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Preliminary Assessment of Instrumental Methods to Evaluate the Purity of Biodiesel Produced from Waste Vegetable Oil

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Michael MacMurdo is a senior pursuing a B.S. degree in Chemistry (biochemistry concentration), with a second major in Philosophy and a minor in Biotechnology. His research was conducted during the summer and fall of 2013 with support from Adrian Tinsley and Center for Sustainability grants, and under the guidance of Dr. Edward Brush (Chemistry). Mike presented his research at the American Chemical Society national meeting in Dallas, Texas in March 2014. Mike plans to pursue his Ph.D. in chemical biology beginning in the fall of 2014.

America's transportation sector is the key link between our growing dependency on oil, and resulting global warming pollution. Petroleum in our cars and trucks accounts for two-thirds of our total oil used and one-third of the U.S. carbon dioxide emissions. Biodiesel is a renewable fuel source and an alternative to petroleum diesel. Biodiesel burns much cleaner than petroleum diesel, producing a significantly lower amount of toxic and greenhouse gas emissions.[1] Whereas, there is only a finite amount of petroleum in the earth, biodiesel is produced from renewable resources and even recycled resources such as waste vegetable oil. Despite the benefits, biodiesel has some problems with gelling at cold temperatures, and efficiency issues and high waste volumes in large scale production.[2] But with the benefits biodiesel provides to human health, the environment, sustainability and the economy, it would be worthwhile to solve these problems so this resource can be utilized to its fullest potential.

Biodiesel is composed of fatty acid methyl esters derived from vegetable oils. The production of biodiesel from BSU waste vegetable oil (WVO) would provide positive impacts for the institution, including financial and educational benefits. BSU would no longer have to pay for the disposal of WVO and would not be as dependent on petroleum diesel, thus reducing the cost of fueling campus diesel vehicles. BSU would be able to use a biodiesel production facility as a learning tool for students in introductory, intermediate and advanced chemistry courses, non-major courses, undergraduate research, STREAMS and K-12 outreach. This would allow students to acquire a better understanding of biodiesel production at the lab and manufacturing scale. BSU could become a regional research, education and outreach center on biodiesel production and its use, especially for those interested in small scale production for their own business or as an educational teaching tool. With such strong benefits for BSU, its students, and the community, it is worthwhile to invest in research that will permit the actualization of this potentially lucrative renewable fuel source.

Biodiesel is produced through a base-catalyzed chemical reaction called transesterification, where the fatty acid glyceryl esters of vegetable oil exchange glycerol for methanol, producing fatty acid methyl esters (biodiesel), Figure 1.[1] The crude biodiesel is purified by washing out byproducts and residual reagents with water. This is a crucial step because any impurities in the biodiesel could lead to serious and expensive engine damage.

Figure 1. The Transesterification Reaction of Vegetable Oil Into Biodiesel
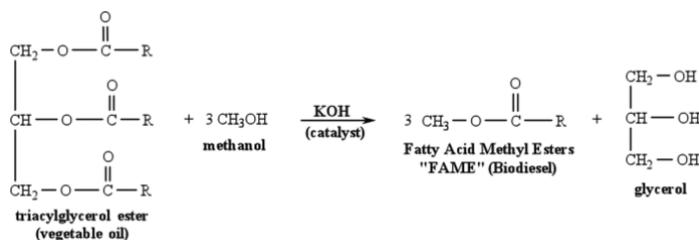
Research in Dr. Brush's group is focused on applying the 12 Principles of Green Chemistry to develop an efficient and cost-effective process for converting WVO into biodiesel for campus use.[3] The focus of this research project has been on Green Chemistry Principle #11 which states the importance of "Real-Time Analysis for Pollution Prevention." This means that it is important to analyze the chemical substances at each stage of a chemical process to determine if hazardous products are being produced, and to exert appropriate control measures. For the production of biodiesel from WVO, this can be accomplished using the advanced analytical instrumentation available in the BSU chemistry department. This instrumentation enables the precise assessment of the purity of biodiesel samples, and identity of any contaminants. Furthermore, the data from this project will be valuable in supporting the ongoing biodiesel research projects being carried out by BSU undergraduate chemistry research students.

