be recommended by the glove supplier.

Skin protection: Risk of contact use skin protection. It is a good industrial hygiene practice to minimize skin contact.

Hygiene measures: always observe good personal hygiene measures, such as washing after handling the material before eating drinking and/or smoking. Routinely wash work clothing and protective equipment to remove contaminants.

Environmental exposure controls: environmental manager must be informed of all major spillages.

Physical and chemical properties

Appearance: Gold, amber, light brown, dark brown, black and green beads

Odor: odorless

Odor threshold: Not available

Physical state: Solid (bead)

pH: neutral aqueous slurry

Melting point: not available

Freezing point: not available

Boiling point: not available

Flash point: not available

Evaporation rate: not available

Flammability (solid, gas): not available

Flammability limit

Upper flammability limit: not available.

Lower flammability limit: not available

Vapor pressure: not available

Vapor density (air =1): not available

Specific Gravity: 1.15 to 1.35

Solubility in water: insoluble

Solubility (other): not available

Partition coefficient (n0octanol/water): not available

Auto ignition temperature: not available

Decomposition temperature: not available

Stability and reactivity

Conditions to avoid

Considered stable under normal conditions

Avoid heat

Materials to avoid: Incompatible with strong oxidizing substances. Contact with strong oxidizers, especially nitric acid, may produce low molecular weight organics that may form explosive mixtures

Hazardous decomposition products: At elevated temperatures: Benzene compounds, carbon oxides, styrene, sulfur oxides

Possibility of hazardous reactions: not available

Toxicological information

Acute toxicity: no evidence of acute toxicity

Carcinogenicity: no evidence of carcinogenic effects

Teratogenicity: no evidence of reproductive effects

Mutagenicity: no evidence of mutagenic effects

Ecological information

Ecotoxicity: no data available

Mobility: The product is insoluble in water and will sediment in water systems

Persistence and degradability: The product is not readily biodegradable

Bio-accumulative potential: potential to bio-accumulate is low

Other adverse effects: no data available

Disposal considerations

General information: Dispose of waste and residues in accordance with local authority requirements

Disposal method: no specific disposal method required

Container: Since emptied containers retain product residue, follow label warnings even after container is emptied

Transport information

DOT: not regulated

TDG: not regulated

IATA: not regulated

IMDG: not regulated

Regulatory Information

Canadian controlled Products Regulations: This product has been classified according to the hazard criteria of the Canadian Controlled Products Regulations Section 33 and the MSDS contains all required information WHMIS Classification - This is not a WHMIS controlled product

Mexican Dangerous Statement: This product is not dangerous according to Mexican regulations

Applicable International laws and regulations: This substance meets OECD polymer definition and is therefore exempt from REACH registration

Inventory Status: This product or all components are listed or exempt from listing on the following inventory: TSCA, DSL

US Regulations CERCLA Hazardous Substance List (40 CFR 302.4): not regulated

SARA Title III-Section 302 Extremely Hazardous Substances (40 CFR 355, Appendix A): Not

Not regulated Section 311/312 (40 CFR 370): Acute (Immediate) Chronic (delayed) Fire reactive Pressure Generating

Section 313 Toxic Release Inventory (40C CFR 372): Not regulated

Found: http://www.dudadiesel.com/msds/dudalite.pdf

Raw Data

11/6/13 High Velocity Run Data

Table 6: 11/6/13 Optical Density

Run	Time	Optical Density @ 570 nm
1	0	0.067
2	1.5	0.07
3	3	0.069
4	4.5	0.068
5	6	0.07
6	7.5	0.069
7	9	0.093
8	10.5	0.067
9	12	0.067
10	13.5	0.069
11	15	0.075
12	16.5	0.067
13	18	0.067
14	19.5	0.071
15	21	0.066
16	22.5	0.069
17	24	0.078
18	25.5	0.068
19	27	0.066
20	28.5	0.069
21	30	0.065
22	31.5	0.073
23	33	0.07
24	34.5	0.068
25	36	0.063
26	37.5	0.064
27	39	0.069
28	40.5	0.085
29	42	0.093
30	43.5	0.103
31	45	0.111
32	46.5	0.122
33	48	0.121
34	49.5	0.123

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.048	0.053	0.048	0.046	0.047	0.046	0.049	0.048	0.047	0.047	0.048
B	0.059	0.047	0.046	0.047	0.046	0.046	0.049	0.049	0.050	0.049	0.047	0.046
C	0.048	0.079	0.067	0.070	0.069	0.068	0.070	0.069	0.093	0.067	0.067	0.054
D	0.048	0.069	0.075	0.067	0.067	0.071	0.066	0.069	0.078	0.068	0.066	0.046
E	0.047	0.069	0.065	0.073	0.070	0.068	0.063	0.064	0.069	0.085	0.093	0.046
F	0.047	0.103	0.111	0.122	0.121	0.123	1.064	1.223	0.499	0.057	0.047	0.047
G	0.047	0.047	0.048	0.047	0.048	0.049	0.047	0.046	0.047	0.049	0.049	0.049
H	0.047	0.048	0.049	0.048	0.048	0.048	0.049	0.049	0.049	0.049	0.048	0.058

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:23 PM 11/7/2013

Wavelength Combination: !Lm1
Mean Temperature: 26.5
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 16: Optical Density - 11/6/13 - Minute 20 after adding Working Reagent

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.048	0.048	0.053	0.048	0.046	0.047	0.046	0.049	0.048	0.047	0.046	0.048
B	0.059	0.047	0.046	0.047	0.046	0.046	0.049	0.049	0.050	0.049	0.047	0.047
C	0.047	0.080	0.067	0.070	0.068	0.068	0.070	0.069	0.094	0.067	0.068	0.054
D	0.047	0.070	0.075	0.068	0.068	0.072	0.067	0.069	0.079	0.069	0.067	0.046
E	0.047	0.070	0.065	0.073	0.071	0.069	0.063	0.065	0.070	0.085	0.094	0.046
F	0.047	0.103	0.112	0.124	0.122	0.124	1.085	1.248	0.509	0.057	0.047	0.047
G	0.047	0.047	0.048	0.047	0.048	0.049	0.047	0.046	0.047	0.049	0.049	0.049
H	0.047	0.048	0.049	0.048	0.048	0.048	0.049	0.049	0.049	0.049	0.048	0.058

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:25 PM 11/7/2013

Wavelength Combination: !Lm1
Mean Temperature: 26.5
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 17: Optical Density - 11/6/13 - Minute 21

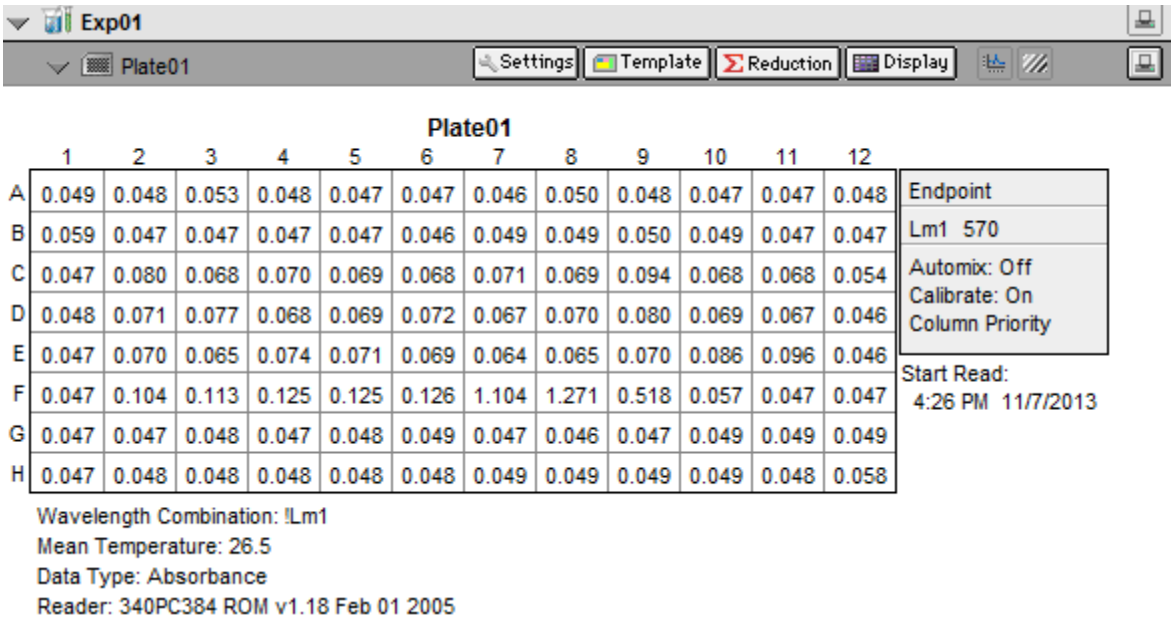


Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.048	0.053	0.048	0.047	0.047	0.046	0.050	0.048	0.047	0.047	0.048
B	0.059	0.047	0.047	0.047	0.047	0.046	0.049	0.049	0.050	0.049	0.047	0.047
C	0.047	0.080	0.068	0.070	0.069	0.068	0.071	0.069	0.094	0.068	0.068	0.054
D	0.048	0.071	0.077	0.068	0.069	0.072	0.067	0.070	0.080	0.069	0.067	0.046
E	0.047	0.070	0.065	0.074	0.071	0.069	0.064	0.065	0.070	0.086	0.096	0.046
F	0.047	0.104	0.113	0.125	0.125	0.126	1.104	1.271	0.518	0.057	0.047	0.047
G	0.047	0.047	0.048	0.047	0.048	0.049	0.047	0.046	0.047	0.049	0.049	0.049
H	0.047	0.048	0.048	0.048	0.048	0.048	0.049	0.049	0.049	0.049	0.048	0.058

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:26 PM 11/7/2013

Wavelength Combination: !Lm1
Mean Temperature: 26.5
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 18: Optical Density - 11/6/13 - Minute 22

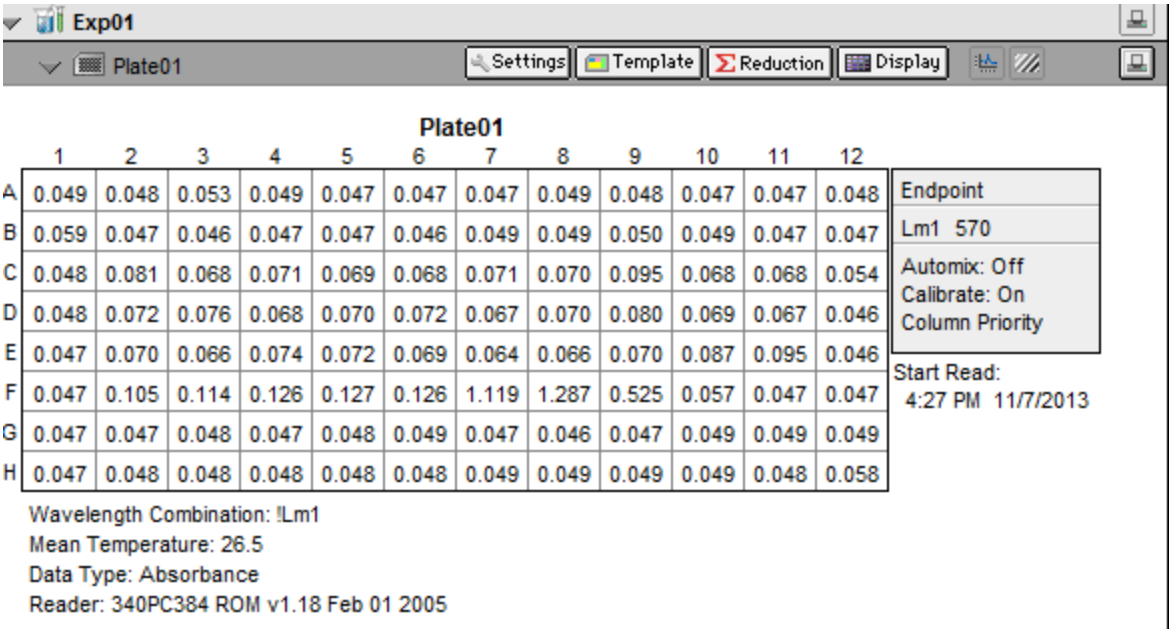


Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.048	0.053	0.049	0.047	0.047	0.047	0.049	0.048	0.047	0.047	0.048
B	0.059	0.047	0.046	0.047	0.047	0.046	0.049	0.049	0.050	0.049	0.047	0.047
C	0.048	0.081	0.068	0.071	0.069	0.068	0.071	0.070	0.095	0.068	0.068	0.054
D	0.048	0.072	0.076	0.068	0.070	0.072	0.067	0.070	0.080	0.069	0.067	0.046
E	0.047	0.070	0.066	0.074	0.072	0.069	0.064	0.066	0.070	0.087	0.095	0.046
F	0.047	0.105	0.114	0.126	0.127	0.126	1.119	1.287	0.525	0.057	0.047	0.047
G	0.047	0.047	0.048	0.047	0.048	0.049	0.047	0.046	0.047	0.049	0.049	0.049
H	0.047	0.048	0.048	0.048	0.048	0.048	0.049	0.049	0.049	0.049	0.048	0.058

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:27 PM 11/7/2013

Wavelength Combination: !Lm1
Mean Temperature: 26.5
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 19: Optical Density - 11/6/13 - Minute 23

modify the template to include additional standards, unknowns, and controls.

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.048	0.053	0.048	0.046	0.047	0.047	0.049	0.048	0.047	0.047	0.048
B	0.059	0.047	0.047	0.047	0.047	0.046	0.049	0.049	0.050	0.049	0.048	0.047
C	0.047	0.081	0.068	0.071	0.070	0.069	0.071	0.070	0.096	0.069	0.069	0.054
D	0.048	0.072	0.077	0.068	0.071	0.072	0.068	0.071	0.080	0.070	0.068	0.046
E	0.047	0.071	0.066	0.074	0.072	0.070	0.064	0.066	0.071	0.087	0.096	0.046
F	0.047	0.106	0.115	0.127	0.128	0.127	1.131	1.301	0.532	0.057	0.047	0.047
G	0.047	0.047	0.048	0.047	0.048	0.049	0.047	0.046	0.047	0.049	0.049	0.049
H	0.047	0.048	0.049	0.048	0.048	0.048	0.049	0.049	0.049	0.049	0.048	0.058

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:28 PM 11/7/2013

Wavelength Combination: !Lm1
Mean Temperature: 26.5
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 20: Optical Density - 11/6/13 - Minute 24

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.048	0.048	0.053	0.048	0.046	0.047	0.046	0.049	0.050	0.047	0.047	0.048
B	0.059	0.047	0.046	0.047	0.046	0.046	0.049	0.049	0.050	0.049	0.047	0.046
C	0.047	0.081	0.068	0.071	0.070	0.069	0.072	0.070	0.096	0.069	0.069	0.054
D	0.047	0.072	0.077	0.069	0.071	0.073	0.068	0.071	0.081	0.070	0.068	0.046
E	0.047	0.071	0.066	0.074	0.072	0.070	0.064	0.066	0.071	0.087	0.097	0.046
F	0.047	0.106	0.115	0.128	0.140	0.128	1.141	1.312	0.537	0.057	0.047	0.047
G	0.047	0.047	0.048	0.047	0.048	0.049	0.047	0.046	0.047	0.049	0.049	0.049
H	0.047	0.048	0.048	0.048	0.048	0.048	0.049	0.049	0.049	0.049	0.048	0.058

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:29 PM 11/7/2013

Wavelength Combination: !Lm1
Mean Temperature: 26.5
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 21: Optical Density - 11/6/13 - Minute 26

