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Eco-friendly sonoluminescent determination of free glycerol in biodiesel samples

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ABSTRACT

This paper proposes a flow-batch methodology for the determination of free glycerol in biodiesel that is notably eco-friendly, since non-chemical reagents are used. Deionized water (the solvent) was used alone for glycerol (sample) extractions from the biodiesel. The same water was used to generate water-cavitation sonoluminescence signals, which were modulated by the quenching effect associated with the amount of extracted glycerol. The necessarily reproducible signal generation was achieved by using a simple and inexpensive piezoelectric device. A linear response was observed for glycerol within the 0.001–100 mg/L range, equivalent to 0.004–400 mg/kg free glycerol in biodiesel. The lowest measurable concentration of free glycerol was estimated at 1.0 μg/L. The selectivity of the proposed method was confirmed by comparing the shape and retention of both real and calibration samples to standard solution chromatograms, presenting no peaks other than glycerol. All samples (after extraction) are greatly diluted; this minimizes (toward non-detectability) potential interference effects. The methodology was successfully applied to biodiesel analysis at a high sampling rate, with neither reagent nor solvent (other than water), and with minimum waste generation. The results agreed with the reference method (ASTM D6584-07), at a 95% confidence level.

1. Introduction

The commercialization of bioenergy is beginning providing an effective way to overcome the problems of both petroleum scarcity (through fossil fuel depletion), and environmental degradation. Biodiesel fuels have chemical properties that are very similar to conventional diesel; as such, there is no need for motor adaptations or new equipment to enable their use when blending (diesel/biodiesel), or substituting diesel fuel [1–3].

Glycerol is a natural product of biodiesel refinery: for every 9 kg of biodiesel, 1 kg of crude glycerol is produced as a co-product [4]. Glycerol is soluble in water (> 500 g/L), slightly soluble in esters (biodiesel), almost colorless, odorless, viscous, hygroscopic, and has a high boiling point. Glycerol is widely used in the pharmaceutical, chemical, and food industries [5–7], being neither toxic, nor volatile, and nor flammable at low temperatures [8,9].

In general, a result of inadequate processing through insufficient separation of the glycerin phase or incomplete water-washing after phase separation, free glycerol may be present in the biodiesel. Glycerol is the major impurity found in commercial biodiesel; it can occlude fuel filters, impairing engine performance, cause fuel tank damage, and it can also release acrolein into the environment [10–13].

International regulations specify a limit for free glycerol in biodiesel of 0.020% (w/w) [14,15]. Considering the increasing use of biofuels, the need for fast, precise, reliable, and inexpensive analytical methods for the determination of free glycerol in biodiesel is clear. Several methods for determining free glycerol content in biodiesel have been developed based on chromatographic, amperometric, and spectrophotometric techniques [16–21]. However, these procedures are generally laborious, expensive, and typically use various chemicals, generating large amounts of waste. The development of alternative analytical methods, which require neither solvents nor reagents, and use both automation and miniaturization, has minimized substantially the adverse impacts once sustained by the environmental [22].

Flow-batch systems [23] explore programed (in-flow) multicommutation to achieve very high sampling rates, and discrete (or batch) analytical precision. It can accommodate markedly different analytical procedures without significantly changing the physical configuration of the system, and modifying operational parameters becomes a function of programed valve-switch timing control.

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During the last few years, many new analytical procedures using the sonochemical effect for a wide range of applications have emerged, especially those involving degradation, nanoparticle formation, emulsification, and polymerization processes [24–28]. There are three classes of sonochemical reactions: homogeneous liquid sonochemistry; heterogeneous liquid–liquid, or liquid–solid sonochemistry; and sonocatalysis (which overlaps both) [28]. The underlying mechanism of sonochemistry is cavitation. The sonoluminescence (SL) occurs when brief light emissions occur due to the collapse of cavitation bubbles in a liquid irradiated with ultrasound. During a typical bubble collapse, high temperature and pressure are reached, which can generate highly reactive species (radicals). In the present case, ultrasonic waves cause water decomposition, and produce $OH^\bullet$ radicals [28–31].

$$H_2O \leftrightarrow OH^\bullet + H^\bullet \tag{1}$$

Light emission occurs from a transition

$$OH^* \ (A^2\Sigma^+) \rightarrow OH(X^2\Pi) + h\nu \tag{2}$$

which is characteristic of an excited hydroxyl deactivation. Generally, OH^* is produced by exciting water to a level of $^1A_2$ [30].

