GLYCEROL SENSING IN BIODIESEL USING TURBIDIMETRY

A. Zawadzki, D. S. Shrestha

ABSTRACT. *Free glycerol and total glycerol are the key quality parameters of biodiesel. Turbidity caused by emulsion formation when biodiesel is mixed with water was investigated as a method to sense free glycerol (FG) and bound glycerol (BG). Turbidity was measured as the absorbance at 600 nm. Six batches of biodiesel samples were spiked with 0%, 0.01%, 0.02%, and 0.03% of FG prepared with glycerol-ethanol solution. A small amount of deionized water was added, and the prepared sample was shaken in a shaker. The turbidity was measured at 3 min intervals at up to 27 min of shaking. Initially, the turbidity increased rapidly with shaking, but it soon leveled off. A linear relationship was found between FG and the square root of turbidity measured after 12 min of shaking (R^2 = 0.87). Although ANOVA showed a significant difference in mean measured turbidities with different levels of FG and BG, a mathematical relationship could not be derived relating BG to turbidity measurement. It is pointed out that the variation in turbidity could have come from unmeasured sterols rather than from BG itself. This method of predicting FG from turbidity was shown to be useful in screening biodiesel batches that need further glycerol removal through additional water washing or other techniques in a biodiesel production process. An absorbance value of less than 0.12 corresponded to an FG value of less than 0.02% with a 95% confidence interval. Four production samples of biodiesel showed that the absorbance limit of 0.12 is adequately conservative because the turbidity of the production samples was found to be slightly higher for the same amount of free glycerol compared to the spiked samples. This observation was ascribed to the presence of other impurities in the production samples, which were maintained at zero level for the control samples.*

Keywords. *Biodiesel, Glycerol, Sensor, Turbidimetry.*

Free and total glycerol in biodiesel affect the fuel quality in several ways. Fuel with excessive free glycerol usually causes problems with glycerol settling in storage tanks, creating a viscous mixture that can plug fuel filters and create combustion problems in engines (Van Gerpen et al., 2004). Residual glycerides in the fuel indicate lack of completeness of the transesterification reaction into mono-alkyl esters. High levels of mono-, di-, and triglycerides can cause injector deposits and adversely affect cold weather operation. According to the National Renewable Energy Laboratory, out of 32 B100 samples surveyed nationwide for quality, one failed for free glycerol content and ten failed for total glycerol content (Alleman et al., 2006). According to ASTM Standard D6751, biodiesel should contain a maximum of 0.02% free glycerol (FG) and a maximum of 0.24% total glycerol by weight.

Different methods have been proposed for measuring free and total glycerol in biodiesel. A commercially available kit for an enzymatic method to determine glycerol content involves mixing of specific reagents followed by spectrophotometric measurement (Sigma-Aldrich, 2003). Free glycerol concentration in the sample is directly proportional to absorbance at 540 nm of guinoneimine dye. However, the distribution of the kit was discontinued by the enzyme vendor for unknown reasons, and no further information is available on the cause of the discontinuation. Bondioli and Bella (2005) applied periodate oxidation of glycerol followed by an enzymatic reaction, and the use of dye for glycerol detection. Both methods involved the use of multiple reagents and a spectrophotometer. A commercially available "pHLip" test allows visual biodiesel quality testing. In this test, a red liquid is mixed with biodiesel, and when the biodiesel contains glycerides or glycerol or is rancid, the added fuel becomes turbid and the liquid changes its color. The test is qualitative and subjective in nature and requires purchasing the test kit.

The only specified method in ASTM Standard D6751 for measuring free and total glycerol levels is test method D6584, which entails the use of a gas chromatograph (GC). This method requires a relatively expensive GC instrument, derivatizing reagents, and trained personnel for operation. Additionally, the testing process can be time-intensive if the sample is shipped to an external laboratory. Therefore, there is a pressing need for a rapid and inexpensive estimation method of free glycerol levels in biodiesel that can be used as a field test to screen biodiesel batches that are distinctly within or above specification limits. Such a quick field test should be affordable, portable, and easy to use.