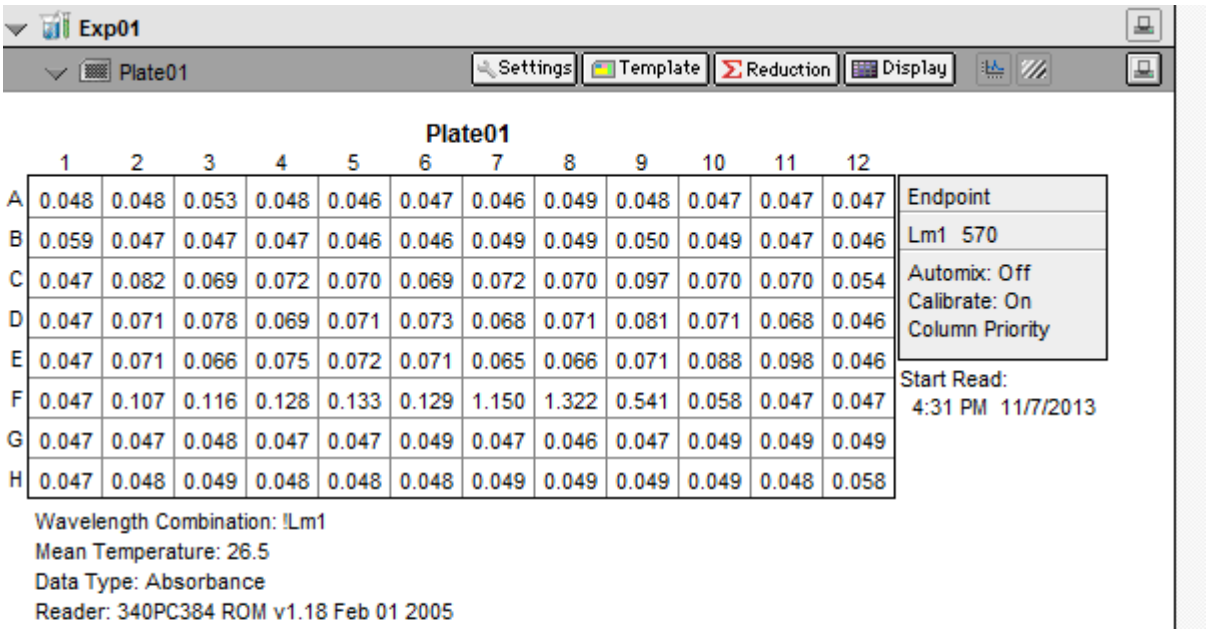


Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.048	0.048	0.053	0.048	0.046	0.047	0.046	0.049	0.048	0.047	0.047	0.047
B	0.059	0.047	0.047	0.047	0.046	0.046	0.049	0.049	0.050	0.049	0.047	0.046
C	0.047	0.082	0.069	0.072	0.070	0.069	0.072	0.070	0.097	0.070	0.070	0.054
D	0.047	0.071	0.078	0.069	0.071	0.073	0.068	0.071	0.081	0.071	0.068	0.046
E	0.047	0.071	0.066	0.075	0.072	0.071	0.065	0.066	0.071	0.088	0.098	0.046
F	0.047	0.107	0.116	0.128	0.133	0.129	1.150	1.322	0.541	0.058	0.047	0.047
G	0.047	0.047	0.048	0.047	0.047	0.049	0.047	0.046	0.047	0.049	0.049	0.049
H	0.047	0.048	0.049	0.048	0.048	0.048	0.049	0.049	0.049	0.049	0.048	0.058

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority
Start Read:
4:31 PM 11/7/2013

Wavelength Combination: !Lm1
Mean Temperature: 26.5
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 22: Optical Density - 11/7/13 - Minute 27

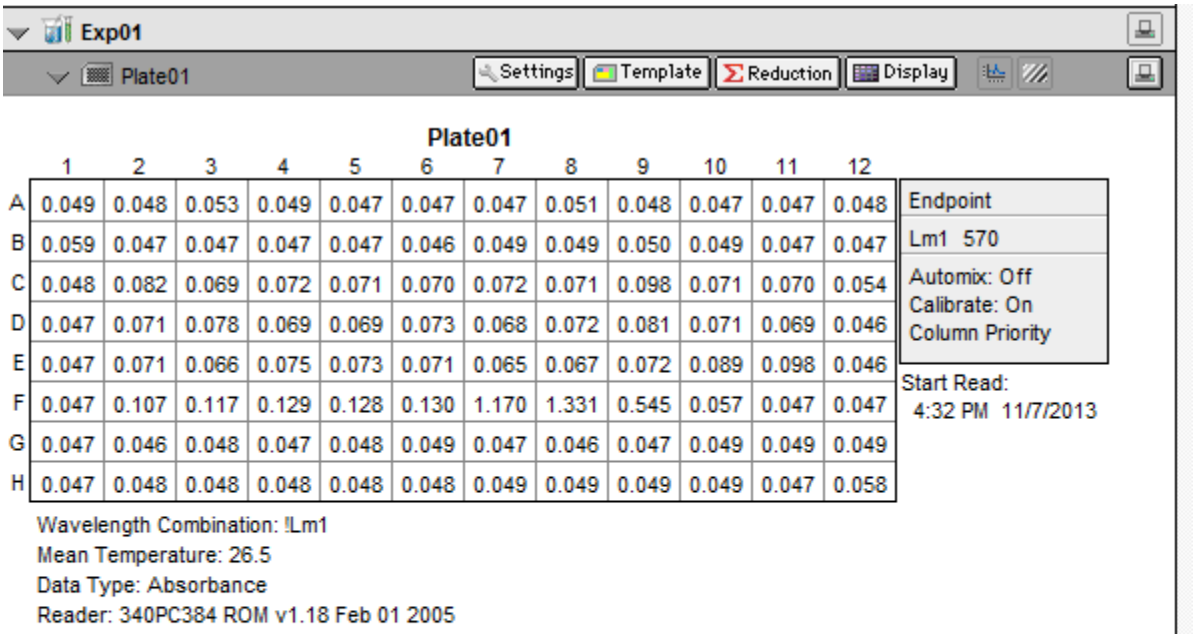


Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.048	0.053	0.049	0.047	0.047	0.047	0.051	0.048	0.047	0.047	0.048
B	0.059	0.047	0.047	0.047	0.047	0.046	0.049	0.049	0.050	0.049	0.047	0.047
C	0.048	0.082	0.069	0.072	0.071	0.070	0.072	0.071	0.098	0.071	0.070	0.054
D	0.047	0.071	0.078	0.069	0.069	0.073	0.068	0.072	0.081	0.071	0.069	0.046
E	0.047	0.071	0.066	0.075	0.073	0.071	0.065	0.067	0.072	0.089	0.098	0.046
F	0.047	0.107	0.117	0.129	0.128	0.130	1.170	1.331	0.545	0.057	0.047	0.047
G	0.047	0.046	0.048	0.047	0.048	0.049	0.047	0.046	0.047	0.049	0.049	0.049
H	0.047	0.048	0.048	0.048	0.048	0.048	0.049	0.049	0.049	0.049	0.047	0.058

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority
Start Read:
4:32 PM 11/7/2013

Wavelength Combination: !Lm1
Mean Temperature: 26.5
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 23: Optical Density - 11/6/13 - Minute 28

11/18/13 High Velocity Run Data

Table 7: 11/18/13 Optical Density

Time	Optical Density @ 570 nm
0	0.529
3	0.472

6	0.529
9	0.531
12	0.532
15	0.555
18	0.521
21	0.565
24	0.569
27	0.557
30	0.521
33	0.415
36	0.558
39	0.508
42	0.521
45	0.56
48	0.512

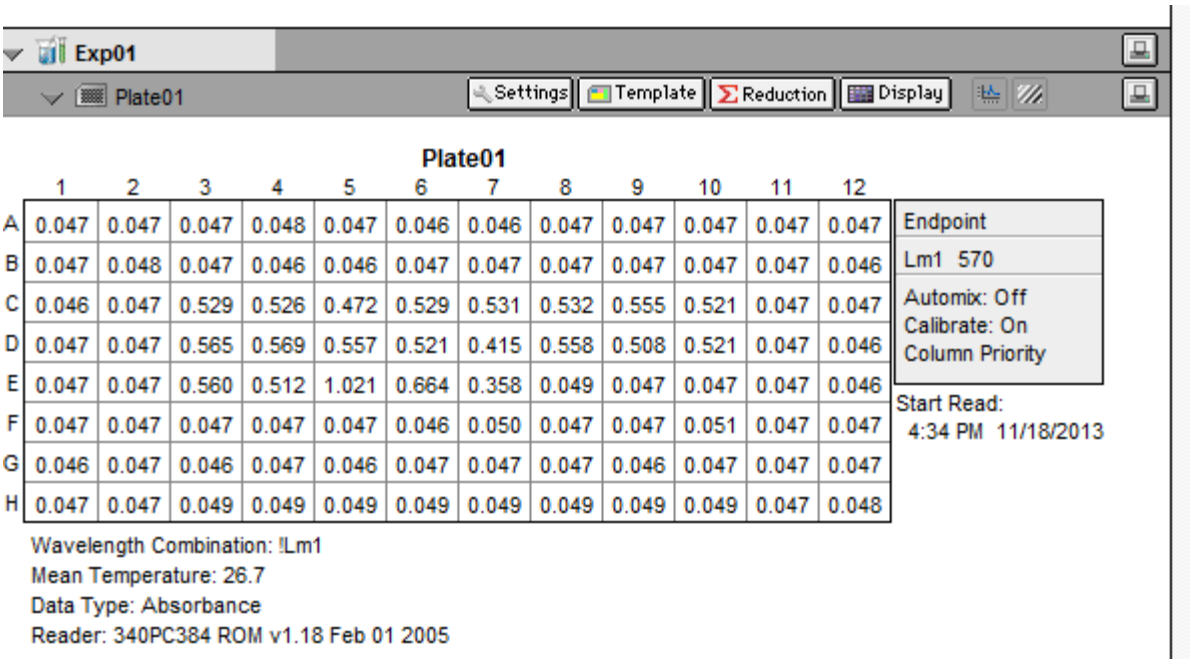


Figure 24: Optical Density - 11/18/13 - Minute 20 after adding Working Reagent

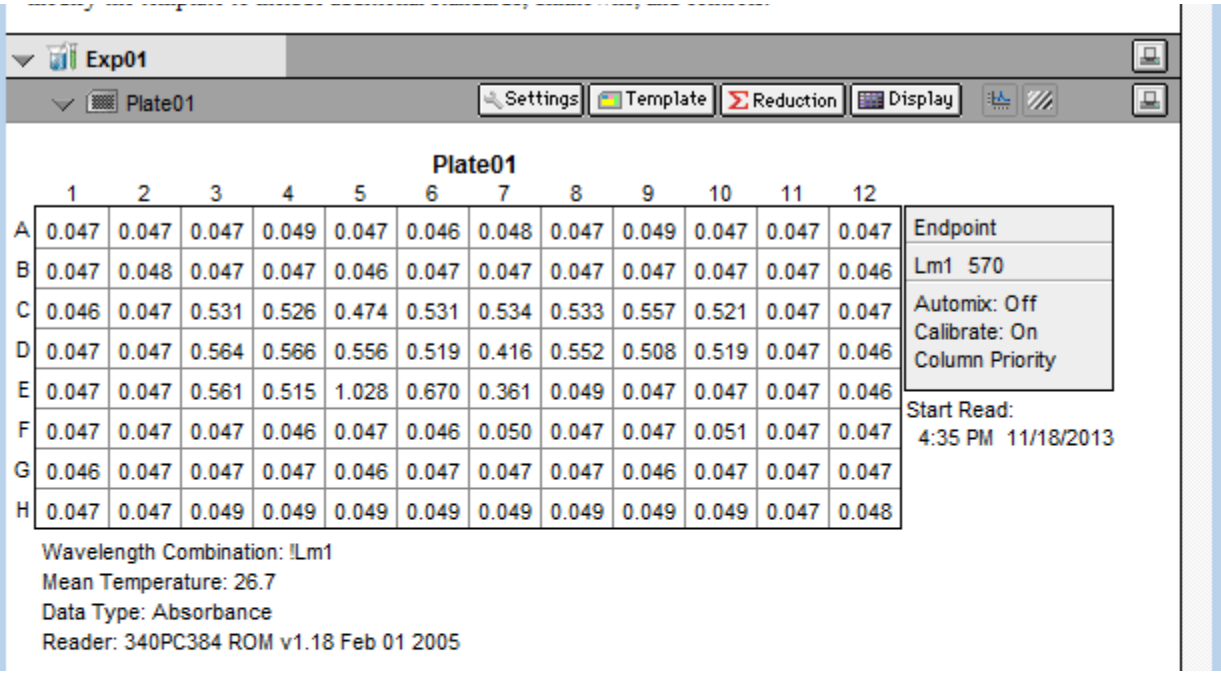


Figure 25: Optical Density - 11/18/13 - Minute 21

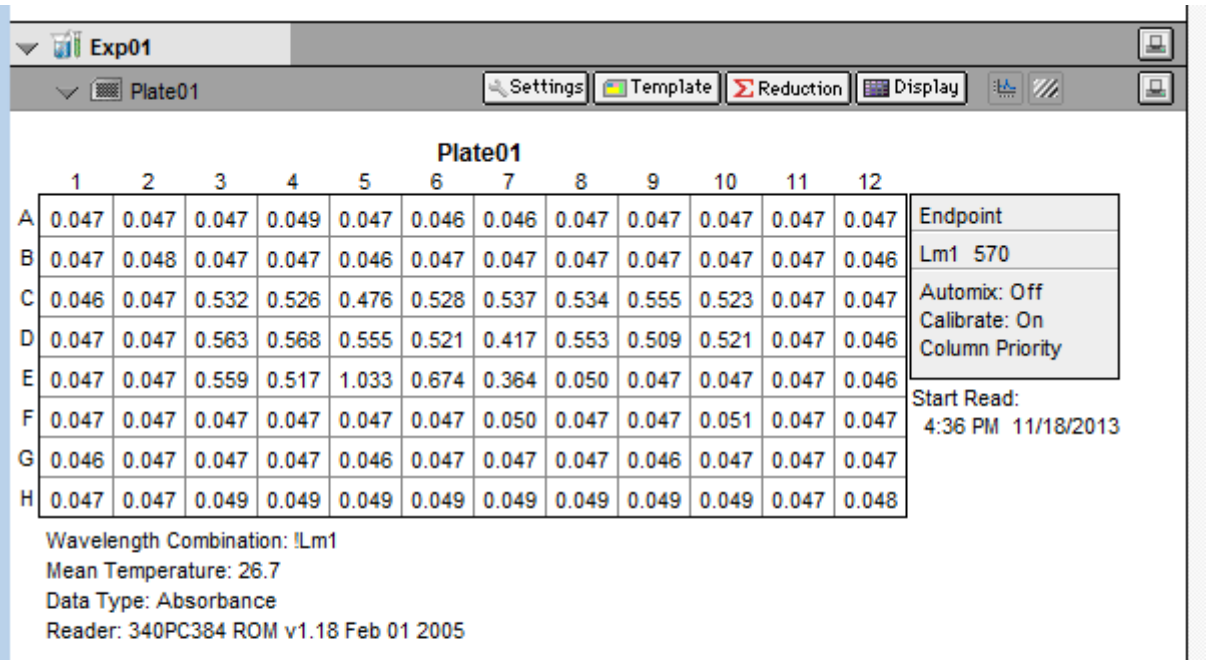


Figure 26: Optical Density - 11/18/13 - Minute 22

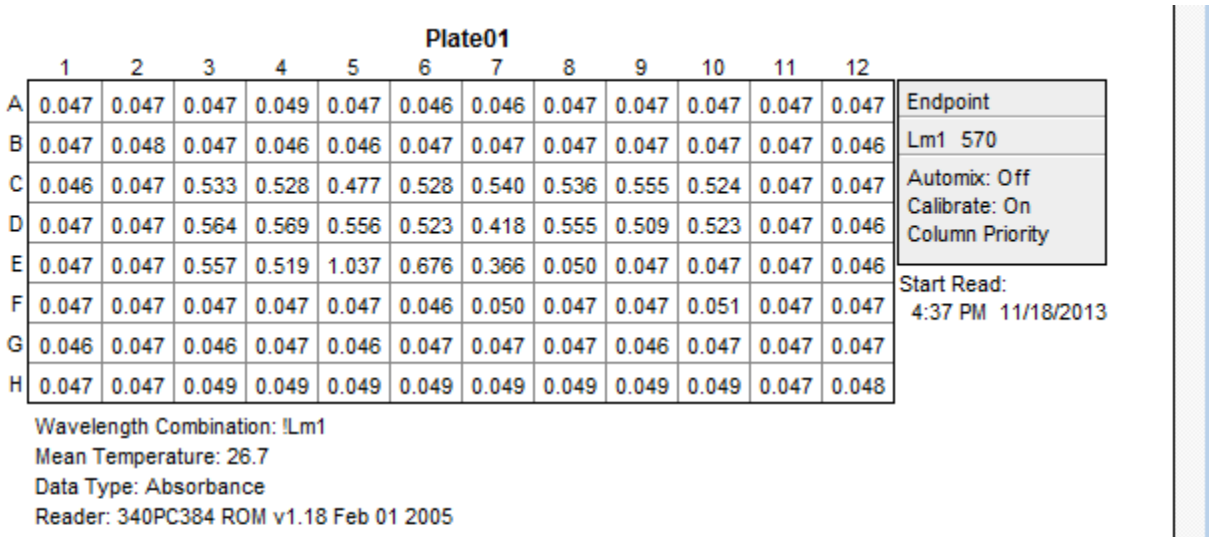


Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.047	0.047	0.049	0.047	0.046	0.046	0.047	0.047	0.047	0.047	0.047
B	0.047	0.048	0.047	0.046	0.046	0.047	0.047	0.047	0.047	0.047	0.047	0.046
C	0.046	0.047	0.533	0.528	0.477	0.528	0.540	0.536	0.555	0.524	0.047	0.047
D	0.047	0.047	0.564	0.569	0.556	0.523	0.418	0.555	0.509	0.523	0.047	0.046
E	0.047	0.047	0.557	0.519	1.037	0.676	0.366	0.050	0.047	0.047	0.047	0.046
F	0.047	0.047	0.047	0.047	0.047	0.046	0.050	0.047	0.047	0.051	0.047	0.047
G	0.046	0.047	0.046	0.047	0.046	0.047	0.047	0.047	0.046	0.047	0.047	0.047
H	0.047	0.047	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.047	0.048