Depending on the type of surface-active solutes, sonoluminescence intensity can either be enhanced (e.g., with low concentrations of ionic surfactants), or quenched (e.g., with certain aliphatic alcohols). SL quenching in water with aliphatic alcohols, including glycerol, may be related to the air/water interface concentration of solute molecules on the inside surface of the collapsing bubble. The most commonly accepted mechanism of SL quenching describes bubble collapse thermal energy, being consumed by endothermic bond breaking reactions of the solute molecules. A lower temperature inside the bubble occurs, producing a lower number of excited-state molecules, and consequently leading to a lower sonoluminescent intensity [28,32,33].

This paper proposes a notably eco-friendly flow-batch (FB) method for sonoluminescent determination of free glycerol in biodiesel. The proposed flow-batch sonoluminescent (FBS) system uses an online glycerol extraction method (from biodiesel) with deionized water as the single extraction solvent. The same water was also employed to generate analytical signals through water-cavitation sonochemiluminescence, which are modulated by quenching, a result proportional to the amount of extracted glycerol in the water. A reproducible water-cavitation signal generation was achieved using a simple and inexpensive piezoelectric device. This approach is an environmentally safe methodology, and is the first to completely avoid the use of any chemical reagents whatsoever during the entire analytical process of free glycerol determination in biodiesel samples.

2. Experimental

2.1. Solution and samples

Stock solution was prepared by dissolving 0.10002 g glycerol (99.5%, Mallinckrodt®) in 1000 ml of water. The glycerol-containing homogenized soybean biodiesel samples from different lots were purchased from the Petrobras® refinery located in *Bahía Blanca* City, in Argentina. Distilled-deionized water (18 MΩ/cm) was used exclusively throughout the entire study.

2.2. Flow-batch assembly

A schematic diagram of the flow-batch system used for sonoluminescent determination of free glycerol is shown in Fig. 1.

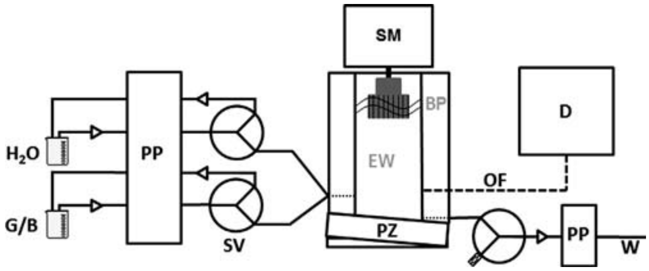

Fig. 1. Flow diagram of the flow-batch system for free glycerol sonoluminescent determination. SV—solenoid valve (three-way); PP—peristaltic pump; SM—stepper motor; BP—biodiesel phase, EW—extractive water, PZ—piezoelectric device; OF—optical fiber; D—lab-built luminometer; W—waste; H_2O: water; G/B—glycerol standard/biodiesel sample.

The FBS system consists of three three-way solenoid valves ($V_{G/B}$, $V_{H_2O,}$ and V_W) (model 161T031, NResearch®) polyethylene tubing connectors with 0.8 mm i.d., and a peristaltic pump (model M312, Gilson®) equipped with six pumping channels, and Tygon® pumping tubes (1.29 and 2.06 mm i.d.).

The lab-constructed flow-batch chamber was built in PTFE with ca. 2.0 mL of total volume. The chamber has a fixed stirrer on the top, which is turned by a stepper motor (MDN3, 200 rpm, 9 V DC), and has a piezoelectric device (taken from a commercial ultrasonic nebulizer ASPEN® NU400 [34]) at the bottom of the chamber. Fig. 2 displays the circuit for controlling the piezoelectric frequency. The chamber was connected by a fiber optic (P400-025-UV/SR, Ocean Optics®) probe to a lab-constructed luminometer, with a photomultiplier tube (PMT), (CE-A59/RX, 33-29-71 PMT, Bausch &Lomb®). The PMT was operated at 700 V, and the emission of photons was registered at 425 nm. The analytical signals were recorded by using a Data Chart 1250 Paperless Recorder (Monarch Instrument®).

A microcomputer connected with a USB interface (USB6009, National Instruments®) was used to control the FBS system, running software developed in LabView® 8.0 graphics language (National Instruments®, Austin, TX, USA).

2.3. Analytical procedure

The operating steps carried out by the FBS system are shown in Table 1.

Before starting the analysis, the channels are filled with the appropriate fluids. The valves $V_{G/B}$, V_{H_2O} and V_W are initially switched OFF, so that the solutions are continuously pumped to load the channels and are continuously returned to their respective containers. Valves $V_{G/B}$ and V_{H_2O} are then simultaneously switched ON for 2 s, and the solutions are pumped toward the flow-batch (FB) chamber to fill the channels between the valves and the chamber. Then, V_W is switched ON, and the excess solution in the chamber is aspirated as waste for 5 s.