Washing with water followed by heating the fuel until it clarifies is a common way to purify biodiesel. The aim of the heating step is to evaporate the remaining alcohol and water. The speed of the clarification process may vary from one batch to another. The factors influencing the clarification time include the glycerol and glycerides content. Conversely to the drying process, if glycerol and glycerides are present when biodiesel is mixed with water, the fuel becomes turbid. Increase in fuel turbidity upon mixing with water could be ex-

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plained, at least partially, by the presence of glycerol and glyceride impurities in biodiesel.

Upon mixing biodiesel with water, an emulsion is formed. An emulsion is usually defined as a system of two immiscible liquids, one being dispersed in the other in the form of small droplets (Formo et al., 1979). Emulsion formation causes turbidity of the sample, and quantitative estimation of the emulsion can be done using turbidimetry. Robinson (1995) pointed out that turbidity can be measured by the light not scattered by particles. Shape, size, refractive index, and concentration of the particles influence light scattering. Two identical samples of equal analyte concentration will scatter light equally if they form the same number and size distribution of particles. The particle distribution depends on several experimental conditions, including temperature, degree of agitation, and the length of time that the precipitates are allowed to stand. Ingle and Crouch (1988) defined turbidity quantitatively. Turbidimetric methods measure the decrease that occurs in the transmitted radiation because of particle scattering. The attenuation by scattering follows the relationship:

$$t = \frac{1}{b} \ln \frac{\phi_0}{\phi} \quad (1)$$

where φ is transmitted radiant intensity, φ_0 is incident radiant intensity, b is path length, and t is turbidity (cm^{-1}). Notice that $\ln(\varphi_0/\varphi)$ can be measured directly using a spectrophotometer, and turbidity measurement resembles absorbance measurement using Beer's law. As pointed out by Ingle and Crouch (1988), light scattering from a turbid solution may interfere with absorption measurements, while absorbing species interfere with turbidimetric measurement. Additionally, to obtain precise and accurate results, factors such as pH, temperature, and concentration of the reagents as well as order of mixing, ionic strength, and time before measurement must be carefully reproduced.

The objective of this article is to investigate the use of turbidimetry to determine the free and bound glycerol content in biodiesel. The use of turbidimetry in measuring glycerol provides a cost-effective, quick, and portable way of evaluating biodiesel for its glycerol content.

Materials and Methods

Biodiesel batches from different feedstocks (four from the brassica group and two from the soybean group) were prepared at the biodiesel laboratory of the Department of Biological and Agricultural Engineering, University of Idaho. The four brassica feedstocks were from mustard, rapeseed, canola, and camelina. The biodiesel was then thoroughly water washed and dried to ensure complete removal of free glycerol, soap, and alcohol. Quality parameters were tested for each batch of biodiesel using standard methods. Free and total glycerol in the biodiesel was measured according to ASTM method D6584 using a gas chromatograph (Chemstation 6890N, Agilent Technologies, Santa Clara, Cal.) with a flame ionization detector. Soap content was measured according to AOCS method Cc17-95, and acid value was measured according to ASTM method D974-80.

Turbidity of the biodiesel and deionized water mixture was measured as the absorbance at 600 nm using a single-beam general-purpose spectrophotometer (model DU520, Beckman Coulter, Fullerton, Cal.). Biodiesel from the feedstocks used in the experiments showed negligible absorbance starting from about 600 nm up to the near-infrared band (Zawadzki et al., 2005). This wavelength was selected because of low interference from absorption of biodiesel components, and it was also within the visible range applicable for the development of a low-cost sensor. In addition, the 600 nm wavelength is typically used for applications in biological samples, e.g., measuring light not scattered by microbial cultures (Madigan et al., 2002). Therefore, we decided to use this wavelength for the measurement of turbidity of the biodiesel samples. Standard 1 cm polystyrene cuvettes were used to hold the biodiesel samples during measurements.