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:37 PM 11/18/2013

Wavelength Combination: !Lm1
Mean Temperature: 26.7
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 27: Optical Density - 11/18/13 - Minute 23

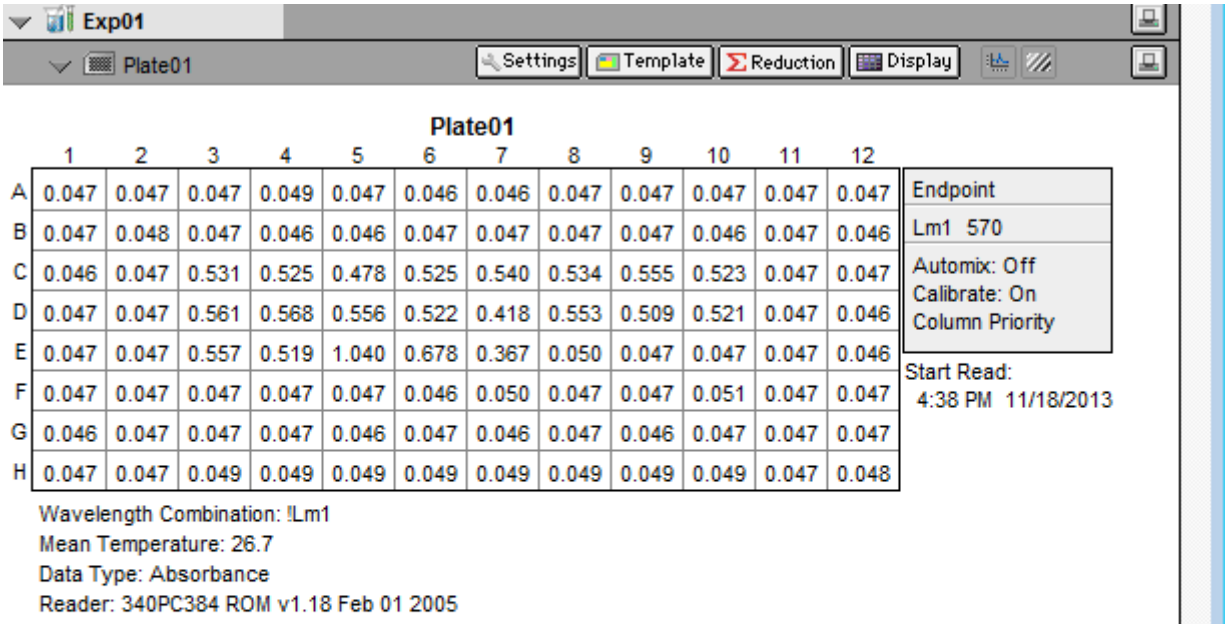


Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.047	0.047	0.049	0.047	0.046	0.046	0.047	0.047	0.047	0.047	0.047
B	0.047	0.048	0.047	0.046	0.046	0.047	0.047	0.047	0.047	0.046	0.047	0.046
C	0.046	0.047	0.531	0.525	0.478	0.525	0.540	0.534	0.555	0.523	0.047	0.047
D	0.047	0.047	0.561	0.568	0.556	0.522	0.418	0.553	0.509	0.521	0.047	0.046
E	0.047	0.047	0.557	0.519	1.040	0.678	0.367	0.050	0.047	0.047	0.047	0.046
F	0.047	0.047	0.047	0.047	0.047	0.046	0.050	0.047	0.047	0.051	0.047	0.047
G	0.046	0.047	0.047	0.047	0.046	0.047	0.046	0.047	0.046	0.047	0.047	0.047
H	0.047	0.047	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.047	0.048

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:38 PM 11/18/2013

Wavelength Combination: !Lm1
Mean Temperature: 26.7
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 28: Optical Density - 11/18/13 - Minute 24

11/19/13 High Velocity Run Data

Table 8: 11/19/13 Optical Density

Time	Optical Density @ 570 nm
0	0.266
3	0.226
6	0.233
9	0.232
12	0.233

15	0.212
18	0.223
21	0.224
24	0.226
27	0.195
30	0.177
33	0.159
36	0.218
39	0.2
42	0.192
45	0.174
48	0.205
51	0.196

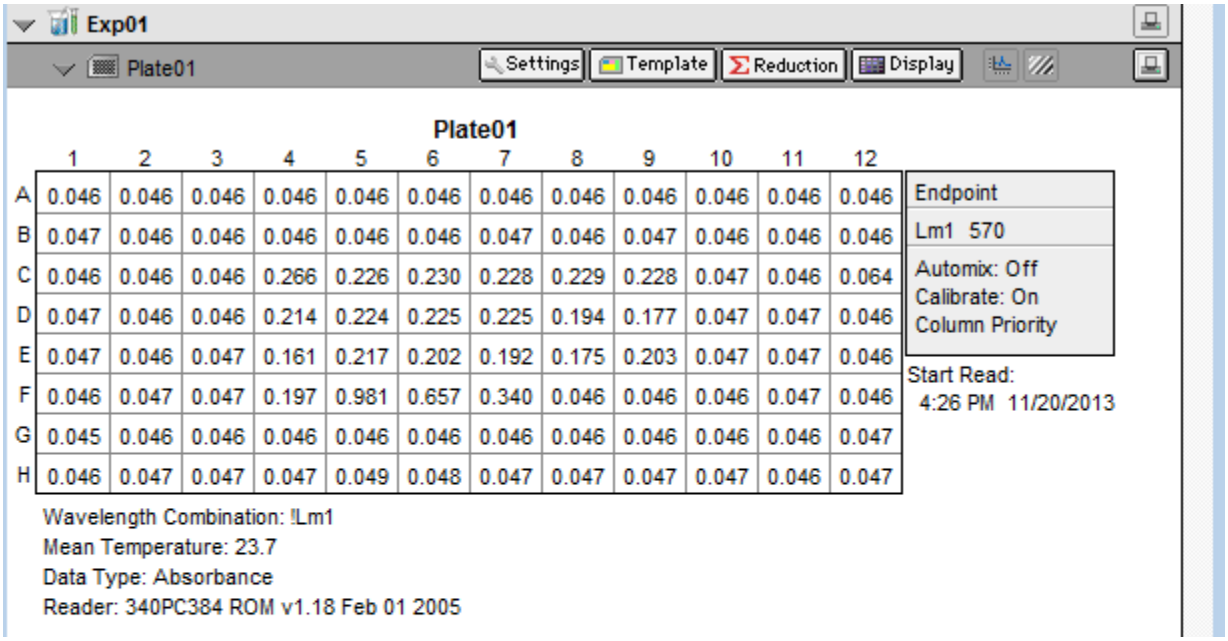


Figure 29: Optical Density - 11/19/13 - Minute 20 after adding Working Reagent

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.047	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.046	0.046
B	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046
C	0.046	0.047	0.046	0.268	0.225	0.228	0.229	0.231	0.230	0.047	0.046	0.064
D	0.047	0.046	0.047	0.216	0.224	0.223	0.225	0.195	0.177	0.047	0.047	0.046
E	0.047	0.046	0.046	0.161	0.217	0.201	0.193	0.174	0.204	0.047	0.047	0.046
F	0.046	0.047	0.047	0.197	0.989	0.662	0.343	0.046	0.047	0.046	0.047	0.046
G	0.046	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.048	0.047	0.047	0.049	0.049	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:27 PM 11/20/2013

Wavelength Combination: !Lm1
Mean Temperature: 23.7
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 30: Optical Density - 11/19/13 - Minute 21

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.046	0.047	0.047	0.047	0.046	0.046	0.047	0.047	0.047	0.047	0.046
B	0.047	0.047	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046
C	0.046	0.047	0.046	0.266	0.226	0.229	0.233	0.232	0.233	0.047	0.046	0.064
D	0.047	0.046	0.046	0.212	0.223	0.224	0.226	0.195	0.177	0.047	0.047	0.046
E	0.047	0.046	0.047	0.159	0.218	0.200	0.192	0.174	0.205	0.047	0.047	0.046
F	0.046	0.047	0.047	0.196	0.989	0.663	0.344	0.046	0.047	0.046	0.047	0.046
G	0.045	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.049	0.049	0.047	0.047	0.047	0.047	0.046	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:28 PM 11/20/2013

Wavelength Combination: !Lm1
Mean Temperature: 23.7
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 31: Optical Density - 11/19/13 - Minute 22

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046
B	0.047	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046
C	0.046	0.047	0.046	0.267	0.227	0.228	0.235	0.233	0.233	0.047	0.046	0.064
D	0.047	0.047	0.046	0.215	0.224	0.226	0.227	0.196	0.178	0.047	0.047	0.046
E	0.047	0.046	0.047	0.159	0.218	0.200	0.192	0.174	0.206	0.047	0.047	0.046
F	0.046	0.047	0.047	0.197	0.992	0.666	0.346	0.046	0.047	0.046	0.047	0.046
G	0.046	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.049	0.049	0.047	0.047	0.047	0.047	0.046	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:29 PM 11/20/2013

Wavelength Combination: !Lm1
Mean Temperature: 23.7
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 32: Optical Density - 11/19/13 - Minute 23

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.046	0.047	0.048	0.046	0.046	0.046	0.046	0.047	0.046	0.046
B	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046
C	0.046	0.047	0.046	0.269	0.226	0.228	0.236	0.234	0.233	0.047	0.046	0.064
D	0.047	0.047	0.046	0.212	0.224	0.226	0.228	0.197	0.179	0.047	0.047	0.046
E	0.047	0.046	0.046	0.160	0.220	0.200	0.193	0.175	0.206	0.047	0.047	0.046
F	0.046	0.047	0.047	0.196	0.996	0.669	0.348	0.046	0.046	0.046	0.047	0.046
G	0.045	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.049	0.049	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:30 PM 11/20/2013

Wavelength Combination: !Lm1
Mean Temperature: 23.7
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 33: Optical Density - 11/19/13 - Minute 24

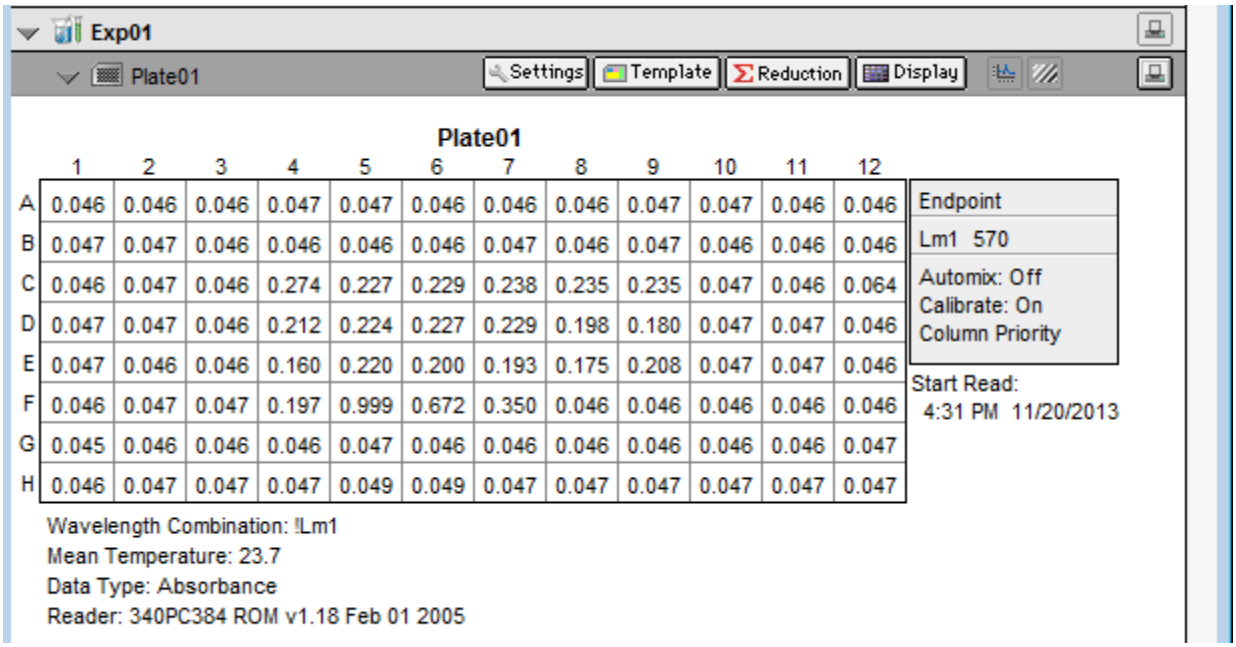


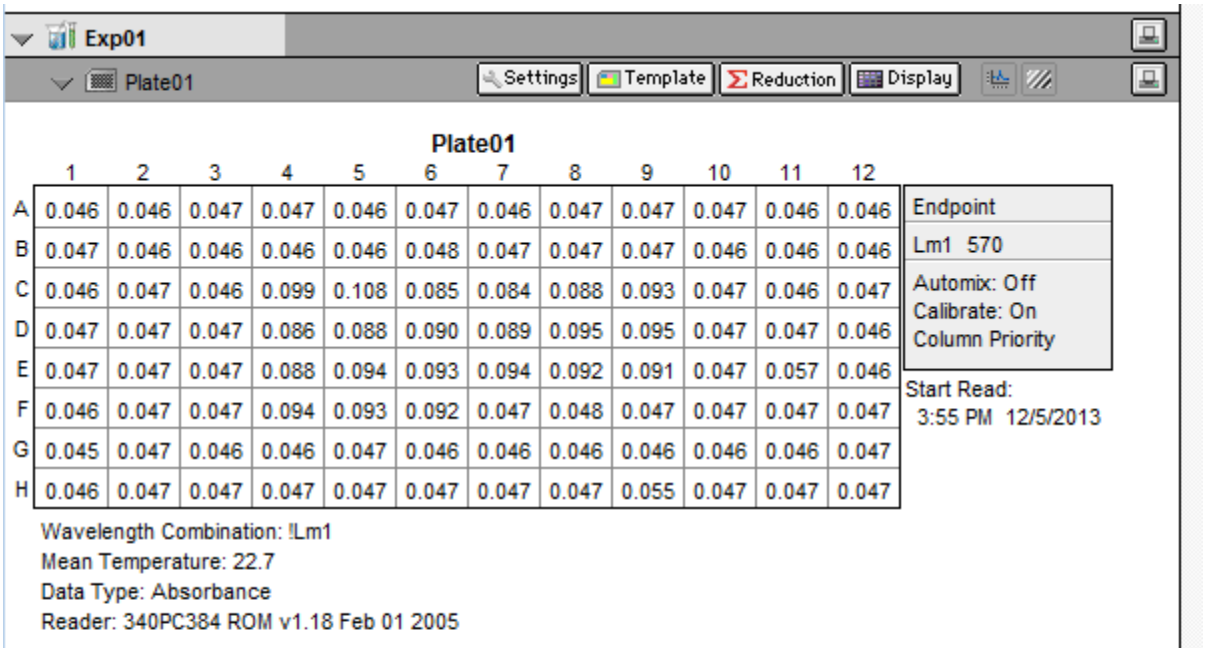
Figure 34: Optical Density - 11/19/13 - Minute 24