In order to achieve our goal of developing an efficient chemical process to produce large quantities of pure biodiesel while eliminating waste, it is essential to know not only the precise purity of the biodiesel product, but also the identification and amount of byproducts. A further complication is that biodiesel is produced from many similar fatty acid glycerol esters, such that the biodiesel product is actually a blend of very similar fatty acid methyl esters. The focus of our research was limited to obtaining quantitative data on impurities and byproducts, as this information would allow us to assess the efficiency of our production method, identify where waste was being produced, and determine how the process could be improved. Furthermore, by perfecting our analysis methods BSU could serve as a regional analytical center performing quality assurance tests on biodiesel made by other educational institutions, small businesses, or farms.

This project is significant to our research group as the data will assist current and future research students in producing biodiesel of the highest purity, help assure that the biodiesel is not hazardous for use, and will inspire confidence in using the biodiesel in BSU vehicles. Currently, research students are relying on qualitative methods of biodiesel analysis that do not have the sensitivity or accuracy needed to identify and quantitate hazardous byproducts. These byproducts could damage an engine, or be hazardous to human health.

The instrumental methods we have selected to characterize biodiesel purity are certified by the American Society for Testing and Materials (ASTM).[4] These quality assurance guidelines are required to prevent damage to the environment, human health, and to diesel engines. These guidelines help us establish our own analytical methods, and evaluate the efficiency of the biodiesel process being developed in our research group. The following instruments were evaluated as part of this research project: Gas Chromatograph/Flame Ionization detector (GC-FID), Nuclear Magnetic Resonance spectrometer (NMR), and Fourier Transform Infrared Spectrometer (FTIR).

Description of Instrumentation
Gas Chromatograph/FID Detector (GC-FID). Gas chromatography is the most widely used method for biodiesel analysis, and the most accurate for minor byproducts such as methanol, glycerol and fatty acid glyceryl esters.[5] Different chemical analytes pass through a heated chromatographic column at different rates depending on their chemical and physical properties, and their interaction with the column's stationary phase. An elution profile is produced that is compared to reference standards in order to identify the product distribution, and all byproducts. The presence of glycerol in the elution profile from a biodiesel sample may indicate problems in the water extraction step of the synthesis process, while the presence of glyceryl esters will indicate an incomplete transesterification reaction. Two types of capillary chromatography columns are typically used, a fused silica 100% dimethylpolysiloxine capillary column, or a fused silica column coated with (5%-Phenyl)-methylpolysiloxine.[5]

The flame ionization detector (FID) is the most sensitive gas chromatographic detector for hydrocarbons; organic compounds containing only carbon, hydrogen and oxygen (like biodiesel). The FID has a wide linear range (6-7 orders of magnitude) and limits of detection in the low picogram or femtogram range, making this gas chromatographic detector the best suited for many carbon containing compounds

GC-FID method development for a new type of analyte is time-intensive. This starts with a chemical pre-treatment step to derivatize the biodiesel sample components through silylation, using methylsilyl trifluoroacetamide (MSTFA). This commonly used approach ensures that the chemical characteristics of all analytes of interest, especially glycerol compounds, are compatible with the conditions in the heated chromatog-

raphy column. The oven temperature is programed to ensure effective separation of the analytes. Typically an oven ramp program is used based upon standard ASTM methods that serve as guidelines, but trial and error are needed to improve separation.[6]

Nuclear Magnetic Resonance Spectrometry (NMR). NMR is the most widely used method for <u>qualitative</u> analysis in determining the structure of organic compounds. However, NMR has also shown excellent potential for the <u>quantitative</u> analysis of biodiesel analytes.[5] NMR spectrometry measures the frequency at which hydrogen and other nuclei resonate when put in a magnetic field, and can provide valuable information about the carbon and hydrogen backbone structure of organic molecules. In biodiesel analysis we focus on specific signals associated with unique hydrogen atoms in the analyte molecules. Overall, NMR can be used to identify and quantitate the presence of glycerol, methanol, fatty acid glycerol esters, and fatty acid methyl esters (biodiesel).