For a full-factorial experiment of free and total glycerol, samples from each batch in table 1 were spiked with four levels of glycerol in an ethanol solution to bring the free glycerol levels to 0%, 0.01%, 0.02%, and 0.03%. Samples containing each glycerol level were repeated three times for each batch, so 12 samples were prepared from each batch.

A spiked sample was prepared in three steps. In the first step, 1 mL of glycerol was dissolved into 9 mL of ethanol to make 10% (v/v) glycerol stock solution. In the second step, x mL of stock solution (x = 0, 1, 2, or 3) was mixed with $10x$ mL of ethanol in volumetric flasks to make x% (v/v) glycerol-ethanol solution. Finally, 100 µL of glycerol-ethanol solution was added to 9.9 mL of biodiesel samples to make 0.01%, 0.02%, or 0.03% free glycerol in the biodiesel. Adding the same amount of ethanol solution eliminated the influence of ethanol as a factor in the experiments.

The biodiesel samples were mixed with water using a reciprocating water bath (model 1217, Sheldon Manufacturing, Cornelius, Ore) with the stroke length of 64.7 mm. Oscillation was set to 100 rpm, and the biodiesel samples were mixed with water under uniform operating conditions. Low-density polyethylene cuvette caps were used to prevent the biodiesel from spilling during shaking. Holders for the cuvettes were fabricated using hard foam at the University of Idaho machine shop in order to hold 24 cuvettes horizontally for one series of measurements. At the beginning of mixing, 200 µL of deionized water was placed directly onto 2 mL of biodiesel. The cuvettes were manually transferred to the spectrophotometer, and the reading of absorbance was recorded. The samples were then put back into the shaker for further mixing. Turbidity of the samples was relatively stable during the measurement process (about 10 min for 24 cuvettes). Absorbance readings were recorded after every 3 min of shaking up to 27 min. The absorbance after x min of shaking was designated tx. All measurements were performed at normal room temperature (25°C).

For comparison of turbidity measurements between spiked and non-spiked samples, four additional samples of biodiesel were made. They were prepared from vegetable oil

Table 1. Bound glycerol for six batches of biodiesel (BME = brassica, SME = soybean). Acid value, free glycerol, and soap were zero for all samples.

Sample	Bound Glycerol (%)
BME1	0.0379
BME2	0.2846
BME3	0.0284
BME4	0.0635
SME1	0.0652
SME2	0.0285

and methanol following the normal base-catalyzed biodiesel production procedure with sodium methoxide as catalyst. Free glycerol was measured for these samples using GC, and these measurements were used to compare the turbidity with the spiked samples.

Results and Discussion

Average turbidity of the biodiesel samples, as the absorbance at 600 nm, was plotted as a function of shaking time for samples with different levels of free glycerol. Although the absorbance curves were different, they all initially increased at a rapid rate and eventually hit a plateau at a certain level (fig. 1). In general, the plateau was higher for the higher level of glycerol. Turbidity measured after 9 to 12 min had the greatest separation in absorbance values, and hence the turbidity measured after 12 min ($t12$) was considered for further analysis.

Two-way ANOVA was carried out for FG and BG from the control group with absorbance as response variable. The analysis showed a significant group mean difference for FG ($F_{5,48}$ = 313.73; P < 0.0001), BG ($F_{3,48}$ = 20.01; P < 0.0001), and the interaction of FG and BG ($F_{15,48}$ = 3.93; P < 0.0001). In order to determine if all of the means were significantly different, the Bonferroni method of multiple comparisons (Johnson and Wichern, 2002) was used for free and bound glycerol separately. Multiple comparison on free glycerol showed that the mean turbidity values of all groups were significantly different at a 95% confidence interval (fig. 2). However, all group means were not significantly different for bound glycerol. This indicated that much of the variability of absorbance was coming from free glycerides. This was further confirmed from the much higher F-value for free glycerol (F = 313.73) compared to the F-value for bound glycerol (F = 20.01). This led to the conclusion that turbidity measurement was more sensitive for measuring FG than BG.