12/4/13 High Velocity Run Data

Table 9: 12/4/13 Optical Density

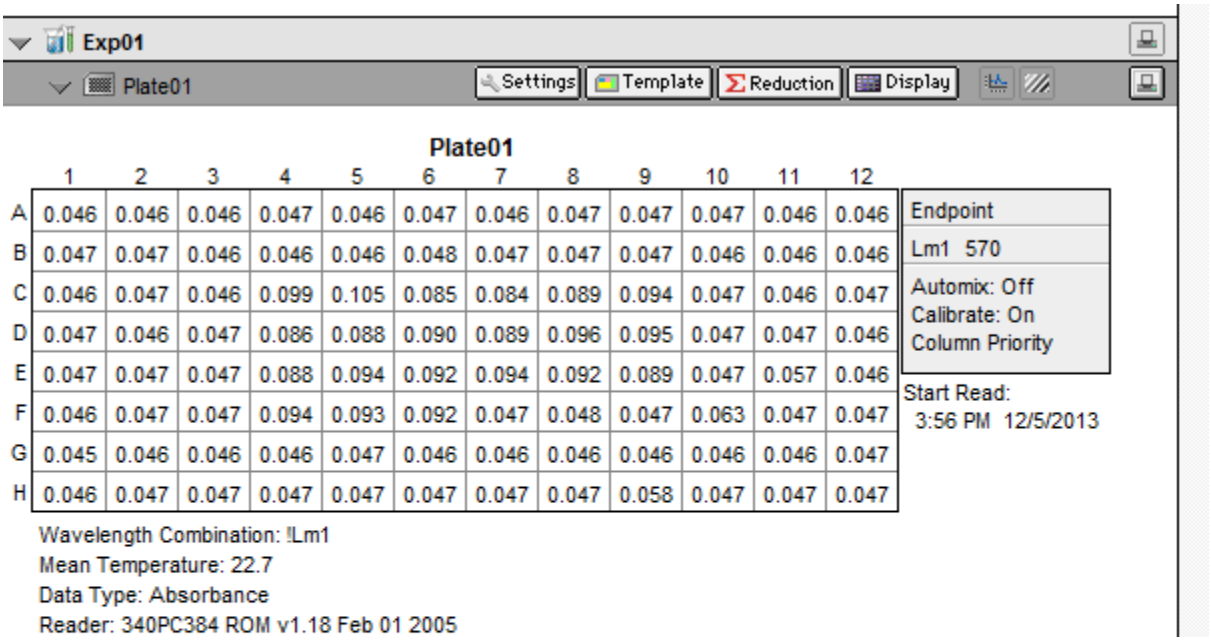
Time	Optical Density @ 570 nm
0	0.099
3	0.106
6	0.085
9	0.084
12	0.087
15	0.093
18	0.087
21	0.088
24	0.09
27	0.089
30	0.096
33	0.095
36	0.088
39	0.093
42	0.092
45	0.094
48	0.092
51	0.091
54	0.095

57	0.093
60	0.092



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.047	0.047	0.046	0.047	0.046	0.047	0.047	0.047	0.046	0.046
B	0.047	0.046	0.046	0.046	0.046	0.048	0.047	0.047	0.047	0.046	0.046	0.046
C	0.046	0.047	0.046	0.099	0.108	0.085	0.084	0.088	0.093	0.047	0.046	0.047
D	0.047	0.047	0.047	0.086	0.088	0.090	0.089	0.095	0.095	0.047	0.047	0.046
E	0.047	0.047	0.047	0.088	0.094	0.093	0.094	0.092	0.091	0.047	0.057	0.046
F	0.046	0.047	0.047	0.094	0.093	0.092	0.047	0.048	0.047	0.047	0.047	0.047
G	0.045	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.055	0.047	0.047	0.047

Figure 35: Optical Density - 12/4/13 - Minute 20 after adding Working Reagent



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.046	0.047	0.046	0.047	0.046	0.047	0.047	0.047	0.046	0.046
B	0.047	0.047	0.046	0.046	0.046	0.048	0.047	0.047	0.047	0.046	0.046	0.046
C	0.046	0.047	0.046	0.099	0.105	0.085	0.084	0.089	0.094	0.047	0.046	0.047
D	0.047	0.046	0.047	0.086	0.088	0.090	0.089	0.096	0.095	0.047	0.047	0.046
E	0.047	0.047	0.047	0.088	0.094	0.092	0.094	0.092	0.089	0.047	0.057	0.046
F	0.046	0.047	0.047	0.094	0.093	0.092	0.047	0.048	0.047	0.063	0.047	0.047
G	0.045	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.058	0.047	0.047	0.047

Figure 36: Optical Density - 12/4/13 - Minute 21

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.047	0.047	0.046	0.047	0.046	0.047	0.047	0.047	0.047	0.046
B	0.047	0.047	0.046	0.046	0.046	0.048	0.047	0.047	0.047	0.046	0.046	0.046
C	0.046	0.047	0.046	0.099	0.106	0.085	0.084	0.087	0.093	0.047	0.046	0.047
D	0.047	0.047	0.047	0.087	0.088	0.090	0.089	0.096	0.095	0.047	0.047	0.046
E	0.047	0.047	0.047	0.088	0.093	0.092	0.094	0.092	0.091	0.047	0.057	0.046
F	0.046	0.047	0.047	0.095	0.093	0.092	0.047	0.048	0.047	0.047	0.047	0.047
G	0.045	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.060	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
3:57 PM 12/5/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.7
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 37: Optical Density - 12/4/13 - Minute 22

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.047	0.047	0.046	0.047	0.046	0.047	0.047	0.047	0.047	0.046
B	0.047	0.047	0.046	0.046	0.046	0.048	0.047	0.047	0.047	0.046	0.046	0.046
C	0.046	0.047	0.046	0.099	0.106	0.085	0.085	0.087	0.093	0.047	0.046	0.047
D	0.047	0.047	0.047	0.087	0.088	0.090	0.089	0.096	0.094	0.047	0.047	0.046
E	0.047	0.047	0.047	0.088	0.093	0.092	0.095	0.092	0.090	0.047	0.057	0.046
F	0.046	0.047	0.047	0.095	0.094	0.093	0.047	0.048	0.047	0.047	0.047	0.046
G	0.046	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.060	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
3:58 PM 12/5/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.7
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 38: Optical Density - 12/4/13 - Minute 23

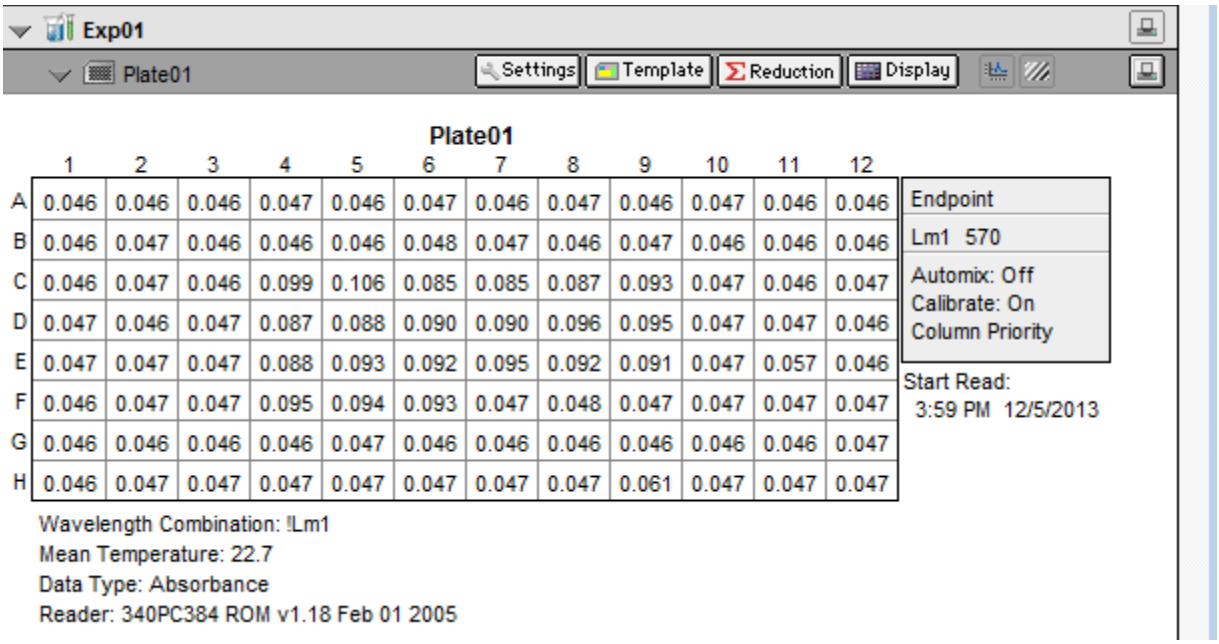


Figure 39: Optical Density - 12/4/13 - Minute 24

12/5/13 High Velocity Run Data

Table 10: 12/5/13 Optical Density – Dilution 2

Time	Optical Density @ 570 nm (2)
0	0.159
5	0.158
10	0.177
15	0.145
20	0.17
25	0.155
30	0.145
35	0.151
40	0.138
45	0.153
50	0.138
55	0.164
60	0.137
65	0.142
70	0.157
75	0.143
80	0.135
85	0.162
90	0.193

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.047	0.047	0.047	0.046	0.046	0.046	0.046	0.047	0.047	0.047	0.046
B	0.047	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046
C	0.047	0.047	0.047	0.162	0.159	0.174	0.147	0.173	0.046	0.047	0.047	0.047
D	0.050	0.047	0.046	0.154	0.144	0.149	0.141	0.155	0.047	0.047	0.047	0.046
E	0.047	0.047	0.047	0.140	0.162	0.139	0.144	0.158	0.053	0.047	0.047	0.048
F	0.047	0.046	0.047	0.147	0.138	0.161	0.194	0.047	0.047	0.047	0.047	0.052
G	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.047	0.047	0.057
H	0.047	0.050	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
6:45 PM 12/5/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.6
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 40: Optical Density - 12/6/13 - Minute 20 after adding Working Reagent

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

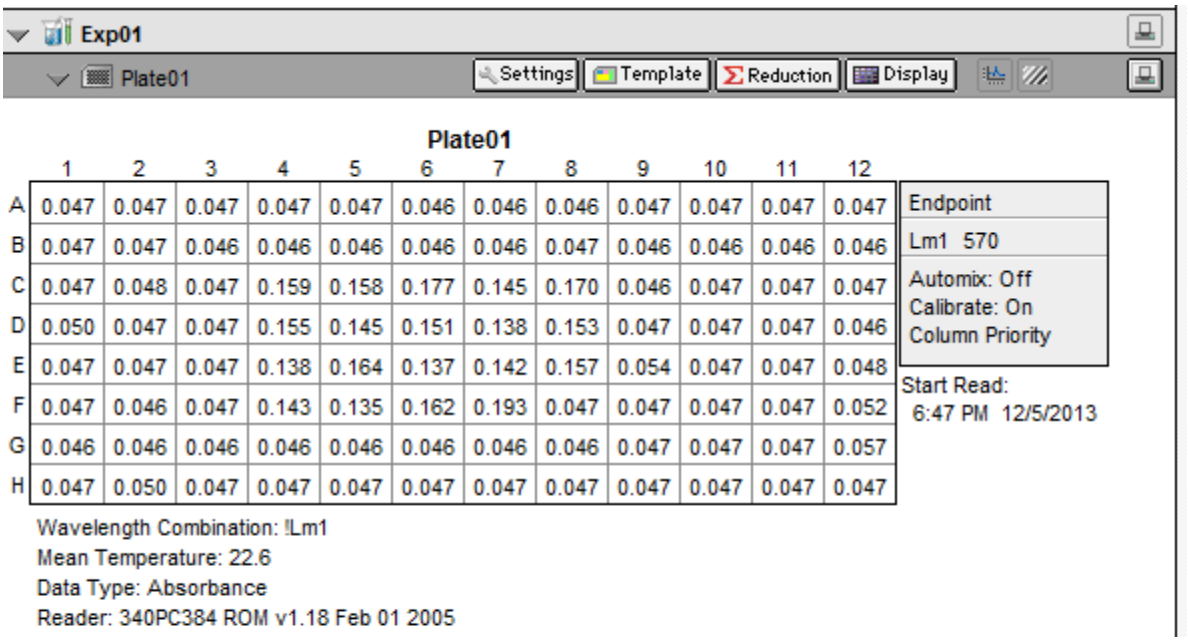
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.047	0.047	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.046
B	0.047	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.047	0.046
C	0.047	0.048	0.047	0.160	0.158	0.177	0.146	0.170	0.046	0.047	0.047	0.047
D	0.050	0.047	0.046	0.154	0.144	0.152	0.139	0.153	0.047	0.047	0.047	0.046
E	0.047	0.047	0.047	0.138	0.160	0.137	0.143	0.156	0.054	0.047	0.047	0.048
F	0.047	0.046	0.047	0.142	0.137	0.162	0.193	0.047	0.046	0.047	0.047	0.052
G	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.047	0.047	0.057
H	0.047	0.050	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.048	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
6:46 PM 12/5/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.6
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 41: Optical Density - 12/5/13 - Minute 21



Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

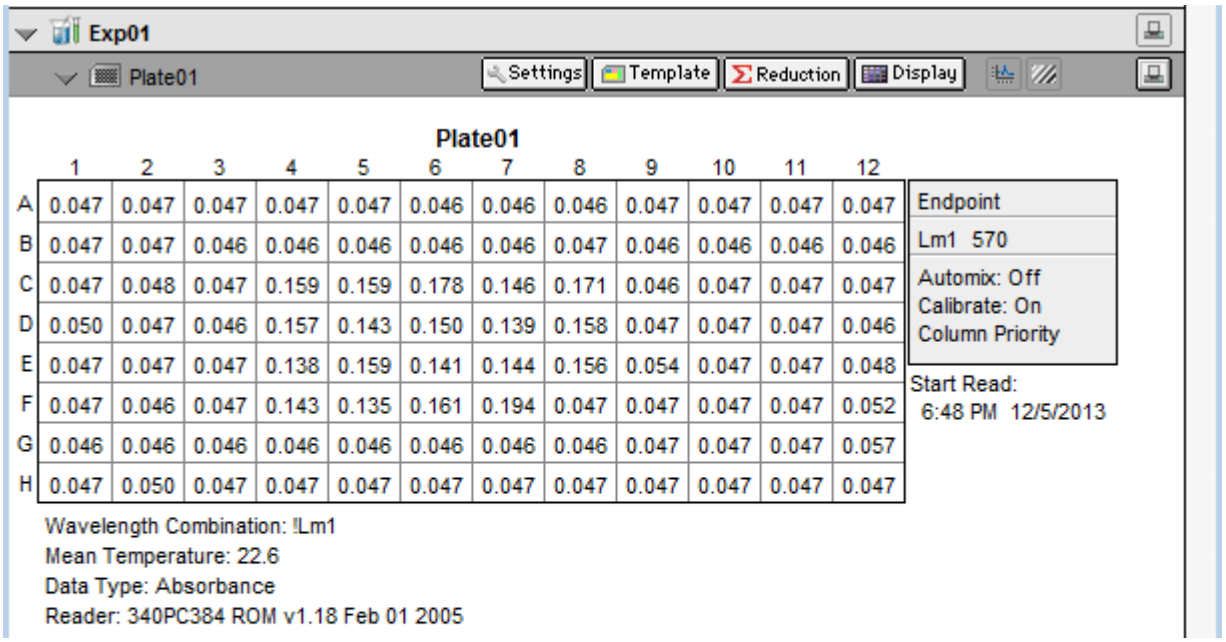
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.047	0.047	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.047
B	0.047	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046
C	0.047	0.048	0.047	0.159	0.158	0.177	0.145	0.170	0.046	0.047	0.047	0.047
D	0.050	0.047	0.047	0.155	0.145	0.151	0.138	0.153	0.047	0.047	0.047	0.046
E	0.047	0.047	0.047	0.138	0.164	0.137	0.142	0.157	0.054	0.047	0.047	0.048
F	0.047	0.046	0.047	0.143	0.135	0.162	0.193	0.047	0.047	0.047	0.047	0.052
G	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.047	0.047	0.057
H	0.047	0.050	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
6:47 PM 12/5/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.6
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 42: Optical Density - 12/5/13 - Minute 22



Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.047	0.047	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.047
B	0.047	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046
C	0.047	0.048	0.047	0.159	0.159	0.178	0.146	0.171	0.046	0.047	0.047	0.047
D	0.050	0.047	0.046	0.157	0.143	0.150	0.139	0.158	0.047	0.047	0.047	0.046
E	0.047	0.047	0.047	0.138	0.159	0.141	0.144	0.156	0.054	0.047	0.047	0.048
F	0.047	0.046	0.047	0.143	0.135	0.161	0.194	0.047	0.047	0.047	0.047	0.052
G	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.047	0.047	0.057
H	0.047	0.050	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
6:48 PM 12/5/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.6
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 43: Optical Density - 12/5/13 - Minute 23

12/6/13 High Velocity Run Data

Table 11: 12/6/13 UO Mixture Optical Density

Time	Optical Density @ 570 nm
0	0.278
3	0.325

6	0.343
9	0.348
12	0.331
15	0.355
18	0.363
21	0.275
24	0.367
27	0.365
30	0.352
33	0.347
36	0.366
39	0.332
42	0.351
45	0.363
48	0.367
51	0.378
54	0.392
57	0.271