Fourier Transform Infrared Spectrometry (FTIR). The FTIR is another routine instrument used by organic chemists to conduct qualitative structural analysis, and typically goes hand-in-hand with NMR. FTIR provides information on the types of chemical bonds in a sample as related to specific functional groups that can indicate the presence of methanol, water, and fatty acid esters. This is the simplest of the three instrumental methods, analysis is very rapid, and no special sample preparation is required. FTIR is most valuable for the rapid screening of samples prior to detailed analysis by GC and NMR. FTIR has been used for quantitative analysis of biodiesel and related analytes, but the accuracy of these methods is questionable.

Assessment Protocol
The goal of this project was to assess and evaluate the effectiveness of each instrument in the qualitative and quantitative determination of biodiesel, methanol, glycerol and fatty acid glyceryl esters. This assessment was based on a six point rubric that covers the difficulty, cost, speed, accuracy, precision, and versatility of the instrumental method.

Difficulty considers the challenge involved with learning the instrument and procedure, analysis preparations, running the instrument, and evaluating the results. *Cost* is concerned with the expenses needed for any chemical reagents and expendable supplies, instrument maintenance, and upgrades needed for the instrument. This attribute does NOT include the cost of purchasing the instrument. *Speed* refers to the time required for sample preparation, analysis, and evaluation of the results. *Accuracy* considers the detection limitations, signal to noise ratio, and possible interference involved in the analysis, as well

as any means to verify the accuracy. *Precision* looks for reproducible results and maintenance of consistency. *Versatility* is concerned with the volume of data each instrument can provide and if the instrument can be used for multiple purposes or just one.

The six point rubric was applied to each instrument by grading each attribute on a 5 point scale that considered how much and often the attribute is displayed:

5: The particular attribute is positive at all times by the instrument.

4: The particular attribute is mostly positive at all times or fully positive at most times.

3: The particular attribute is half positive at all times or fully positive half of the time.

2: The particular attribute is slightly positive at all times or fully positive few times.

1: The particular attribute is always negative at all times.

Methods
Fourier Transform Infrared Spectrometer (FTIR). Samples of biodiesel were examined using the PerkinElmer Spectrum 2 Infrared Spectrometer along with the Spectrum Touch interface software. This software includes a guided procedure based off the European biodiesel standard procedure, EN14078 meant for biodiesel blend analysis. It utilizes a calibration curve to quantify the percent by volume of biodiesel using Beer's law. The software walks the user through the procedure and the Spectrum QUANT analysis software quickly computes calibration and experimental data.

Preparation involved making calibration standards of pure biodiesel using 1, 3, 5, 7 and 9% by volume samples in hexane solvent. Two samples of synthetic biodiesel were prepared at 4% by volume to assess the accuracy of the calibration curve. The FTIR was prepared for analysis by installing the flow cell apparatus with a 1 mm path length. The Spectrum software was used to calibrate the exact path length for highest accuracy.

Using the FTIR and TouchSoft EN14078B procedures, the calibration standards were scanned with the EN14078B calibration method, and the experimental samples were scanned with the nonstandard method. The data produced was saved and analyzed using the Spectrum QUANT program. The analysis software configured the FTIR to use the ester carbonyl absorbance band at 1720 cm^{-1}, and built a calibration curve with the biodiesel reference data. Using the completed calibration curve, biodiesel experimental samples were evaluated with the

same program to determine the concentration of diluted test samples, as compared to the actual concentration.

Nuclear Magnetic Resonance (NMR). The biodiesel samples for NMR quantification were prepared in triplicate using maleic acid as an internal reference standard and an equal molar amount of biodiesel analyte (16.87 mmoles). When making stock solutions, the mass needed for biodiesel was calculated using the methyl ester of oleic acid as a model compound with molecular weight of 296.5 g/mol. The biodiesel stock solution was diluted with deuterated acetone solvent, and the maleic acid stock solution diluted in DMSO solvent. These stock solutions were used to create the NMR samples by mixing them with deuterated acetone (and TMS if needed) into a standard 5mm NMR tube.