Deionized water is then inserted into the chamber, and the piezoelectric device is activated for 5 s in order to obtain the sonoluminescence signals through water cavitation. An optical fiber (P400-025-UV/SR Ocean Optics®) is used to transmit the emitted photons from the FB chamber to the detector.

The online (in chamber) preparation of calibration solutions (0.001–100 mg/L glycerol, being equivalent to 0.004–400 mg/kg free glycerol in biodiesel) are performed using the stock solution. In these preparations, only valve-switch-timing controls ($V_{G/B}$ and V_{H_2O}) are employed to insert differing amounts of stock solution, and water into the chamber. While the solutions are pumped into the chamber, the stepper motor is switched ON. Afterward, the stepper motor is stopped and the piezoelectric device is activated to sonicate the standard working solutions for 5 s. The SL signals are then recorded, with decreases in intensity being proportional to the increases in the concentration of glycerol. A wash cycle

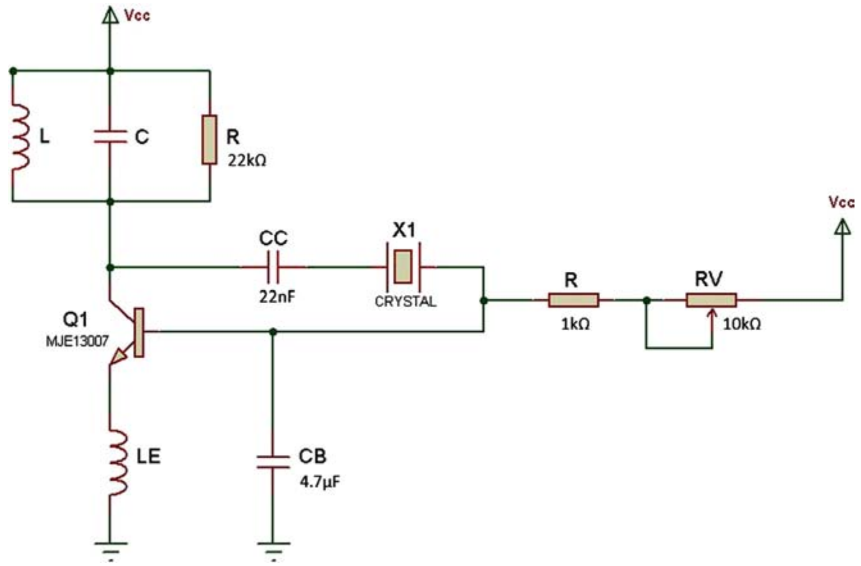

Fig. 2. Electronic circuit for piezoelectric frequency control.

Table 1
Operation of the FBS system for extraction and determination of free glycerol in biodiesel.

Step	Event	Time (s)	Volume (µL)	Pump rotation speed (rpm)	Pump tube (mm i.d.)
1	Biodiesel ($V_{G/B}$)	1.0	14.8	10.0	1.29
	Water (V_{H_2O})	3.9	985.2	48.0	2.06
2	Stirrer time[a]	240	–	0.0	–
3	Detection	5.0	–	0.0	–
4	Waste (V_W)	4.2	–	48.0	2.06
5[b]	Water (V_{H_2O})	2.9	690.5	48.0	2.06
6[b]	Waste (V_W)	4.2	–	48.0	2.06

[a] Extraction time of the free glycerol in biodiesel.
[b] Chamber cleaning.

(with water) is performed after each measurement. The piezoelectric device is turned ON during the wash step.

The free glycerol extraction from the biodiesel samples was carried out (using water) inside the FB chamber (Fig. 1) as described by Lima et al. [21]. After extraction, the stepper motor is stopped and the piezoelectric device is activated to sonicate the solutions for 5 s. The analytical signals are then recorded.

2.4. *Reference method*

The chromatographic procedure was carried out according to the reference method (D6584-07 “Standard Test Method for the Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography”) [35]. A gas chromatograph, (Agilent Technologies® 6890 GC) with a flame ionization detector (FID), a programmed temperature vaporizing (PTV) inlet (CIS 4, GERSTEL®), Dual Rail MPS 2 robotic sampler with 10 µL on-column syringe, and an 80 µL sideport syringe with a diluter module (GERSTEL®) were used. An Rtx-Biodiesel TG Restek column (10 m × 0.32 mm i.d.) was also used. Each sample solution was injected in triplicate, with a free glycerol retention time of 4.1 min, and the concentrations were calculated from a calibration curve.