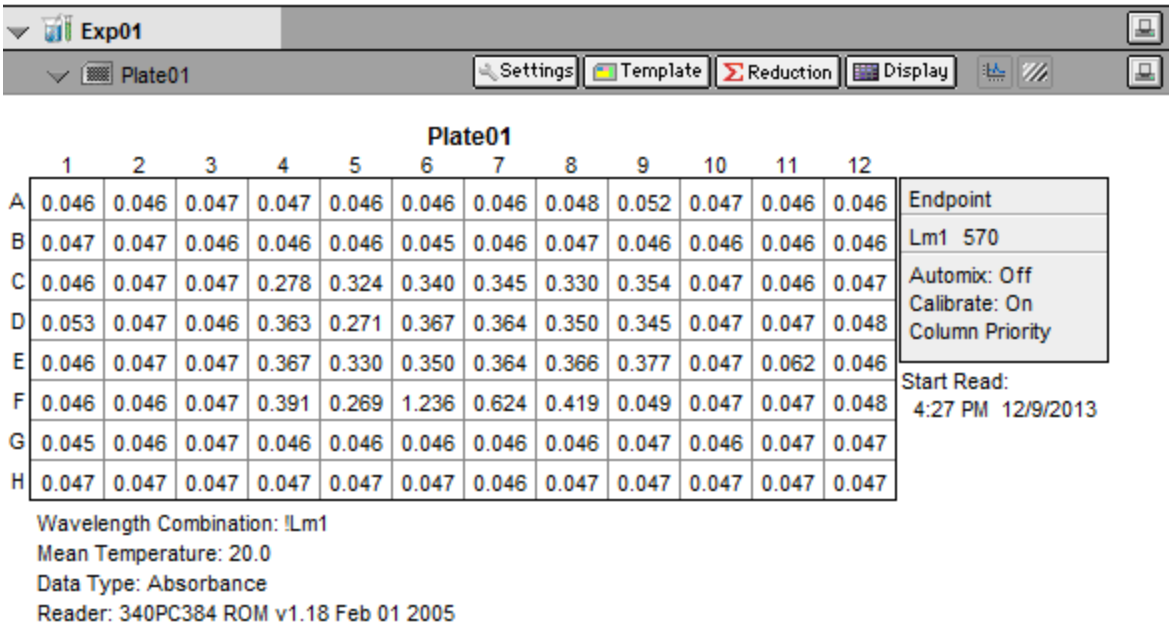


Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.047	0.047	0.046	0.046	0.046	0.048	0.052	0.047	0.046	0.046
B	0.047	0.047	0.046	0.046	0.046	0.045	0.046	0.047	0.046	0.046	0.046	0.046
C	0.046	0.047	0.047	0.278	0.324	0.340	0.345	0.330	0.354	0.047	0.046	0.047
D	0.053	0.047	0.046	0.363	0.271	0.367	0.364	0.350	0.345	0.047	0.047	0.048
E	0.046	0.047	0.047	0.367	0.330	0.350	0.364	0.366	0.377	0.047	0.062	0.046
F	0.046	0.046	0.047	0.391	0.269	1.236	0.624	0.419	0.049	0.047	0.047	0.048
G	0.045	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.047	0.047
H	0.047	0.047	0.047	0.047	0.047	0.047	0.046	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
4:27 PM 12/9/2013

Wavelength Combination: !Lm1
Mean Temperature: 20.0
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 44: Optical Density - 12/9/13 - Minute 20 after adding Working Reagent

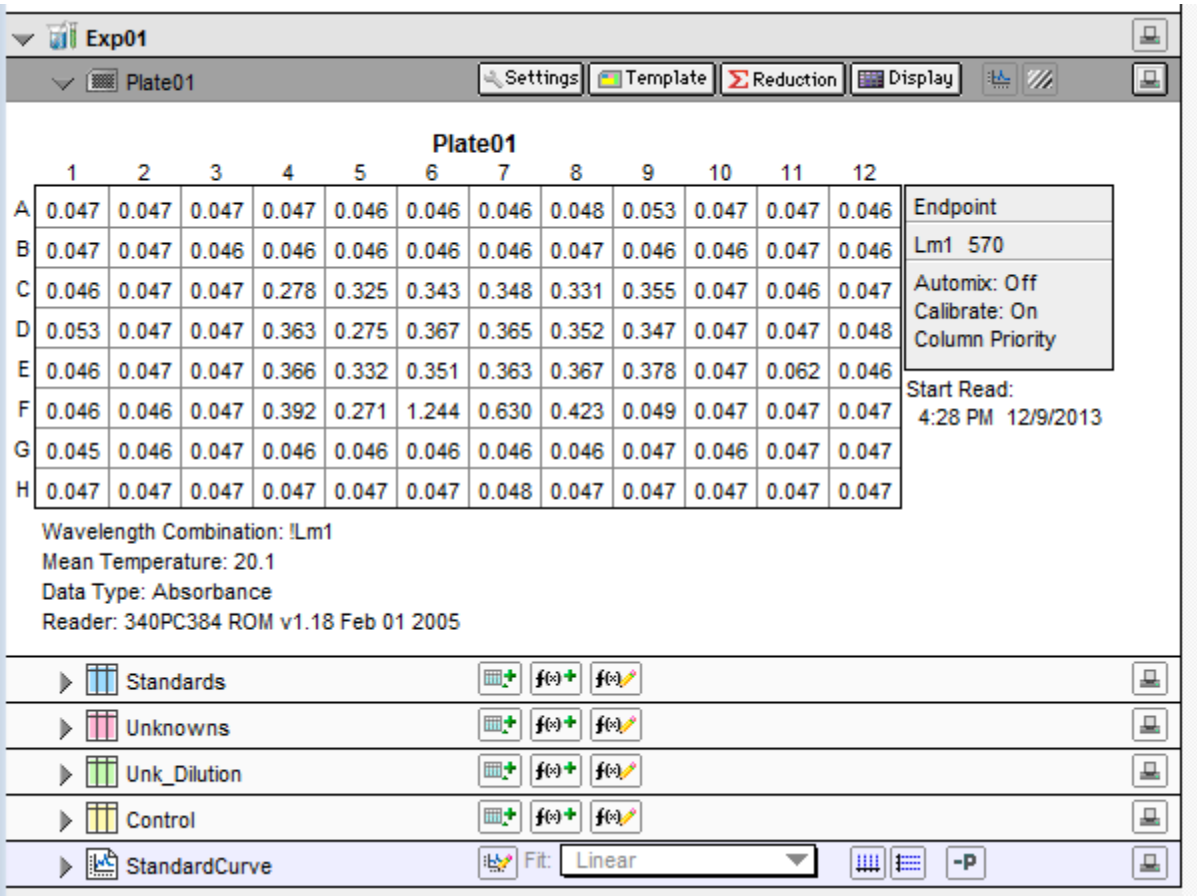


Figure 45: Optical Density - 12/9/13 - Minute 21

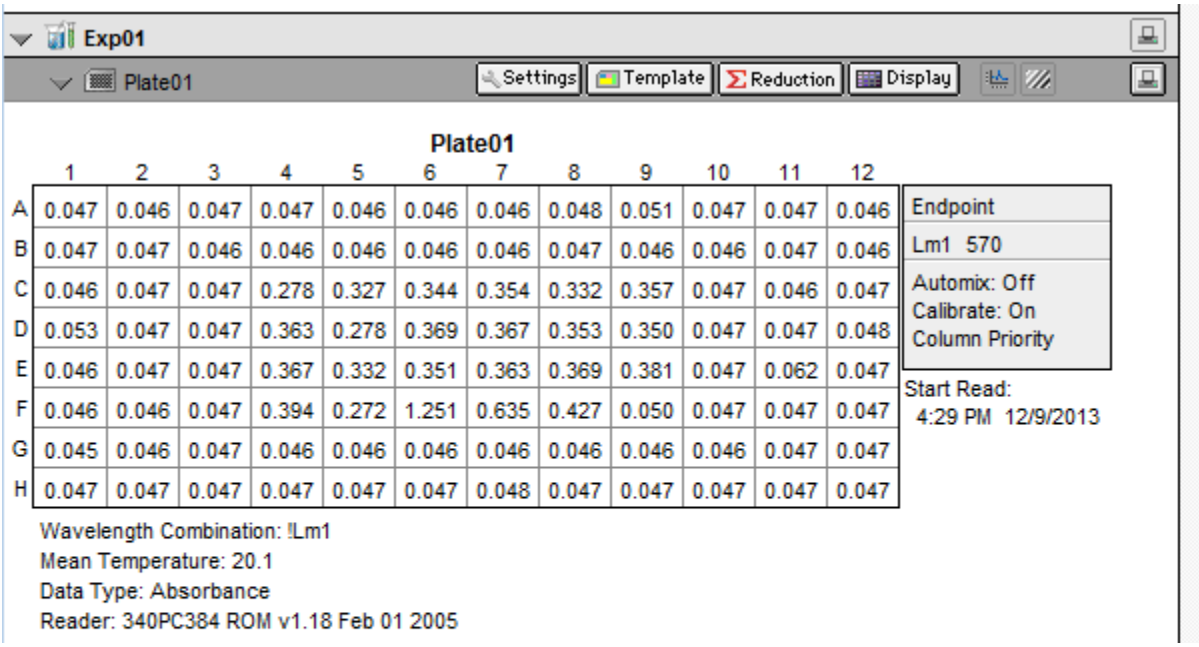


Figure 46: Optical Density - 12/9/13 - Minute 22

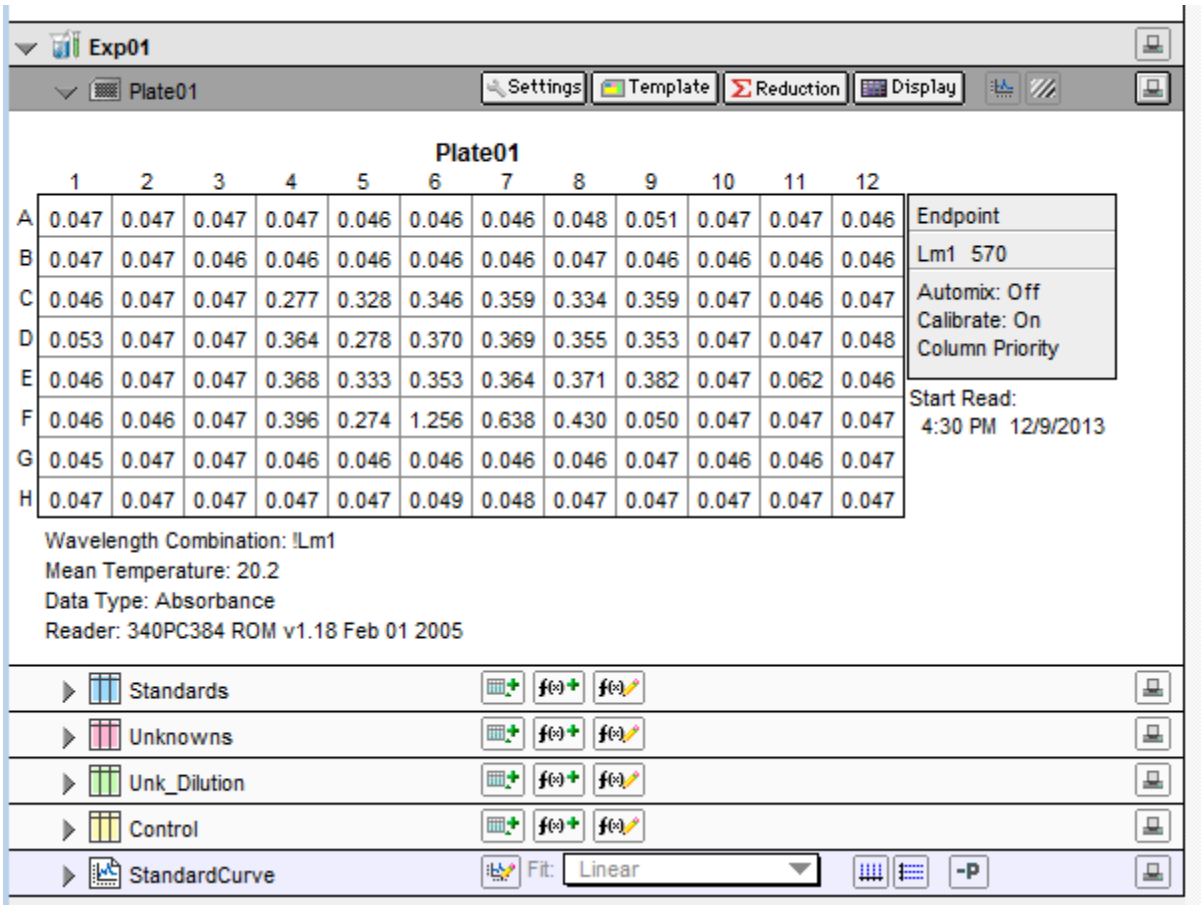


Figure 47: Optical Density - 12/5/13 - Minute 23

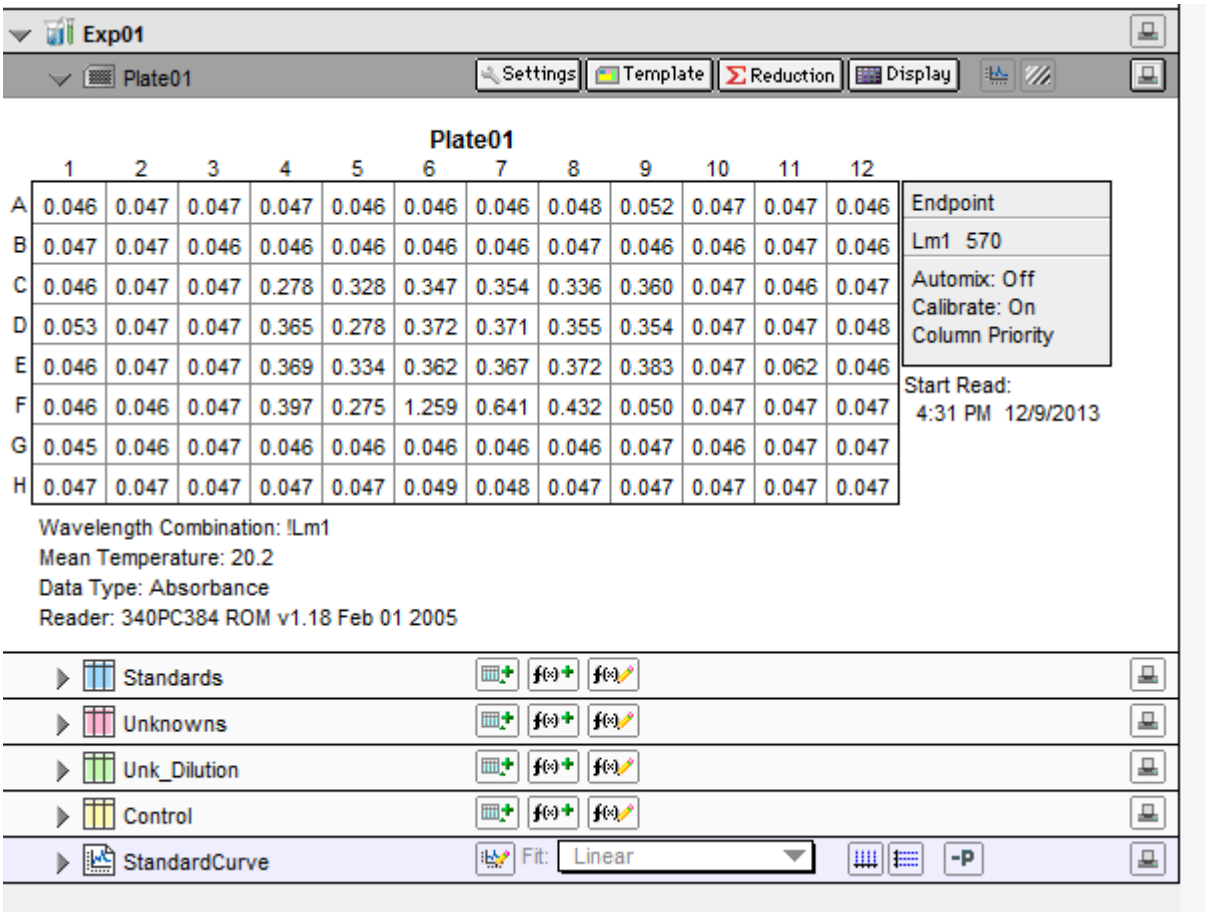
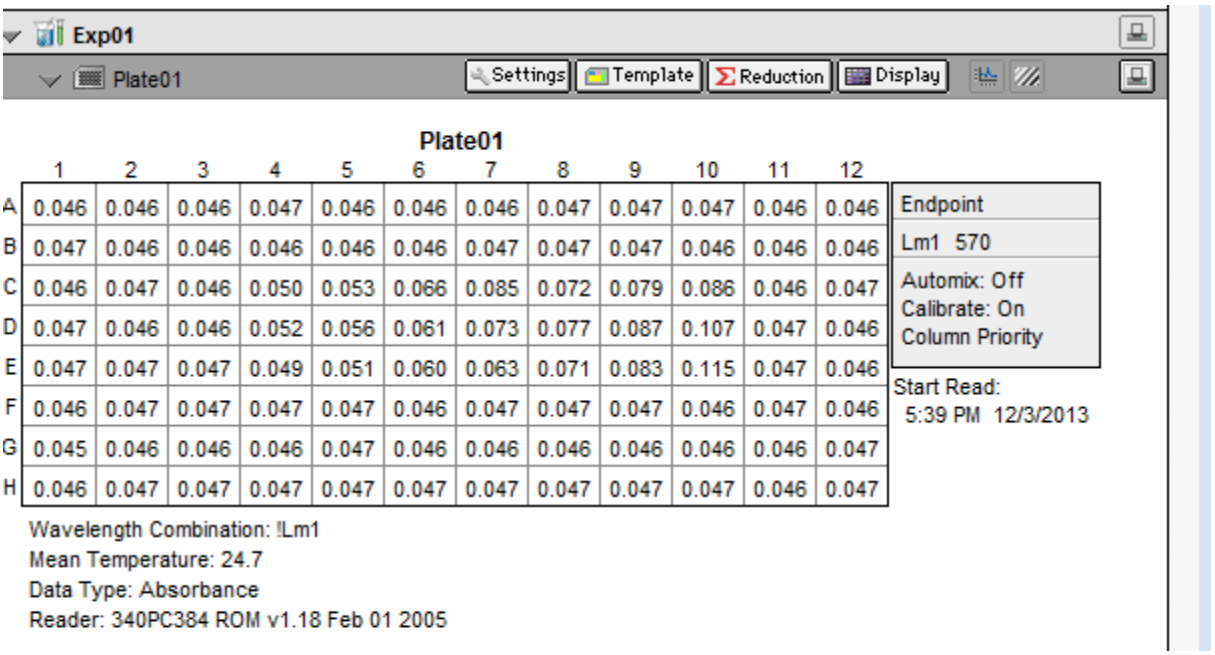