After collecting an H-NMR spectrum and setting the TMS reference signal to 0.0 ppm, the signal for the maleic acid vinyl protons at 6.3 ppm were integrated to a value of 2H. The biodiesel methyl ester peak was observed at 3.7 ppm, and the validation methylene peak at 2.3 ppm. The biodiesel percent yield was calculated using Equation 1, where x is the integration signal for the methyl ester group, and y is the value of the maleic acid vinyl proton integration signal.[5]

$$\text{Eq 1: } \% \ yield = \left(\frac{2(x)}{3(y)}\right) \text{x100}$$

The resulting value is the percent yield of biodiesel found in the formula in relation to how much was expected, represented by the maleic acid signal. To validate the results, Equation 2 was used to calculate the validation percent yield, where z is the methylene peak integration signal and y represents maleic acid.[5]

$$\text{Eq 2: } validation \ \% \ yield = \left(\frac{(z)}{(y)}\right) \text{x100}$$

The percent yield validates the experiment if it is nearly identical to the previous percent yield. Major deviations from the yield may indicate poor sample preparation, a bad scan, or poor integration.

Gas Chromatograph/FID Detector (GC-FID). The PerkinElmer GC-FID Clarus 580 was prepared with a 15 meter fused silica capillary column with an internal diameter of 0.32 mm, and a 0.1 mm film thickness of 5% phenylpolydimethyl-

siloxane bonded and cross linked phase internal coating. The column also included a 2 meter guard column with 0.53 mm internal diameter.

For the preliminary assessment, one standard was prepared using commercial stock solutions (each in pyridine solvent): 500,000 ppm glycerin (100 uL); 5,000,000 ppm monoolein (200 uL), diolein (100 uL), and triolein (100 uL); 1,000,000 ppm butanetriol (100 uL); and 8,000,000 ppm tricaprin (100 uL). The standard solution mixture was derivatized by adding 100 ml of N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), shaken, and left to stand at room temperature for 15-20 minutes. The sample was diluted with 8 ml of heptane and the vial shaken to mix the contents.[6]

The solution was then transferred to a glass GC vial, which was sealed shut with a TFE-fluorocarbon-lined cap. These solutions were placed in the GC-FID auto injector tray and a chromatogram of each sample was obtained using the programed instrumental method recommended in the ASTM procedure, displayed in Table 1.[6] The only modification made to this method was to the injection technique. Without any programmable temperature setting for the injector inlet, the inlet was kept at 50ºC throughout the method.

Table 1. Detailed Procedure for the GC Instrument Settings Used During the Analysis

Injector	
Cool on Column	50°C
Sample Size	1uL
Column Temperature Program	
Initial Temperature	50°C (hold 1 min)
Rate 1	15°C per min to 180°C
Rate 2	7°C per min to 230°C
Rate 3	30°C per min to 380°C (hold 10 min)
Detector	
Type	Flame Ionization Detector
Temperature	380°C
Carrier Gas	
Type	Helium
Flow Rate	3 mL/min

Preliminary Assessment: Results and Discussion
The scores for this preliminary assessment are as shown in Table 2, where the FTIR displays moderate efficiency and the NMR near perfect efficiency. The GC was determined to be difficult and expensive to operate, and requires more work before any other grades can be assigned.

Table 2. Assessment of Each Instrument with Respect to the Six Attributes Graded on a 5 Point Scale (5 = Positive…1 = Negative)

	IR	NMR	GC
Difficulty	5	5	-
Speed	4	5	-
Cost	3	5	1
Accuracy	1	5	-
Precision	5	5	-
Versatility	3	5	-

Fourier Transform Infrared Spectrometer (FTIR). In reviewing the performance of the FTIR during biodiesel analysis, we found success in the areas of difficulty, speed, and precision; moderate success with cost and versatility; and failure with accuracy. Looking first at difficulty, the FTIR was an easy instrument to use due to a heavily guided procedure allowing for a short learning curve. With the procedure prompt walking the user through the experiment, and a comprehensive manual, the experimenter will have no problem with setup of the instrument apparatus or mastering its proper use. The manual also acts as a concise guide for using the Spectrum QUANT software, which makes the construction of a calibration curve and quantification of the experimental data an easy process. Considering all of these findings this instrument scores a 5 for difficulty.

Considering speed, the analysis is fairly quick, ranging between 20 minutes and 2 hours. The procedure time runs longer if a calibration curve needs to be produced, where most of the time is spent preparing and analyzing each calibration standard. With a curve in place the procedure is no more than 20 minutes, which will almost always be the case since the calibration curve will rarely need replacement. For an analysis where most of the time is spent performing dilutions, the FTIR scores a 5 for procedure speed.