From the absorbance plot, it was observed that the differences in absorbance between 0%, 0.01%, 0.02%, and 0.03% glycerol at 12 min were not linear. A square root transformation of absorbance was used to linearize the differences. Regression analysis was carried out to establish the relationship between FG and turbidity measured after 12 min ($t12$). The equation of the best-fit line was found to be:

$$FG = -0.0022 + 0.0394\sqrt{t12} \qquad (2)$$

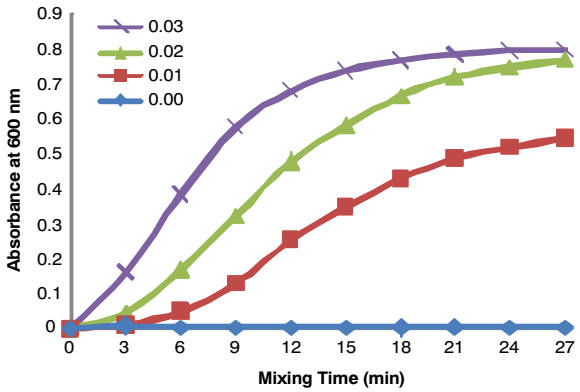

Figure 1. Average turbidity curve for MME spiked with different level of FG. Absorbance measured after 9 or 12 min had the greatest separation.

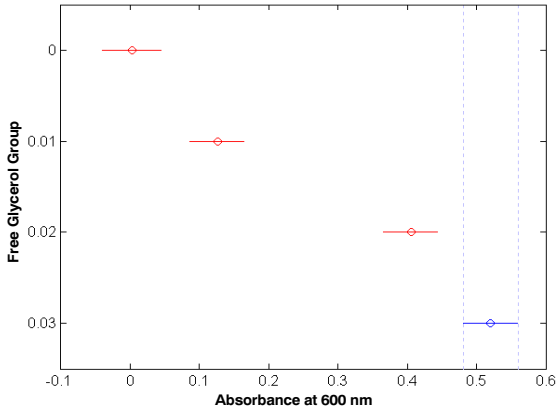

Figure 2. Multiple comparison of group means using Bonferroni method.

The coefficient of determination (R^2) for the regression line was 0.87, and the root mean squared error (RMSE) was 0.004%. A residual plot did not reveal any noticeable pattern in error. A similar model that was used to predict BG from $t12$ had an R^2 value that was not significantly different from zero. This indicated that turbidity and BG do not have a simple relationship, as in equation 2. ANOVA showed that all group means for different level of BG were not the same. There may have been some higher-order relationship that could not be revealed in this experiment, or there may not have been any effect of BG on turbidity, as BG is not readily soluble in water. The significance in ANOVA may have come from some unmeasured variable not considered in this experiment, such as sterols.

It is also possible for some of the biodiesel to hydrolyze after water mixing and shaking. However, we see primarily two reasons why free fatty acids (or simply fatty acids) would have a much smaller effect on absorbance compared to sterols. First, fatty acids with long carbon chains are primarily non-polar. The solubility of 18-carbon chain length fatty acid is 0.0003 g per 100 g of water at 20°C (Gunstone, 1958). Non-polar lipids and lipid molecules are essentially hydrophobic and practically insoluble in water. Therefore, it is less likely that water and fatty acids will form an emulsion to affect turbidity significantly. In addition, the fatty acids are transparent for 600 nm light and are not likely to affect the turbidity themselves. The second reason is that, even if there were any bias in turbidity measurement due to the free fatty acids, the amount of water and the amount of shaking were kept constant over all free glycerol levels. The only difference among samples was the levels of free and total glycerol. The differences arising from fatty acids would be approximately equal and will not cause significant bias. They are expected to have a negligible random error.