Figure 48: Optical Density - 12/5/13 - Minute 24

Optimizing Dilution

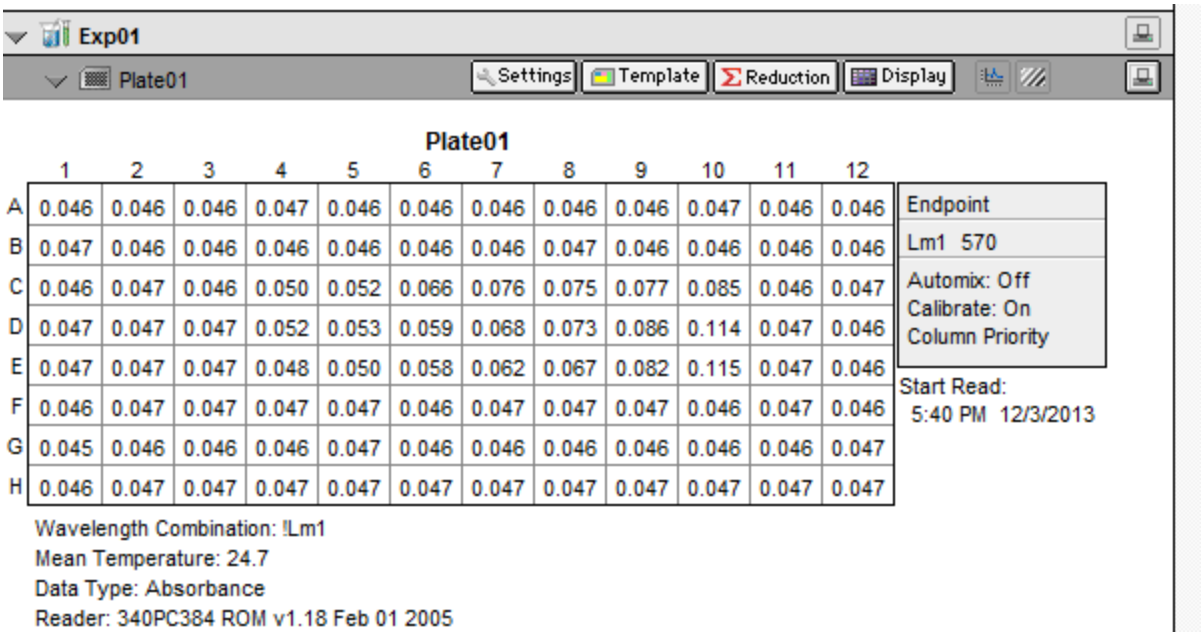
Table 12: Optimizing Dilution

dilution (microL in 20 ml water)	500 microL Optical Density @ 570 nm	100 microL Optical Density @ 570 nm	filtered 500 microL Optical Density @ 570 nm
5	0.049	0.051	0.049
10	0.052	0.054	0.052
20	0.058	0.059	0.064
40	0.062	0.069	0.075
50	0.069	0.073	0.074
100	0.083	0.086	0.078
200	0.116	0.117	0.084



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.046	0.046
B	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.047	0.047	0.046	0.046	0.046
C	0.046	0.047	0.046	0.050	0.053	0.066	0.085	0.072	0.079	0.086	0.046	0.047
D	0.047	0.046	0.046	0.052	0.056	0.061	0.073	0.077	0.087	0.107	0.047	0.046
E	0.047	0.047	0.047	0.049	0.051	0.060	0.063	0.071	0.083	0.115	0.047	0.046
F	0.046	0.047	0.047	0.047	0.047	0.046	0.047	0.047	0.047	0.046	0.047	0.046
G	0.045	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.046	0.047

Figure 49: Optical Density - Optimizing Dilution - Minute 20 after adding Working Reagent



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046
B	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046
C	0.046	0.047	0.046	0.050	0.052	0.066	0.076	0.075	0.077	0.085	0.046	0.047
D	0.047	0.047	0.047	0.052	0.053	0.059	0.068	0.073	0.086	0.114	0.047	0.046
E	0.047	0.047	0.047	0.048	0.050	0.058	0.062	0.067	0.082	0.115	0.047	0.046
F	0.046	0.047	0.047	0.047	0.047	0.046	0.047	0.047	0.047	0.046	0.047	0.046
G	0.045	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Figure 50: Optical Density - Optimizing Dilution - Minute 21

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.046	0.046
B	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.047	0.046	0.046	0.046
C	0.046	0.047	0.046	0.049	0.052	0.064	0.075	0.074	0.078	0.084	0.046	0.047
D	0.047	0.047	0.047	0.051	0.054	0.059	0.069	0.073	0.086	0.117	0.047	0.046
E	0.047	0.047	0.047	0.049	0.052	0.058	0.062	0.069	0.083	0.116	0.047	0.046
F	0.046	0.047	0.047	0.047	0.047	0.046	0.047	0.047	0.047	0.046	0.047	0.046
G	0.045	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
5:41 PM 12/3/2013

Wavelength Combination: !Lm1
Mean Temperature: 24.7
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 51: Optical Density - Optimizing Dilution - Minute 22

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046
B	0.047	0.046	0.046	0.047	0.046	0.046	0.047	0.047	0.047	0.046	0.046	0.046
C	0.046	0.047	0.046	0.049	0.051	0.068	0.076	0.074	0.077	0.085	0.046	0.047
D	0.047	0.046	0.046	0.052	0.054	0.060	0.072	0.077	0.086	0.117	0.047	0.046
E	0.047	0.047	0.047	0.049	0.052	0.058	0.063	0.069	0.083	0.116	0.047	0.046
F	0.046	0.047	0.047	0.046	0.047	0.046	0.047	0.047	0.047	0.046	0.047	0.047
G	0.045	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
5:42 PM 12/3/2013

Wavelength Combination: !Lm1
Mean Temperature: 24.7
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 52: Optical Density - Optimizing Dilution - Minute 23

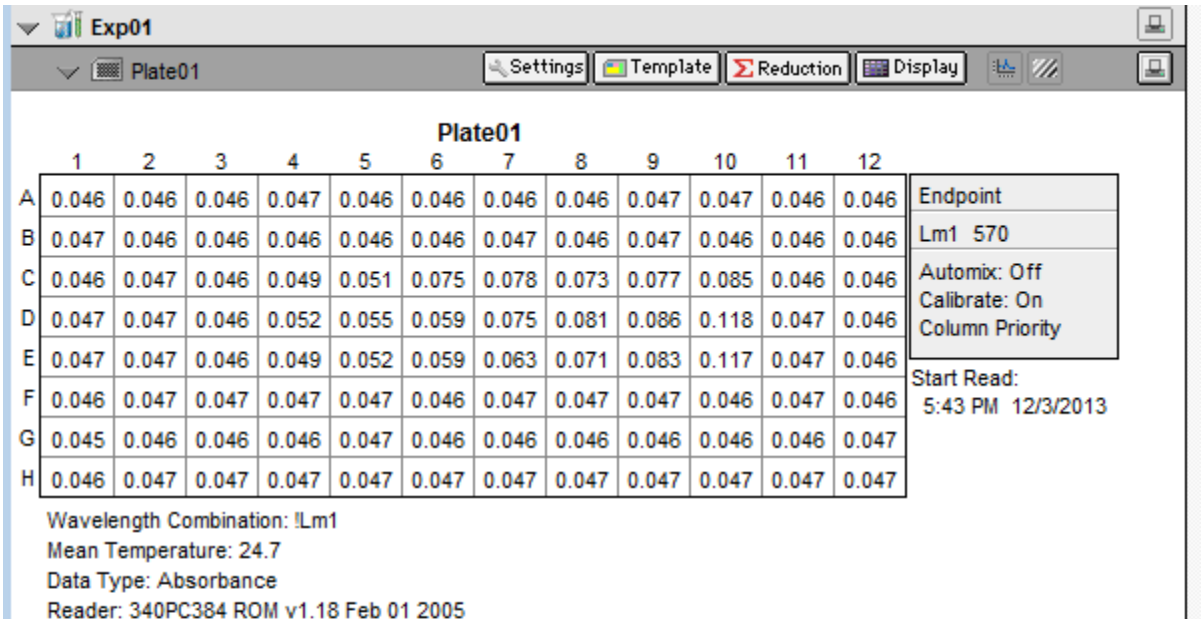


Figure 53: Optical Density - Optimizing Dilution - Minute 24

Extended Run

Table 13: Extended Run

Time (Hour)	Time	Optical Density @ 570nm
0	bottle	0.345
0.1	7:00 PM	0.067
1	8:00:00 PM	0.08
2	9:00:00 PM	0.076
3	10:00:00 PM	0.072
7	2:00 AM	0.073
11	6:00 AM	0.073
15	10:00 AM	0.068
19	2:00 PM	0.069
23	6:00 PM	0.07
27	10:00 PM	0.08
28	*11:00 PM*	0.073
29	*12:00 AM*	0.076
30	1:00 AM	0.071
31	2:00 AM	0.075
32	3:00 AM	0.074
33	4:00 AM	0.066
34	5:00 AM	0.069
35	6:00 AM	0.073
36	7:00 AM	0.078

37	8:00 AM	0.083
38	9:00 AM	0.08
39	10:00 AM	0.085
40	11:00 AM	0.087
41	12:00 PM	0.078
42	1:00 PM	0.088
43	2:00 PM	0.081
44	3:00 PM	0.088
45	4:00 PM	0.096
46	5:00 PM	0.104
47	6:00 PM	0.085
48	7:00 PM	0.093
49	8:00 PM	0.083
50	9:00 PM	0.122
51	10:00 PM	0.096
52	11:00 PM	0.086
53	12:00 AM	0.09
54	1:00 AM	0.092
55	2:00 AM	0.093
56	3:00 AM	0.077
57	4:00 AM	0.075
58	5:00 AM	0.076
59	6:00 AM	0.084
60	7:00 AM	0.098
62	9:00 AM	0.083
63	10:00 AM	0.085
64	11:00 AM	0.077
65	12:00 PM	0.085
66	1:00 PM	0.088
67	2:00 PM	0.08
71	6:00 PM	0.085
75	10:00 PM	0.084
83	6:00 AM	0.091
87	10:00 AM	0.09
91	2:00 PM	0.095
95	6:00 PM	0.087
99	10:00 PM	0.092
103	2:00 AM	0.097
111	10:00 AM	0.093
115	2:00 PM	0.092
119	6:00 PM	0.085
123	10:00 PM	0.085
127	2:00 AM	0.083

131	6:00 AM	0.11
135	10:00 AM	0.083
139	2:00 PM	0.084

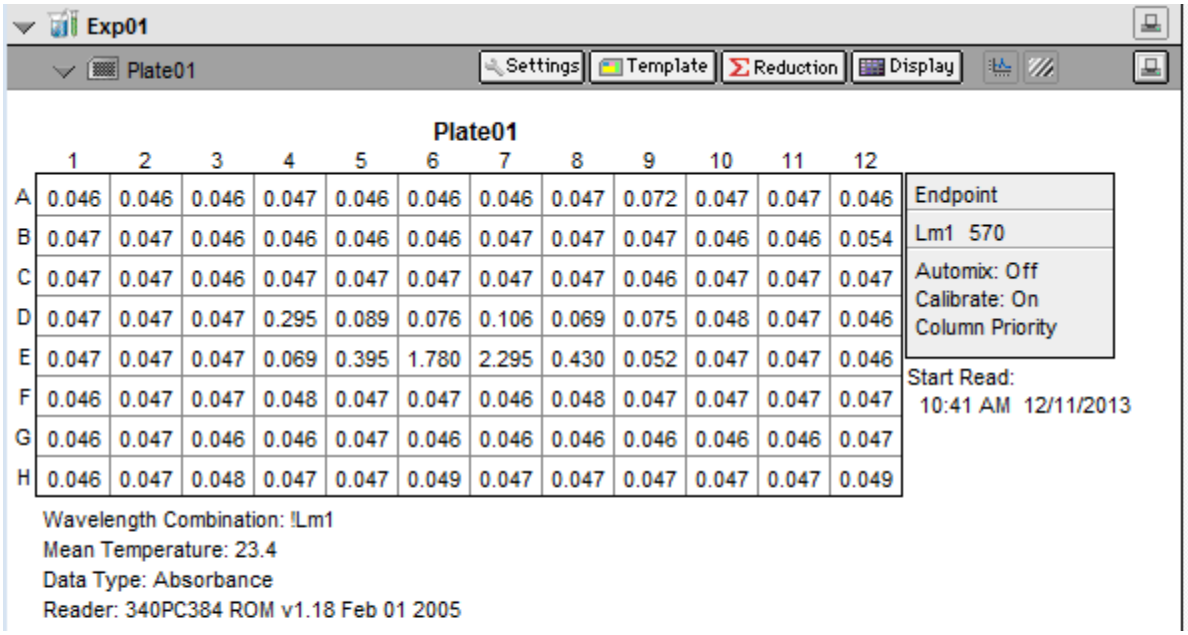


Figure 54: Optical Density Extended Run - 7pm 12/10/13 to 6am 12/11/13 - Minute 20 after adding Working Reagent

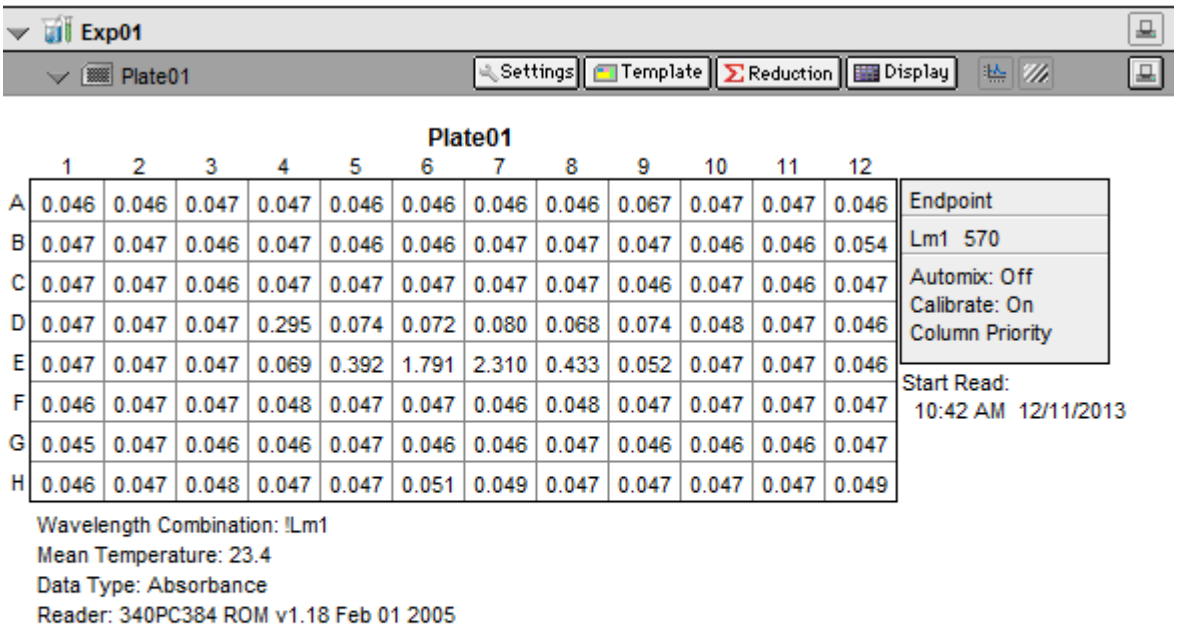


Figure 55: Optical Density Extended Run - 7pm 12/10/13 to 6am 12/11/13 -Minute 21