The instrument shows good precision; however more work needs to be done to properly assess this attribute. When the calibration curve was generated, the linear fit showed a very good correlation coefficient of 0.998. The calibration curve was able to calculate the correct concentration of two different biodiesel reference samples. With a calibration curve generated by analytical grade biodiesel samples, the curve should be able to produce even more precise measurements of experimental samples.

The cost for this procedure is both positive and negative for this instrument. Apart from the actual cost of the analysis kit and software, maintaining the instrument with analytical reagents to build a good calibration curve, glass syringes for injections, and analytical solvents all can add up to become expensive. But if the calibration curve is already in place and there are syringes available, all that is needed are the solvents to dilute the biodiesel and rinse the flow cell between injections. However, if the flow cell is damaged its replacement cost would be very expensive. As a result the FTIR scores a 3 for cost, since most of the cost is usually spent replacing solvents, but this cost can climb if calibration reagents or kit maintenance is needed.

When considering accuracy, the FTIR was unreliable at assessing the purity of synthesized biodiesel samples due to contaminates interfering with the results. The biodiesel methyl esters and bound glycerin contaminates all have a carbonyl functional group that adds to the signal produced at the analysis frequency of 1720 cm^{-1}. This interference makes obtaining an accurate concentration of biodiesel difficult. This is not surprising because this FTIR method was designed to measure the concentration of pure biodiesel blended with petroleum diesel. As a result, the FTIR scores a 1 for analyzing lab-synthesized biodiesel samples, making this instrument very unreliable for quantification of biodiesel purity under these conditions.

The FTIR maintains a moderate amount of versatility for biodiesel analysis. The kit was designed for analyzing biodiesel blended with petroleum diesel, using the 1720 cm^{-1} ester carbonyl absorbance band. This will come in handy for quality control analysis if there is ever a need to blend our biodiesel with petroleum diesel. Since this procedure is very simple, it may be useful as an educational tool to teach about basic quantitative analysis. The FTIR can also be used for qualitative analysis in the determination of unique functional groups from contaminating species that may exist in the sample. However, there is an issue concerning contaminant interference at the analysis frequency of 1720 cm^{-1}, making the instrument unreliable for quantitative analysis of biodiesel for % yield determination. Furthermore, the analysis procedure cannot focus on any absorbance band other than 1720 cm^{-1}, making quantification of any contaminant impossible. With all of this under consideration, the FTIR receives a 3 for versatility.

Nuclear Magnetic Resonance Spectrometer (NMR). The NMR seems to display high success with the areas of difficulty, speed, cost, accuracy, precision, and versatility. The learning curve to use the NMR to assess biodiesel purity is minimal. Essentially, the user needs to only understand how to prep the sample, load it into the instrument, set up and run the analysis, and know which signals need to be integrated on the spectrum

for calculation. With such a high level of simplicity, the NMR scores a 5.

In terms of speed, NMR analysis is rapid. Preparing the sample might take 5 to 10 minutes, each H-NMR scan takes about 10 minutes to complete, and interpreting the results might take 5 minutes. Without the need to run standards for a calibration curve, the experimental time is cut down drastically. Running one sample took about 25 minutes, but this time increases with more scans and samples. As a result, the NMR receives a 5 for speed.

Assuming that an NMR is already available, the cost of using this instrument is very minimal. Purchasing solvents, an internal reference standard, and NMR tubes is the only upkeep needed for this instrument, landing a solid 5 for cost.

Considering accuracy and precision, this instrument is able to very accurately determine the amount of biodiesel in a sample, and precisely reproduce the results. The procedure utilizes an internal quantitative reference standard, which allows the user to determine the amount of biodiesel in a sample with high accuracy. Multiple samples gave near identical results, vouching for the precision of the instrument. The only possible negative aspect is that the limit of detection may not allow the NMR to be accurate enough for quantifying low concentrations of contaminating species in a sample. Both of these aspects receive a 5 as a result of excellent performance.

The NMR shows a high amount of versatility in analyzing biodiesel. This method can very accurately quantify the amount of biodiesel in a sample using an internal reference standard. The NMR can also be used for qualitative analysis, providing information related to the structure of the biodiesel molecule, and about contaminating species as well. It is possible that NMR might be sensitive enough to quantify contaminating species, but more work is needed to assess this. With the ability to detect and identify a number of analytes, the NMR receives a 5 for versatility.