3. Results and discussion

3.1. *Study of the piezoelectric frequency*

In order to generate reproducible SL signals, we investigated three operating frequencies for the piezoelectric device: (a) 320 kHz, (b) 358 kHz, and (c) 385 kHz. For this purpose, three glycerol solutions 1.0, 10, and 30 mg/L were used. No SL production at 320 kHz was observed, whereas at 385 kHz the analytical signals were no reproducible. The best frequency for the study was found while operating in a continuous mode of 358 kHz, which corroborates with the work of Sunartio et al. [29].

3.2. *Variation of the temperature caused by piezoelectric*

A study of possible piezoelectric-caused temperature variations in the standard solutions and samples was carried out (before and after) for the sonication time of 5 s. As the cavitation time is short, and the piezoelectric frequency is low, no significant temperature variation was observed. Each experiment was made in triplicate at room temperature (20 ± 1 °C). The thermocouple (MAX6675® with sensitivity of 41 µV/°C) was removed from the FB chamber during sonication to avoid damage.

3.3. *Location of the optical fiber*

As indicated in Fig. 1, the optical fiber is located at the inferior lateral of the mixing chamber, in front of the aqueous phase (extractive water). Since the biodiesel phase (immiscible fraction) is located on top of the mixing chamber, there is no light scattering. In addition, the system no needs no time for phase separation; emulsion is avoided by a slow rotation speed (200 rpm), as was done by Lima et al. [21].

3.4. *Analytical curve*

A calibration curve was obtained for $I = 0.302\ (\pm 0.011) - 0.101 \log C\ (\pm 0.003)$, $r = 0.996$, in which I is the relative SL intensity, and C is the glycerol concentration in g/L. A linear response was observed within the range of 0.001–100 mg/L glycerol, which is equivalent to 0.004–400 mg/kg free glycerol in biodiesel. Compared to the previously published works [12,18–21], the proposed method provides a lower measurable concentration of glycerol (1.0 μg/L).

3.5. Determination of the free glycerol content in the real samples

Table 2 shows the results of the determinations of the free glycerol content in commercial biodiesel samples. The precision of the proposed method was estimated determining the relative standard deviation (RSD) for the repeated measurement of authentic triplicates of different real samples, acquired and analyzed on different days, by both the reference and proposed methods at 1.5% [36]. Accuracy was evaluated comparing results from the proposed method with those of a reference method. For this purpose, an ordinary least squares (OLS) fitting was obtained, and the estimated intercept (a) and slope (b) were compared (with their ideal values of 0 and 1) using the elliptical joint confidence region (EJCR) test [21,37]. The intercept −(0.02 ± 0.13), and the slope (1.00 ± 0.03) values demonstrate that the joint confidence region certainly contains the theoretical (0, 1) point (α=0.05, 2, $n-2=4$). Fig. 3 shows the EJCR plot for the proposed method. The concentrations of free glycerol obtained by our proposed method for all the biodiesel samples were in a good agreement with those obtained by gas chromatography.

Table 2
Determinations of free glycerol content (mg/L) in commercial biodiesel samples by using the proposed method and the reference method. The values of uncertainty were estimated by using the expression $\pm t_{N-1}\, s/\sqrt{N}$, where N is the number of replicate measurements, t_{N-1} is the statistic parameter often called Student's t (with $N=3$, at 95% level of confidence) and s is the standard deviation.

Samples	Proposed method (mg/L)	Reference method (mg/L)
(1)	3.29 ± 0.06	3.10 ± 0.08
(2)	8.74 ± 0.03	8.80 ± 0.05
(3)	0.12 ± 0.01	0.13 ± 0.01
(4)	0.19 ± 0.04	0.18 ± 0.00
(5)	0.21 ± 0.03	0.22 ± 0.00
(6)	0.20 ± 0.04	0.21 ± 0.01

3.6. Analytical features

Table 3 compares the analytical features of the proposed method with other procedures described in the literature. In relation to the others, the proposed method has a great advantage: no chemical reagents are employed in any phase of analysis. Deionized water alone is used as the solvent for online extraction of glycerol from the biodiesel, as was done by Lima et al. [21]. The same water is then employed as the medium, generating the analytical signal, and the extracted glycerol quenches the sonoluminescence produced during water cavitation. Reproducible signals were generated under robust conditions due to the use of a simple and inexpensive piezoelectric device.