On the other hand, the sterols in oil are found in many variations. They are mainly found as free sterols or linked to glucosides. Plant sterols are also mostly insoluble in water, but the solubility could be much higher. For instance, tocopherol, one of the constituents of vegetable oil, is reported to have a solubility of 20.9 mg L^{-1} (= 0.00209 g per 100 g of water), which is almost 7 times greater than the solubility of 18-carbon chain length fatty acid. With an addition of 10% ethanol (mass basis), the solubility increases to 1.5-fold. More pronounced effects were observed at higher ethanol concentrations. By adding 70% (mass basis) ethanol, solubility was enhanced about 450-fold (Dubbs and Gupta, 1998).

Sterols, like glycerides, consist of polar and non-polar aliphatic chains, so they could have helped emulsion formation. Higher solubility helps polar hydroxyl groups of sterols position themselves on the water-biodiesel interface to form an emulsion that may enhance turbidity.

FG is sparingly soluble in biodiesel and can remain in biodiesel as suspended droplets. On the other hand, FG is readily soluble in water and hygroscopic due to the presence of its three alcoholic hydroxyl groups. Therefore, the glycerol remaining in biodiesel acts as a humectant, attracting polar water molecules when water is mixed with biodiesel. Glycerol hydroxyl groups form hydrogen bonds with water molecules. Since water is not miscible with biodiesel, a suspension of fine water droplets forms and causes the biodiesel to become turbid. FG and BG can both position themselves on the water-biodiesel interface when biodiesel is in contact with water and cause the formation of an emulsion, which makes the biodiesel turbid.

Use of Turbidity Measurement for Initial Quality Screening

In normal biodiesel production, the amount of free glycerol should be quite low. Water washing usually removes free glycerol to below the ASTM D6751 specification of 0.02% (Van Gerpen, 2008). Higher levels of free glycerol in biodiesel ultimately result in a pool of free glycerol collected at the bottom of the storage tank. The glycerol may attract water and produce monoglycerides and free fatty acids during storage. To avoid an involuntary high free glycerol content, final product turbidimetry can be used as a quick check to detect biodiesel with a high level of FG.

Equation 2 was used to predict the average percent FG for a given $t12$ measurement. The 95% confidence interval of predicted FG for a measured $t12$ was then calculated and plotted (fig. 3). ASTM Standard D6751 specifies the maximum amount of FG to be ≤0.02% in biodiesel. From figure 3, it can be seen that if the $\sqrt{t12}$ value is less than 0.35 (or $t12 \leq 0.12$), then the FG is less than 0.02% at a 95% confidence interval.

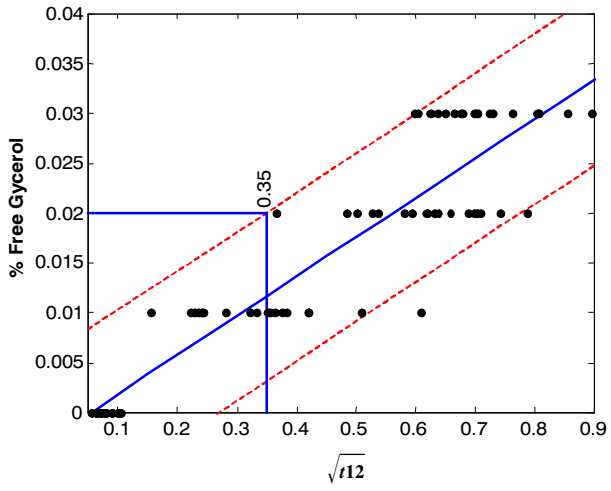

Figure 3. Percent free glycerol as a function of $\sqrt{t12}$. Dashed lines show 95% confidence interval of predicted value; $\sqrt{t12} \leq 0.35$ gives a 95% confidence that the product has FG ≤ 0.02%.