Gas Chromatograph/FID Detector (GC-FID). While there is enough preliminary information to provide an evaluation of the cost aspect of the assessment for this instrument, there is not enough data to evaluate any of the other attributes. Beginning with difficulty, the GC-FID has a steep learning curve due to the complex nature of this instrument. Furthermore, in order to follow the certified biodiesel analysis procedure the experimenter must become very adept with the numerous facets involved with the instrument. It takes a while to learn the variety of software tools related to the instrument, and even longer to know how to maintain the hardware. Once the complexi-

ties involved with the instrument are grasped, the difficulties faced with instrument use and maintenance become easier to handle. Troubleshooting issues with the experiment operation are complicated, and could range from temperature programming to issues with the hardware, where the only means to address these issues is through trial and error. In some cases, a specialist may be needed to solve certain problems that are too complicated to fix alone. There is still much more that needs to be done with this instrument for biodiesel analysis before a final assessment can be given.

The operating cost of the GC is high compared to other instruments for biodiesel analysis. The column specific for the procedure costs $380, chemical reagents cost about $300, and the parts needed to install the column to the GC costs another $100. This instrument needs a lot of cost investment for biodiesel analysis that require specific parts and reagents. Since it is so expensive to prepare the GC for the procedure, and to purchase reagents, a score of 1 was assigned to the cost.

Regarding the accuracy and precision, much more data is needed before any grade or assessment can be made. Nonetheless, these aspects are projected to score high, and make all the difficulty and cost worth the trouble, as the GC is typically an accurate and precise instrument in normal circumstances.

Conclusions
Fourier Transform Infrared Spectrometer (FTIR). Due to its poor accuracy with biodiesel analysis, the FTIR is not useful for analyzing experimental samples of biodiesel for purity from contamination. However, it can play an effective role with qualitative analysis for contamination without using the kit, by looking for functional groups unique to contaminates found in biodiesel samples. It also will play an effective role with the quality assurance of biodiesel blended with petroleum diesel when that stage of research is reached. This method can also be used for educational purposes in BSU chemistry classes, where students can learn about the FTIR by using the kit to analyze samples of blended biodiesel fuel purchased from local gas stations.

Nuclear Magnetic Resonance Spectrometer (NMR). The NMR plays a vital role in biodiesel analysis, by providing very effective and efficient qualitative and quantitative analysis. This instrument is simple, cost effective, rapid, accurate, precise, and versatile, providing a large amount of sample information.

Gas Chromatograph (GC). This evaluation is still ongoing, but it is clear that this instrument is difficult to learn and operate.

Future Work

In moving forward with this project we need to continue our evaluation of the GC-FID by optimizing the method, including minimizing oven temperature to limit degradation of the GC column. More work will be done with the FTIR and NMR to quantify accuracy and precision. If time permits, I will also conduct research evaluating the use of atomic absorbance spectrometry and high performance liquid chromatography in biodiesel analysis.

Acknowledgements

References Cited

1. Clarke, N.R., Casey, J.P., Brown, E.D., Oneyma, E. and Donaghy, K.J. (2006), "Preparation and Viscosity of Biodiesel from New and Used Vegetable Oil," *J. Chem. Educ.,* 83 (2): 257-259.

2. McCurry, J.D. (2011) "GC/MS Analysis of Trace Fatty Acid Methyl Esters (FAME) in Jet Fuel Using Energy Institute Method IP585," Agilent Technologies, Inc., http://Agilent.com.

3. Anastas, P. T.; Warner, J. C. Green chemistry: theory and practice; Oxford University Press: Oxford [England]; New York, 1998.

4. ASTM International, http://www.astm.org/ABOUT/full_overview.html.

5. Knothe, G. (2001), "Analytical Methods Used in the Production and Fuel Quality Assessment of Biodiesel," *Transactions of the American Society of Agricultural Engineers* 44 (2): 193-200.

6. ASTM International (2000) "Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters By Gas Chromatography," D 6584-00, http://leapwiki.com/mediawiki/images/archive/d/d3/20090403003018!ASTM_D6584_Free_&_Total_Glycerin.pdf**.**