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.066	0.047	0.047	0.046
B	0.047	0.047	0.046	0.046	0.046	0.046	0.047	0.047	0.047	0.046	0.046	0.054
C	0.047	0.047	0.046	0.047	0.047	0.047	0.047	0.047	0.046	0.047	0.047	0.047
D	0.047	0.047	0.047	0.295	0.084	0.074	0.081	0.069	0.075	0.048	0.047	0.046
E	0.047	0.047	0.047	0.070	0.395	1.799	2.319	0.438	0.052	0.047	0.047	0.046
F	0.046	0.047	0.047	0.048	0.047	0.047	0.046	0.048	0.047	0.047	0.047	0.047
G	0.045	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.048	0.047	0.047	0.051	0.049	0.049	0.047	0.047	0.047	0.048

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
10:43 AM 12/11/2013

Wavelength Combination: !Lm1
Mean Temperature: 23.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 56: Optical Density Extended Run - 7pm 12/10/13 to 6am 12/11/13 - Minute 22

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.065	0.046	0.047	0.046
B	0.047	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.047	0.046	0.046	0.054
C	0.046	0.047	0.046	0.047	0.047	0.047	0.047	0.047	0.046	0.047	0.047	0.047
D	0.047	0.047	0.047	0.295	0.076	0.073	0.080	0.069	0.075	0.048	0.047	0.046
E	0.047	0.047	0.047	0.070	0.400	1.804	2.325	0.440	0.052	0.047	0.047	0.046
F	0.046	0.047	0.047	0.048	0.047	0.047	0.046	0.048	0.047	0.047	0.047	0.047
G	0.045	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.048	0.047	0.047	0.051	0.049	0.049	0.047	0.047	0.047	0.048

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
10:44 AM 12/11/2013

Wavelength Combination: !Lm1
Mean Temperature: 23.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 57: Optical Density Extended Run - 7pm 12/10/13 to 6am 12/11/13 -Minute 23

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.046	0.047	0.047	0.046	0.046	0.046	0.055	0.046	0.047	0.046	0.046
B	0.048	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046
C	0.051	0.047	0.342	0.067	0.079	0.074	0.070	0.071	0.071	0.067	0.046	0.046
D	0.047	0.047	0.068	0.069	0.079	0.070	0.074	0.070	0.074	0.072	0.047	0.046
E	0.058	0.047	0.065	0.068	0.072	0.077	0.081	0.077	0.082	0.084	0.047	0.046
F	0.046	0.046	0.077	0.949	0.632	0.341	0.057	0.047	0.046	0.047	0.047	0.047
G	0.045	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.047
H	0.047	0.047	0.047	0.047	0.049	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
2:21 PM 12/12/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 58: Optical Density Extended Run - 7pm 12/10/13 - 12pm 12/12/13 - Minute 20 after adding Working Reagent

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.047	0.047	0.047	0.046	0.046	0.046	0.056	0.047	0.047	0.046	0.046
B	0.048	0.047	0.046	0.047	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046
C	0.051	0.047	0.347	0.067	0.079	0.075	0.072	0.072	0.072	0.068	0.046	0.046
D	0.047	0.047	0.068	0.069	0.080	0.071	0.074	0.071	0.075	0.073	0.047	0.046
E	0.059	0.047	0.066	0.069	0.073	0.078	0.082	0.079	0.084	0.086	0.047	0.046
F	0.046	0.046	0.078	0.960	0.645	0.347	0.058	0.047	0.046	0.047	0.047	0.047
G	0.045	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.047	0.047	0.047	0.047	0.049	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
2:23 PM 12/12/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 59: Optical Density Extended Run - 7pm 12/10/13 - 12pm 12/12/13 - Minute 22

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.047	0.047	0.047	0.046	0.046	0.046	0.055	0.047	0.047	0.046	0.046
B	0.048	0.047	0.046	0.047	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046
C	0.049	0.047	0.345	0.067	0.080	0.076	0.071	0.072	0.072	0.068	0.046	0.046
D	0.047	0.047	0.068	0.070	0.080	0.071	0.075	0.072	0.074	0.076	0.047	0.046
E	0.060	0.047	0.066	0.069	0.072	0.078	0.082	0.079	0.084	0.086	0.047	0.046
F	0.046	0.046	0.078	0.965	0.651	0.349	0.059	0.047	0.046	0.047	0.047	0.047
G	0.045	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.047
H	0.047	0.047	0.047	0.049	0.049	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
2:24 PM 12/12/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.3
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 60: Optical Density Extended Run - 7pm 12/10/13 - 12pm 12/12/13 - Minute 23

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.047	0.047	0.047	0.046	0.046	0.046	0.054	0.047	0.047	0.046	0.046
B	0.048	0.047	0.046	0.047	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046
C	0.049	0.047	0.346	0.067	0.079	0.076	0.071	0.072	0.072	0.068	0.046	0.046
D	0.047	0.047	0.069	0.070	0.080	0.072	0.074	0.071	0.075	0.073	0.047	0.046
E	0.060	0.047	0.066	0.069	0.073	0.078	0.082	0.079	0.084	0.086	0.047	0.046
F	0.046	0.046	0.078	0.969	0.655	0.351	0.058	0.047	0.046	0.047	0.047	0.047
G	0.045	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.047
H	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
2:25 PM 12/12/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.3
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 61: Optical Density Extended Run - 7pm 12/10/13 - 12pm 12/12/13 - Minute 24

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.046	0.047	0.047	0.046	0.046	0.046	0.055	0.047	0.047	0.047	0.046
B	0.048	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046
C	0.050	0.047	0.348	0.067	0.080	0.076	0.072	0.073	0.073	0.068	0.046	0.046
D	0.047	0.047	0.069	0.070	0.080	0.072	0.076	0.071	0.075	0.074	0.047	0.046
E	0.059	0.047	0.066	0.069	0.073	0.078	0.083	0.080	0.085	0.087	0.047	0.046
F	0.046	0.046	0.078	0.975	0.660	0.354	0.059	0.047	0.047	0.047	0.047	0.047
G	0.045	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.047
H	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
2:26 PM 12/12/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 62: Optical Density Extended Run - 7pm 12/10/13 - 12pm 12/12/13 - Minute 25

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.046	0.046
B	0.047	0.047	0.046	0.046	0.046	0.046	0.047	0.047	0.046	0.046	0.046	0.046
C	0.046	0.047	0.046	0.088	0.081	0.088	0.096	0.104	0.085	0.047	0.046	0.047
D	0.047	0.047	0.047	0.093	0.083	0.122	0.096	0.086	0.090	0.047	0.047	0.046
E	0.047	0.047	0.047	1.040	0.671	0.364	0.052	0.047	0.047	0.047	0.047	0.046
F	0.046	0.047	0.047	0.046	0.047	0.046	0.047	0.047	0.047	0.046	0.047	0.046
G	0.045	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.048	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
1:35 AM 12/13/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.3
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 63: Optical Density Extended Run – 1pm 12/12/13 – 12am 12/13/13 - Minute 20

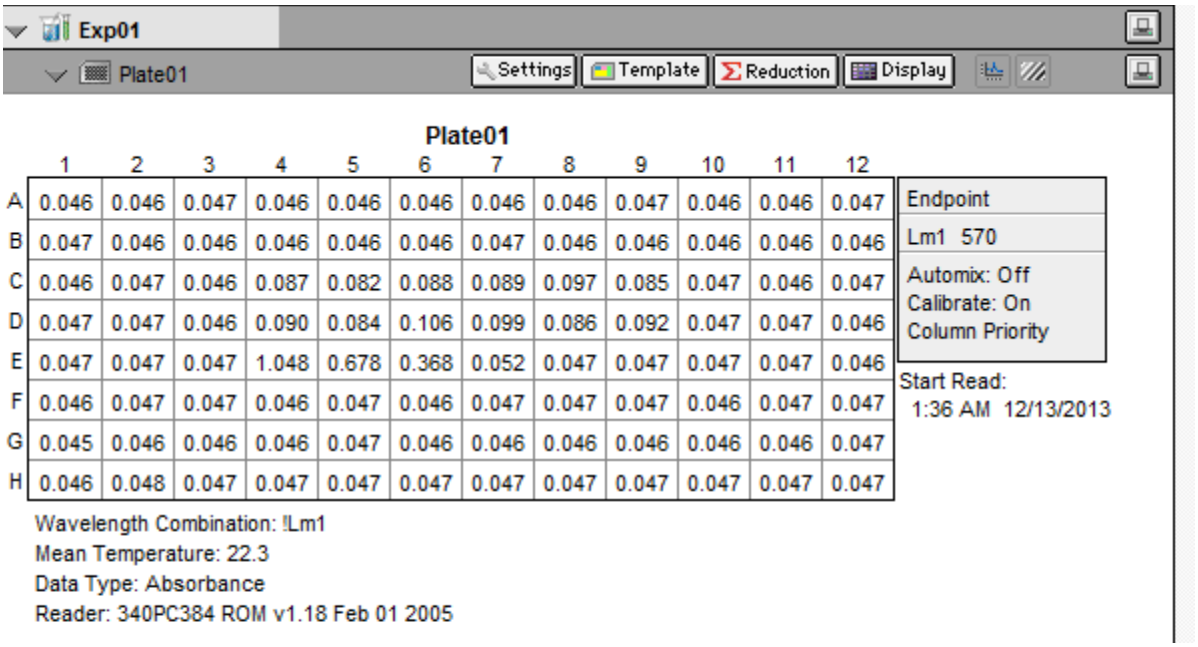


Figure 64: Optical Density Extended Run – 1pm 12/12/13 – 12am 12/13/13 - Minute 21

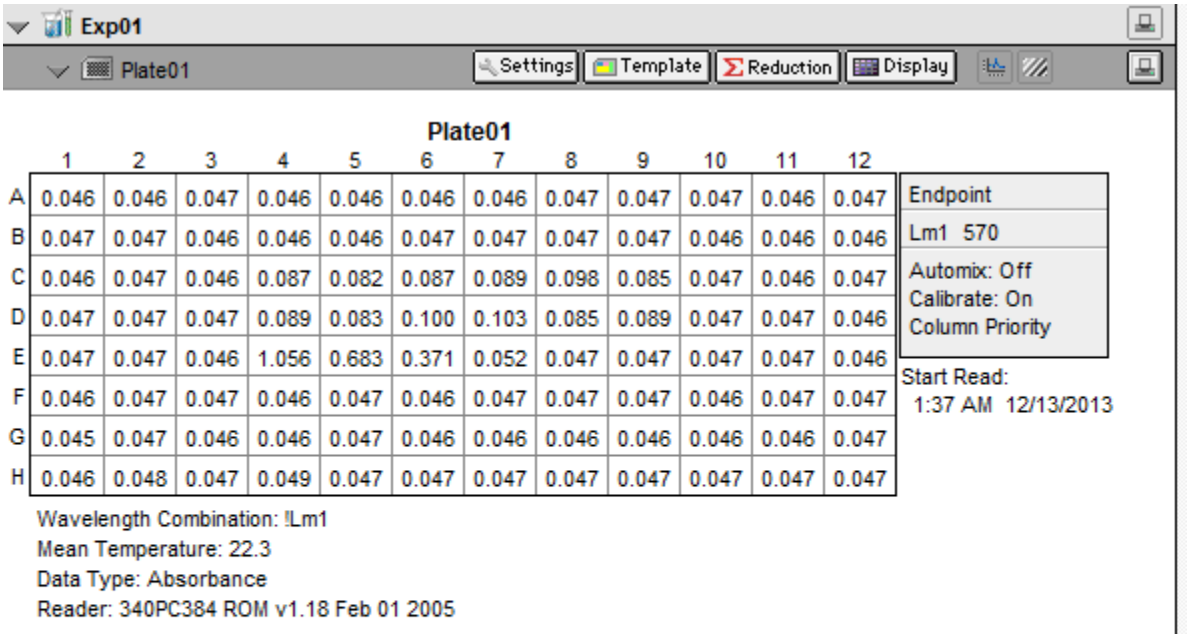


Figure 65: Optical Density Extended Run – 1pm 12/12/13 – 12am 12/13/13 - Minute 22

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.046	0.047
B	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.047	0.047	0.046	0.046	0.046
C	0.046	0.047	0.046	0.087	0.082	0.088	0.089	0.098	0.086	0.047	0.046	0.047
D	0.047	0.046	0.047	0.090	0.084	0.103	0.097	0.085	0.088	0.047	0.047	0.046
E	0.047	0.047	0.046	1.063	0.688	0.374	0.052	0.047	0.047	0.047	0.047	0.046
F	0.046	0.047	0.047	0.046	0.047	0.046	0.046	0.047	0.047	0.046	0.047	0.046
G	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.048	0.047	0.049	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
1:38 AM 12/13/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.3
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 66: Optical Density Extended Run – 1pm 12/12/13 – 12am 12/13/13 - Minutes 23

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.046	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.046	0.047
B	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.047	0.046	0.046	0.046
C	0.046	0.047	0.046	0.088	0.082	0.086	0.090	0.099	0.086	0.047	0.046	0.047
D	0.047	0.046	0.046	0.090	0.084	0.100	0.100	0.086	0.090	0.047	0.047	0.046
E	0.047	0.047	0.047	1.067	0.690	0.376	0.052	0.047	0.047	0.047	0.047	0.046
F	0.046	0.047	0.047	0.046	0.047	0.046	0.047	0.047	0.047	0.046	0.047	0.047
G	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.048	0.047	0.047	0.049	0.047	0.047	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
1:39 AM 12/13/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.3
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 67: Optical Density Extended Run – 1pm 12/12/13 – 12am 12/13/13 - Minute 24

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.046	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.046	0.046
B	0.047	0.046	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046
C	0.046	0.047	0.046	0.094	0.094	0.077	0.075	0.075	0.083	0.047	0.046	0.047
D	0.047	0.046	0.047	0.101	0.084	0.092	0.078	0.083	0.091	0.047	0.047	0.046
E	0.047	0.046	0.046	0.427	1.094	1.460	1.418	0.054	0.047	0.047	0.047	0.046
F	0.046	0.047	0.047	0.046	0.046	0.046	0.046	0.047	0.047	0.046	0.047	0.047
G	0.046	0.046	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.049	0.049	0.049	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
2:03 PM 12/13/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.1
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 68: Optical Density Extended Run – 1am 12/13/13 – 1pm 12/13/13 - Minute 20 after adding Working Reagent

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.047	0.047	0.046	0.046
B	0.047	0.047	0.046	0.046	0.046	0.046	0.047	0.047	0.046	0.046	0.046	0.046
C	0.046	0.047	0.046	0.091	0.094	0.076	0.075	0.075	0.083	0.047	0.046	0.047
D	0.047	0.047	0.046	0.095	0.081	0.085	0.075	0.083	0.088	0.047	0.047	0.046
E	0.047	0.046	0.047	0.424	1.102	1.472	1.430	0.054	0.047	0.047	0.047	0.046
F	0.046	0.047	0.047	0.046	0.046	0.046	0.046	0.047	0.047	0.046	0.047	0.047
G	0.045	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.049	0.047	0.049	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
2:04 PM 12/13/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.1
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 69: Optical Density Extended Run – 1am 12/13/13 – 1pm 12/13/13 - Minute 21