Depending on the severity of consequences, this confidence interval could be adjusted, and the $t12$ value may be set accordingly. A value of $t12$ greater than 0.12 ($\sqrt{t12} = 0.35$) may indicate that water washing was not adequate or that there is something wrong with the production process that needs to be readjusted. This method can be used as a quick check if the product needs further water washing or has inherent problems in the production processes.

To further confirm the results, only the batches spiked with 0.02% FG were used to calculate T-distribution parameters. These samples represented the maximum allowable FG according to ASTM Standard D6751. The mean and standard deviation of the $t12$ value for these 18 samples were 0.39 and 0.12, respectively. The lower 95% confidence interval was calculated as $t12 = 0.13$. This value is close to the 0.12 obtained from the above regression analysis. Taking the conservative value, it was concluded that $t12 \leq 0.12$ would ensure FG ≤ 0.02% at a 95% confidence interval.

A gas chromatograph may not be available in a biodiesel production facility, and it may take days before laboratory test results become available from an external lab. Free glycerol and total glycerol are usually the first parameters that go out of spec if there is a problem in the biodiesel production (Van Gerpen, 2008). Out-of-spec free glycerol will be translated into increased turbidity measurement; hence, this method could be used as a handy, low-cost method to ensure that a biodiesel production process is in order.

Results Validation with Real Samples

All four validation samples met the ASTM D6751 FG specification of 0.02% (table 2). One of them had a $t12$ value of <0.12, which can be considered as a pass for within-limit FG. Three other samples were inside the 95% confidence interval, and hence this method could not reject the hypothesis that FG is within the limit. These samples needed further testing.

Predicted FG was higher than measured FG in all cases (table 2). This may be because the production samples were not as water washed, as a control, and may have had higher levels of impurities. Impurities in biodiesel, such as soap, can add to turbidity. Sensing all these properties at the same time using turbidimetry alone appears very complex and not practical; however, the results obtained using the turbidimetry of samples from a well-established production process could be informative and cost-efficient on a larger scale.

It should be noted that this method has only been tested with freshly made samples and not on samples stored for a long time. Long-stored sample may have different chemical characterization due to oxidation and possible contamination. Further testing of this method is needed to assess its applicability for stored biodiesel and to detect other impurities.

Table 2. Characterization of production samples.

Sample	$t12$	Measured FG	Predicted FG	Comment
1	0.005	0	0.001	Pass
2	0.172	0.008	0.014	Need further testing
3	0.339	0.015	0.021	Need further testing
4	0.311	0.018	0.020	Need further testing

Conclusions

A method for sensing free and bound glycerol in biodiesel samples using turbidity measurement as the absorbance at 600 nm was investigated. Data analysis showed that the influence of both FG and BG on turbidity of biodiesel samples was significant. FG had a linear relation with the square root of absorbance measured after 12 min of shaking. The relation between turbidity and BG was complex and could not be determined in this experiment. Even though the turbidity method did not offer high accuracy in predicting the level of free glycerol, it could serve as a quick, low-cost test to screen production samples with high free glycerol.

In addition to screening of samples, the method could potentially be used to monitor a biodiesel production process. The levels of free and total glycerol are very sensitive to the completeness of the biodiesel reaction. If any process parameter goes wrong, most probably it will be reflected in increased free and total glycerol levels. This in turn will be translated into increased turbidity measurement. Hence, this method could be used as a quick indicator that the production process needs attention.

Turbidity measurement after 12 min of mixing provided adequate information about the glycerol level. The turbidity of real samples was somewhat higher than that of spiked samples for the same level of FG because of other impurities that could cause turbidity. This led to the higher than actual prediction of FG for a given turbidity value. The FG prediction with real sample classification based on a confidence interval was shown applicable in field conditions. Finally, it was concluded that turbidimetry could be used for preliminary sorting of biodiesel that meets ASTM Standard D6751 for free glycerol content.

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