In the process of the biodiesel production, methanol (or ethanol) reacts with the triglyceride (vegetable oil) using a strong alkaline catalyst (generally NaOH or KOH) to give the corresponding mono-alkyl methyl esters (biodiesel), and glycerol in a 9:1 ratio [4]. The reagents (alcohol and catalyst) used in the transesterification reaction remain present in the biodiesel as residuals. After production, the biodiesel is washed exhaustively with water in order to minimize undesirable residues of both reagents and co-products before the commercialization. However, glycerol remains as the major impurity. The selectivity of the proposed method was confirmed by comparing the shape and the retention of real and calibration samples with chromatograms of standard solutions, and no peaks other than glycerol were observed. Extensive (in chamber) dilution of the real samples is always performed after extraction, which greatly minimizes the probability of finding alcohol or catalyst within the limits of detection. Therefore, the effect of other possible interferences in the sonoluminescence needed not be evaluated.

4. Conclusion

The processes of both extraction and determination of glycerol can be controlled by implementing the flow-batch methodology, and the strategy represents an excellent alternative to conventional flow-based procedures. Automatic preparation of the calibration curve, along with the online extraction and detection (using only water) make the proposed system extremely attractive for routine analysis. Waste generation is reduced significantly,

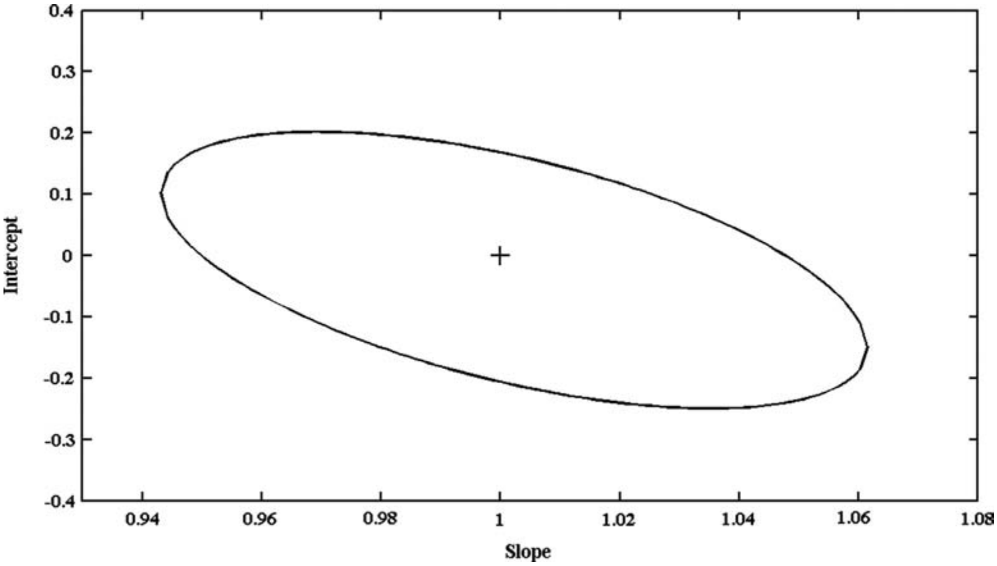

Fig. 3. Elliptical joint confidence region for the slope (b) and intercept (a) corresponding to the free glycerol content determinations of the proposed method. The cross marks the theoretical point ($a=0$, $b=1$).

Table 3
Comparative features of different methods for determination of free glycerol in biodiesel.

	MC [12]	**FIA** [18]	**MC** [19]	**Batch** [20]	**FBF** [21]	**Proposed FBS**
Detection limit [mg kg^{-1}]	4.0	5.0	2.0	2.0	0.144	4.0×10^{-6}
Sampling rate (h^{-1})	34	90	35	4	14	14
Reagents	Water, Acetic acid, Ammonium acetate, Acetylacetone Sodium periodate	Water, Sodium hydroxide	Water, Hydrochloric acid, Ammonium acetate, Sodium hydroxide, Acetylacetone, Sodium periodate	Water, Ethanol, Acetic acid, Ammonium acetate, Potassium periodate, Acetylacetone	Water, Acetic acid, Ammonium acetate, Potassium periodate, Acetylacetone, Isooctane	Water
Extraction	Offline	Offline	Offline	–	Online	Online
Sample (mg)	1000	250	1000	1000	15.4	15.4
Instrumentation	Spectrophotometer	Potentiometer	Spectrofluorometer	Spectrophotometer	Spectrofluorometer	Lab made luminometer

MC—Multicommutation in Flow Analysis; FIA—Flow-Injection Analysis; FBF—Flow-Batch with Fluorescence Detection; FBS—Flow-Batch with Sonoluminescence Detection.

a primary objective of green chemistry. Joining the principles of sonochemistry to the flow-batch methodology provided high sample rates, yet without the use of either chemical reagents or solvents. The new method was successfully applied to biodiesel analysis, and the results agreed with those of the reference method (ASTM D6584-07) at a 95% confidence level.

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