Determination of Free Glycerol in Biodiesel by using PDA UV-Vis Spectrophotometer -Colorimetric method -

SeongHo Gong, Application Scientist, SCINCO CO., LTD., Seoul, Korea,
Tel: (82) -2-545-7300 Fax: (82) -2-548-5984 Email: scinco@scinco.com Web: www.scinco.com

Biodiesel is the typical biofuel, made from vegetable and animal oil, and is currently used for diesel fuel car and heating, blended with diesel fuel or as a pure fuel. Recently, the need of biodiesel has rapidly increased as it is regarded as one of the alternative energies of coal oil. This is generally produced through the ester-transesterfication reaction by adding MeOH to triglyceride as shown in Figure 1 below. The product of the reaction is fatty acid methyl esters (FAME), which are known as biodiesel, and glycerol. As the co-product, glycerol, affects the quality of biodiesel, contents of glycerol are limited in the ASTM. (Total glycerol: below 0.24 %, free glycerol: below 0.02 %)

In this experiment, we determined the free glycerol that is a key element for quality control in the biodiesel. Measurement was performed quickly and accurately by using PDA (Photodiode Array) UV-Vis Spectrophotometer with Quantification mode in LabPro® Plus S/W for data processing.

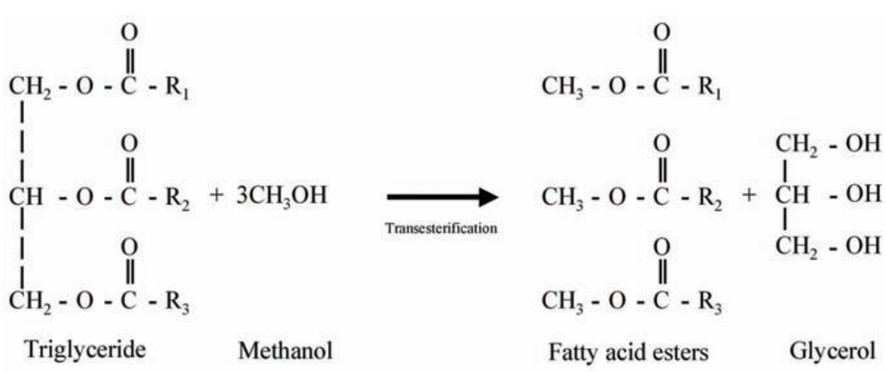

Figure 1. Generation process of the fatty acid esters

Principle

Formaldehyde is generated by the reaction between free glycerol in biodiesel and sodium periodate, and the reaction between acetyl acetone and the generated formaldehyde leads to formation of the 3,5-diacetyl-1,4-dihydrolutidine (see Figure 2) that is yellow complexes. As these complexes are generated proportionally to the amount of the glycerol in the sample, we can determine the glycerol by measuring yellow complexes that have specific absorption band at 410 nm.

Reagents & Apparatus

1. Biodiesel with pretreatment: Refer to reference 1 for pretreatment.
2. 1.6 M Acetic acid stock solution: Dissolve the 9.6 g of acetic acid in 100 mL of distilled water.
3. 4.0 M Ammonium acetate stock solution: Dissolve the 30.8 g of ammonium acetate in 100 mL of distilled water.
4. 0.2 M Acetylacetone solution: Dissolve in a test tube approx. 200 uL (195 mg) of acetylacetone in 5 mL of ammonium acetate stock solution and 5 mL of acetic acid stock solution.
5. 10 mM Sodium periodate solution: Weigh into a test tube approx. 21 mg of sodium meta periodate, add 5 mL of acetic acid stock solution, swirl to dissolved the periodate, and after periodate is completely dissolved, add 5 mL of acetic acid stock solution.
6. Working solvent: Mix equal volumes of distilled water and 95 % ethanol.
7. Glycerol reference stock solution (3 mg/mL): Weigh approx. 150 mg (accuracy ± 0.1 mg) of glycerol into a 50 mL calibrated flask. Dissolve in the working solvent and fill up to the mark.
8. Glycerol reference working solution (0.03 mg/mL): Using a precision pipette, transfer 1 mL of glycerol reference stock solution to a 100 mL calibrated flask. Dilute and fill up to the mark using the same solvent.
9. Water Bath (Enable to maintain temperature at 70 ℃)
10. S-3100 PDA UV-Vis Spectrophotometer
11. LabPro® Plus S/W
12. Cuvette (10 mm pathlength)

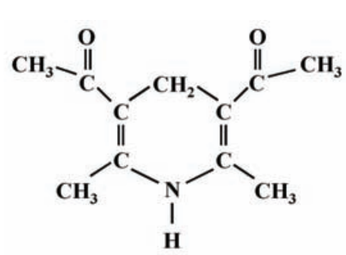

Figure 2. Structure of 3,5-diacetyl-1.4-dihydrolutidine

Procedure

1. Into a series of six 10 mL test tubes, transfer 0.00, 0.25, 0.50, 0.75, 1.00 and 1.25 mL of a 0.036 mg/mL glycerol solution. Dilute with the working solvent in such a way as to get a final volume of 2 mL in each tube as shown in Table 1.
2. Put 0.5 mL of a pretreated biodiesel sample into 10 mL test tube and add 1.5 mL of working solvent into the test tube.
3. Add 1.2 mL of a 10 mM sodium periodate solution to the two former solution (procedure 1, 2) and shake the for 30 s.
4. Add 1.2 mL of a 0.2 M acetylacetone solution to the former solution and put in a water bath thermostated at 70 ℃ for 1 min, stirring manually.
5. After the reaction time, the sample must be immediately cooled in water.
6. Measure the spectra for standards after measurement of spectrum for blank (Std. 1) on Quantification Standard mode.
7. Measure the absorbance of unknown sample on Quantification Sample mode.