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.046	0.047	0.047	0.046	0.046	0.046	0.047	0.047	0.047	0.046	0.046
B	0.047	0.047	0.046	0.046	0.046	0.046	0.047	0.046	0.047	0.046	0.046	0.046
C	0.046	0.047	0.046	0.092	0.093	0.077	0.075	0.076	0.084	0.047	0.046	0.047
D	0.047	0.047	0.047	0.098	0.083	0.085	0.077	0.085	0.088	0.047	0.047	0.046
E	0.047	0.046	0.046	0.420	1.102	1.478	1.437	0.054	0.047	0.047	0.047	0.046
F	0.046	0.047	0.047	0.046	0.046	0.046	0.046	0.047	0.047	0.046	0.047	0.047
G	0.045	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.049	0.049	0.047	0.047	0.047	0.047	0.046	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
2:05 PM 12/13/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.1
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 70: Optical Density Extended Run – 1am 12/13/13 – 1pm 12/13/13 - Minute 22

Exp01

Plate01 | Settings | Template | Reduction | Display

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046
B	0.047	0.046	0.046	0.046	0.045	0.046	0.046	0.046	0.046	0.046	0.046	0.046
C	0.046	0.047	0.046	0.092	0.094	0.077	0.075	0.076	0.084	0.047	0.046	0.047
D	0.047	0.046	0.046	0.096	0.083	0.085	0.076	0.084	0.089	0.047	0.047	0.046
E	0.047	0.046	0.047	0.424	1.107	1.484	1.443	0.054	0.047	0.047	0.047	0.046
F	0.046	0.047	0.047	0.046	0.046	0.046	0.046	0.047	0.047	0.046	0.047	0.046
G	0.046	0.047	0.046	0.046	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.047
H	0.046	0.047	0.047	0.047	0.049	0.049	0.049	0.047	0.047	0.047	0.047	0.047

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
2:06 PM 12/13/2013

Wavelength Combination: !Lm1
Mean Temperature: 22.1
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 71: Optical Density Extended Run – 1am 12/13/13 – 1pm 12/13/13 - Minute 23

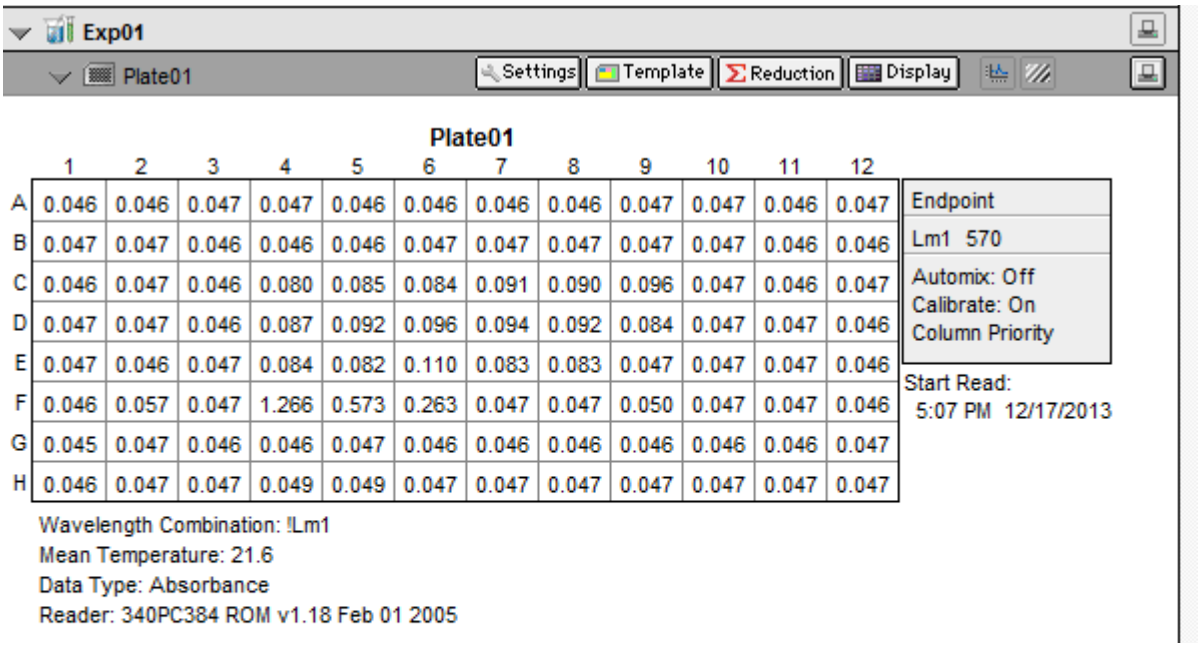


Figure 72: Optical Density Extended Run - 2pm 12/13/13 – 2pm 12/16/13 - Minute 22 after adding Working Reagent

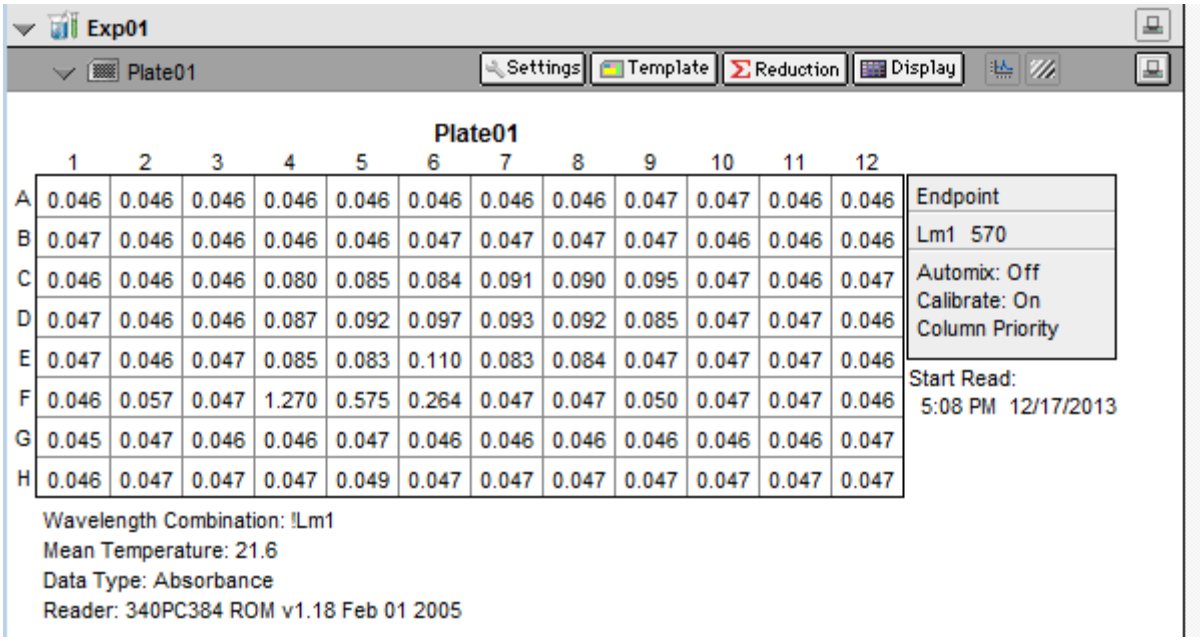


Figure 73: Optical Density Extended Run - 2pm 12/13/13 – 2pm 12/16/13 - Minute 23

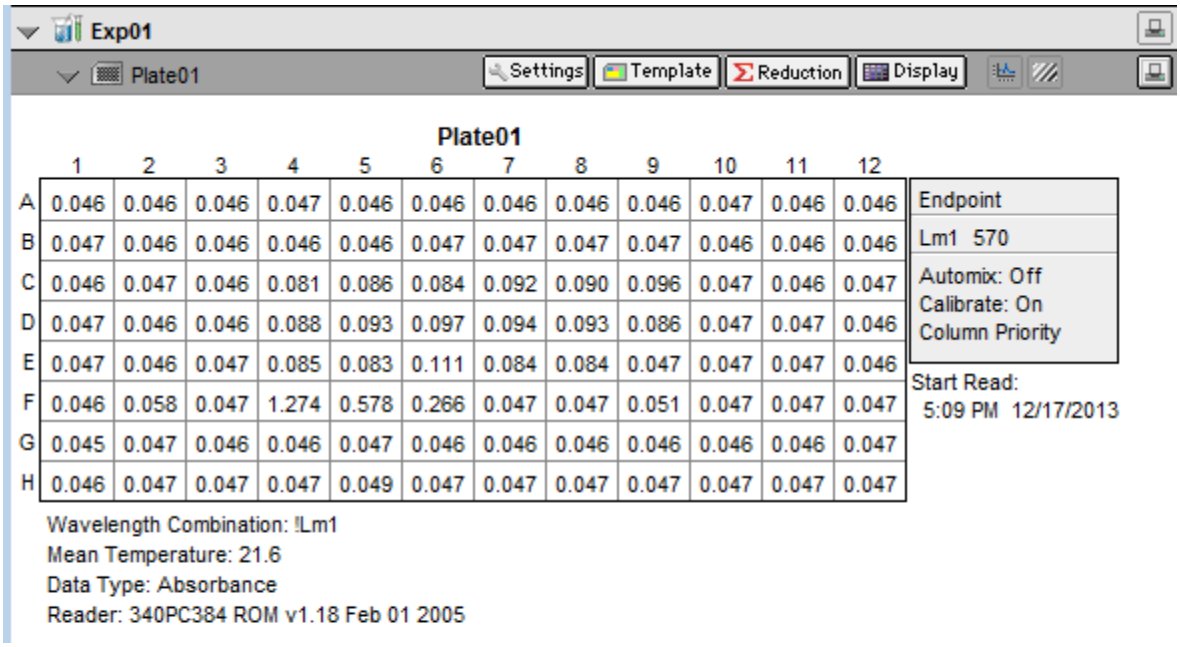


Figure 74: Optical Density Extended Run – 2pm 12/13/13 – 2pm 12/16/13 - Minute 24

Adsorption Isotherm

Table 14: Adsorption Isotherm

grams resin	Optical density @ 570nm
0	0.687
0.01	0.607
0.02	0.764
0.04	0.506
0.06	0.518
0.08	0.775
0.1	0.665
0.12	1.057
0.14	0.453
0.16	0.329
0.18	0.265
0.2	0.207

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.054	0.050	0.048	0.050	0.048	0.047	0.047	0.046	0.068	0.051	0.048	0.062
B	0.075	0.050	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.047	0.063
C	0.046	0.047	0.047	0.679	0.615	0.771	0.499	0.587	0.781	0.048	0.046	0.061
D	0.051	0.047	0.047	0.649	1.056	0.445	0.323	0.261	0.202	0.049	0.050	0.051
E	0.047	0.047	0.050	1.154	0.068	1.268	1.060	0.048	0.053	0.049	0.047	0.056
F	0.049	0.047	0.047	0.980	0.663	0.380	0.065	0.048	0.047	0.047	0.048	0.047
G	0.047	0.050	0.049	0.056	0.052	0.047	0.048	0.047	0.047	0.047	0.047	0.061
H	0.060	0.052	0.048	0.056	0.053	0.049	0.051	0.049	0.049	0.047	0.047	0.048

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
8:57 PM 2/19/2014

Wavelength Combination: !Lm1
Mean Temperature: 23.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 75: Adsorption Isotherm Data - Minute 20 after Loading Working Reagent

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.054	0.050	0.048	0.050	0.048	0.048	0.047	0.046	0.069	0.052	0.048	0.062
B	0.077	0.050	0.046	0.047	0.046	0.046	0.046	0.047	0.046	0.047	0.047	0.063
C	0.046	0.047	0.047	0.682	0.605	0.774	0.503	0.580	0.775	0.048	0.046	0.061
D	0.051	0.048	0.047	0.658	1.047	0.443	0.326	0.263	0.204	0.049	0.050	0.051
E	0.047	0.047	0.050	1.167	0.069	1.272	1.067	0.048	0.053	0.049	0.047	0.056
F	0.049	0.047	0.048	0.989	0.668	0.383	0.065	0.048	0.047	0.047	0.047	0.047
G	0.047	0.050	0.049	0.055	0.052	0.047	0.048	0.047	0.049	0.047	0.047	0.061
H	0.059	0.052	0.048	0.056	0.053	0.049	0.051	0.049	0.049	0.047	0.047	0.048

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
8:58 PM 2/19/2014

Wavelength Combination: !Lm1
Mean Temperature: 23.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 76: Adsorption Isotherm Data - Minute 21

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.054	0.050	0.048	0.050	0.048	0.047	0.047	0.046	0.069	0.052	0.048	0.062
B	0.078	0.049	0.046	0.047	0.047	0.046	0.046	0.047	0.046	0.047	0.047	0.064
C	0.046	0.047	0.047	0.682	0.605	0.778	0.504	0.543	0.775	0.048	0.046	0.061
D	0.051	0.048	0.047	0.658	1.078	0.450	0.327	0.264	0.206	0.049	0.050	0.052
E	0.047	0.047	0.050	1.157	0.069	1.273	1.072	0.048	0.053	0.048	0.048	0.056
F	0.048	0.047	0.047	0.995	0.672	0.384	0.065	0.048	0.047	0.047	0.047	0.047
G	0.047	0.050	0.049	0.054	0.052	0.047	0.048	0.047	0.049	0.047	0.047	0.062
H	0.059	0.052	0.048	0.056	0.053	0.049	0.051	0.048	0.049	0.047	0.047	0.048

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
8:59 PM 2/19/2014

Wavelength Combination: !Lm1
Mean Temperature: 23.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 77: Adsorption Isotherm Data - Minute 22

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.054	0.050	0.048	0.051	0.048	0.047	0.047	0.046	0.069	0.052	0.048	0.062
B	0.078	0.049	0.046	0.047	0.047	0.046	0.046	0.047	0.046	0.047	0.047	0.064
C	0.046	0.047	0.047	0.687	0.607	0.764	0.506	0.518	0.775	0.048	0.046	0.061
D	0.051	0.048	0.047	0.665	1.057	0.453	0.329	0.265	0.207	0.049	0.050	0.052
E	0.047	0.047	0.050	1.138	0.070	1.279	1.077	0.048	0.053	0.048	0.047	0.056
F	0.048	0.047	0.048	1.001	0.675	0.386	0.066	0.048	0.047	0.047	0.047	0.047
G	0.047	0.050	0.049	0.054	0.052	0.047	0.048	0.047	0.049	0.047	0.047	0.062
H	0.059	0.052	0.048	0.056	0.053	0.049	0.051	0.049	0.049	0.048	0.047	0.048

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
9:00 PM 2/19/2014

Wavelength Combination: !Lm1
Mean Temperature: 23.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 78: Adsorption Isotherm Plate Data - Minute 23

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.054	0.050	0.048	0.051	0.048	0.048	0.047	0.046	0.069	0.052	0.048	0.062
B	0.078	0.049	0.047	0.047	0.047	0.046	0.046	0.047	0.046	0.047	0.047	0.064
C	0.046	0.047	0.047	0.686	0.610	0.777	0.508	0.510	0.780	0.048	0.046	0.061
D	0.051	0.048	0.047	0.663	1.064	0.449	0.331	0.267	0.209	0.049	0.051	0.052
E	0.047	0.047	0.050	1.121	0.070	1.280	1.083	0.048	0.053	0.048	0.047	0.056
F	0.048	0.047	0.048	1.007	0.679	0.389	0.066	0.048	0.047	0.047	0.047	0.047
G	0.047	0.050	0.049	0.054	0.052	0.047	0.048	0.047	0.049	0.047	0.047	0.062
H	0.059	0.052	0.048	0.056	0.052	0.049	0.051	0.048	0.049	0.047	0.047	0.048

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
9:01 PM 2/19/2014

Wavelength Combination: !Lm1
Mean Temperature: 23.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 79: Adsorption Isotherm Data - Minute 24

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.054	0.050	0.048	0.050	0.048	0.047	0.047	0.046	0.069	0.052	0.047	0.062
B	0.078	0.049	0.046	0.047	0.047	0.046	0.046	0.047	0.046	0.047	0.047	0.064
C	0.046	0.047	0.047	0.688	0.621	0.775	0.509	0.514	0.781	0.048	0.046	0.062
D	0.051	0.048	0.047	0.662	1.083	0.457	0.332	0.268	0.210	0.049	0.050	0.052
E	0.047	0.047	0.050	1.111	0.070	1.284	1.086	0.048	0.053	0.048	0.048	0.056
F	0.049	0.047	0.048	1.011	0.682	0.390	0.066	0.048	0.047	0.047	0.047	0.047
G	0.047	0.050	0.049	0.054	0.052	0.047	0.048	0.047	0.049	0.047	0.047	0.062
H	0.059	0.052	0.048	0.056	0.052	0.049	0.051	0.049	0.049	0.047	0.047	0.048

Endpoint
Lm1 570
Automix: Off
Calibrate: On
Column Priority

Start Read:
9:02 PM 2/19/2014

Wavelength Combination: !Lm1
Mean Temperature: 23.4
Data Type: Absorbance
Reader: 340PC384 ROM v1.18 Feb 01 2005

Figure 80: Adsorption Isotherm Data - Minute 25