Table 1. Preparation of glycerol standard

	Glycerol working solution	Working solvent
Std. 1	0.00	2.00
Std. 2	0.25	1.75
Std. 3	0.50	1.50
Std. 4	0.75	1.25
Std. 5	1.00	1.00
Std. 6	1.25	0.75

Instrument Parameters

The instrument parameters in S-3100 are as follows: Experimental method is shown at Figure 3.

Experimental setup
Data type: Absorbance
Sampling: Single cell
Mode: Scan no.: 30
Integration no.: 1

Experimental method
Use wavelength: 410nm
Curve dimension: 1
Concentration unit: mg/kg

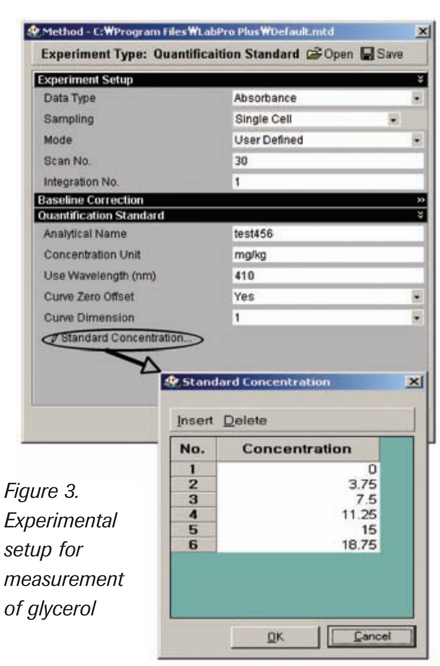

Figure 3. Experimental setup for measurement of glycerol

Result

1. Calibration curve

The spectra of glycerol standards were represented at Figure 4 and data and calibration curve of glycerol standards were shown at Table 2 and Figure 5 respectively. Good calibration curve of which correlation value (R2) is 0.99817 was obtained.

Table 2. Calibration data of glycerol standards

R2 = 0.99817

Function : Y = 0.0524X + -0.0278

Name	Concentration (mg/kg)	AU (410nm)
Standard1	0.00	-0.0063
Standard2	3.75	0.1578
Standard3	7.50	0.3542
Standard4	11.25	0.5431
Standard5	15.00	0.7629
Standard6	18.75	0.9677

2. Contents of free glycerol in biodiesel

We calculate concentration of the free glycerol in unknown sample using calibration curve in Figure 5 and this result is represented at Table 3. We determined the glycerol as 2.11 mg/kg.

Table 3. Concentration of glycerol in unknown sample

Name	Concentration (mg/kg)	AU (410nm)
Sample	2.11	0.0828

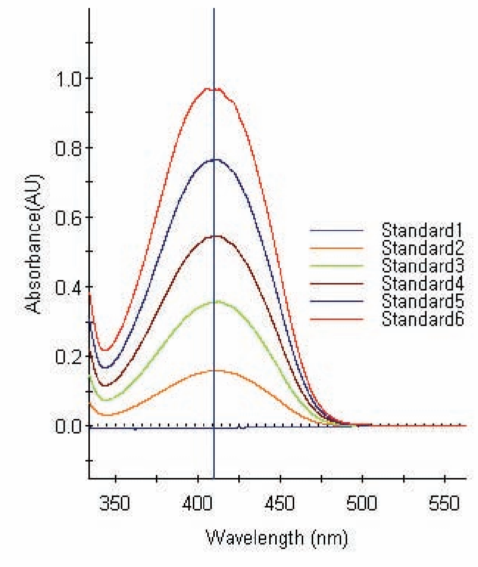

Figure 4. The spectra of glycerol standards

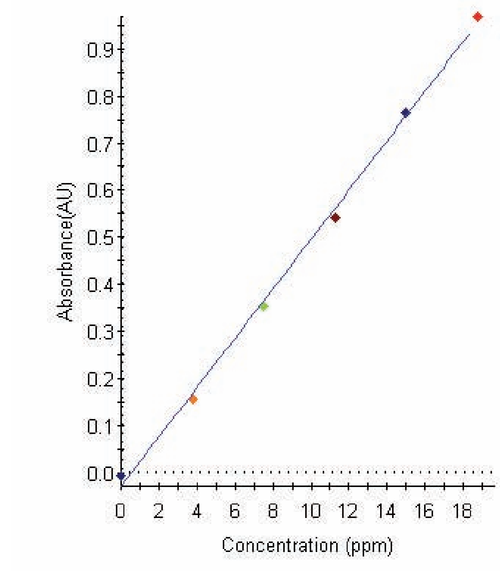

Figure 5. The calibration curve of glycerol standards

Conclusion

Determination of the free glycerol in biodiesel was performed using the S-3100 and LabPro® Plus S/W. Fine calibration curve which has correlation value as 0.99817 was obtained using S-3100 that has superior sensitivity and rapid capability in acquirement of data. We determined the free glycerol in biodiesel by the function, automatic fitting of the calibration curve, in LabPro® Plus Quantification mode at the same time at the end of measurement. As a result of experiment, concentration of free glycerol is 2.11 mg/kg, permitted by ASTM standard.

Reference

1. P, Bondioli, and L. D. Bella,(2005) An Alternative spectrophotometric method for the determination of free glycerol in biodiesel, Eur. J. Lipid Sci. Technol. 107 153-157

2. S. Lee and S. Park (2006) Industrial Biotechnology: Bioconversion of Biomass to Fuel, Chemical feedstock and Polymers, Korean Chem. Eng. Res, Vol. 44, No. 1, 23-34

GCMS Plus for Rapid Analysis of Difficult Samples

Shimadzu Scientific Instruments, Inc.'s (USA) GCMS-QP2010 Plus is equipped with three technologies – AART, FASST, and COAST – that make it ideal for characterizing complex organic mixtures in fields from forensics to food and flavours sciences.

AART (Automatic Adjustment of Retention Time) automatically identifies and quantifies all compounds after a column change without compromise in analysis parameters, ensuring precision and accuracy of target compounds.

FASST (Fast Automated SIM/Scan Type) enables the GCMS-QP2010 Plus to acquire both Scan and SIM data on one peak. At a data acquisition frequency of 50 data points/second in Scan mode and up to 100 in SIM mode, high-quality results are achieved - fast. FASST achieves superior spectra through high-quality quantitative and qualitative analysis, resulting in accurate library search.

COAST (Creation of Automatic SIM (SIM/Scan) Table) allows easy setup of SIM parameters using the SIM table automatic creation function, enabling appropriate parameters to be set automatically when using FASST. Easy setting of compounds and automatic creation of Scan/SIM parameters make high-sensitivity SIM chromatograms simple.

In addition, the GCMS-QP2010 Plus features an extended mass range (1.5 to 1090 m/z) to allow analysis of higher mass compounds, an extended ion source temperature range (from 100° to 300° C) to increase flexibility and reduce maintenance, and dual turbo pumps to increase sensitivity and column flow capacity.

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Viscometer Maker Changes Name

Cambridge Applied Systems (USA), a pioneer in modern viscosity management technology, has changed its name to **Cambridge Viscosity, Inc**.

"Our new name better describes who we are and what we do," said company president Rob Kasameyer. "We also have a new logo and a fresh graphic style that reflects the leading edge qualities of our organisation, our technology and our products."

"The demand for viscosity measurement continues to grow in a world where quality, consistency and productivity are increasingly important," he continued, "we believe that our new identity will help enhance recognition of our uncompromising quality and commitment to leadership in the field."

Cambridge Viscosity manufactures and markets a full range of in-line and in-vessel viscometers for in-process and laboratory use. They utilise a patented self-cleaning electromagnetic sensor technology that makes them very accurate, enables them to hold their calibration and requires very little maintenance. They are known for reliability, repeatability and ease of operation.

With more than 8,000 installations worldwide, Cambridge Viscosity has users that include Fortune 500 companies and their equivalents throughout North America, Asia, Europe and South America.

One of the Company's most successful new systems is the VISCObot robotic viscometer, the world's most advanced automated viscosity analysis tool. It is a fully unattended, high throughput laboratory system that frees up valuable technician hours and saves money on every step of viscosity tests. It can measure viscosity from virtually any type of sample vial and deliver results to any information management system.

Cambridge Viscosity Inc. was founded in 1984 as Cambridge Applied Systems, Inc. It is known for its innovative hardware and software technology, and for its ability to understand and meet the requirements of those who need accurate viscosity measurement to assure successful fluid management.

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Automated ASTM D 445 Testing of Biofuels & Diesels

Biodiesel fuels are required to be certified to ASTM specification D 6751, which states that kinematic viscosity must be measured in accordance with ASTM D 445. This is a time-consuming test to perform in the laboratory.

Now a completely automated system is available. **Rheotek's** (USA) BioVis Automated Viscometer is specifically designed for Biofuels and Diesels and it complies fully with ASTM D 445 in every respect.

The Rheotek BioVis boasts a number of unique features making it the number one choice among laboratory professionals who want expediency without compromising precision or compliance to the method. The instrument is incredibly easy to use and has been designed with operator friendly software.

The instrument will automatically: equilibrate the temperature of the sample in an ASTM Ubbelohde viscometer tube, measure the flow time of the sample, obtain two consecutive flow times, remove the sample and clean and dry the viscometer tube.

The kinematic viscosity will be calculated and displayed on the computer screen. Data can be printed, stored or sent to a LIMS system.

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