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A single-phase spectrophotometric procedure for *in situ* analysis of free glycerol in biodiesel

Mauricio S. Ribeiro [a], Fábio R.P. Rocha [b,*]

[a] Instituto de Química, Universidade de São Paulo, P.O. Box 26.077, 05599-970, São Paulo, SP, Brazil
[b] Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, PO Box 96, 13400-970, Piracicaba, SP, Brazil

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ABSTRACT

Biodiesel can be obtained from renewable sources by catalytic transesterification of vegetable oils or animal fats, yielding alkyl esters of fatty acids and glycerol as a by-product. Glycerol needs to be removed before commercialization of the biofuel because high concentrations can damage motors and produce highly toxic gases during combustion. The aim of this work was to develop a procedure for *in situ* analysis of free glycerol in biodiesel samples exploiting a single-phase system. The time-consuming analyte extraction and phase separation, usually employed in previously proposed procedures, were both avoided. Anhydrous ethanol was used to simultaneously dissolve the biodiesel and the chromogenic reagents and the final solution should contain at least 85% ethanol to ensure the formation of a single phase before the spectrophotometric measurements. A linear response was observed from 20.0 to 400.0 mg kg^{-1} glycerol, described by the equation: $A = 0.0018\,C + 0.0204$, $r = 0.999$, in which C is the concentration of glycerol in mg kg^{-1}. The detection limit was estimated at 2.0 mg kg^{-1} (99.7% confidence level) and the coefficient of variation was 2.1% ($n = 10$). The proposed procedure was successfully applied to biodiesel samples from different fat sources and the results agreed with the reference procedure at the 95% confidence level.

1. Introduction

Biodiesel is a renewable energy source, which can be used as fuel itself or blended with diesel from petroleum. It is widely produced by transesterification of vegetable oil or animal fat with methanol or ethanol and a strong base as catalyst. This reaction yields the mono-alkyl esters of the fatty acids and glycerol as a by-product [1]. Even after the purification steps, the fuel can be partially contaminated with glycerol, triacylglycerols, free fatty acids, residual alcohol and catalyst. Glycerol can settle in fuel tanks, causing clogging of fuel filters and damages to the combustion system. The hydroscopic nature of glycerol may also contribute for tank corrosion and fuel oxidation. In addition, generation of highly toxic gases such as acrolein during fuel combustion causes environmental pollution.

The regulation agencies of Brazil [1], United States [2] and European Community [3] have established the maximum amount of free glycerol in biodiesel as 200 mg kg^{-1} and recommend the analytical methodology based on gas chromatography [2,3]. GC [4] and HPLC [5] alternative procedures have also been reported for biodiesel analysis, usually requiring sample clean-up and analyte derivatization. HPLC with refractometric detection was also proposed, but glycerol extraction is time-consuming (*ca.* 2 h) and large sample amounts are required (4–20 g) due to the poor detection limit [5]. The accuracy of chromatographic procedures can be influenced by baseline drifts and low resolution and they require relatively expensive equipment and trained professionals. On the other hand, the availability of simple, reliable, fast and inexpensive quality testing methods is necessary in view of the high demand for the production of this biofuel and the consequently increasing number of production plants. This especially holds for small-scale production in which expensive instrumentation and skilled analysts typically are not available.

The *in situ* analysis requires practical, fast, and robust procedures, which use stable and low toxicity reagents and require portable instrumentation. These features allow rapid decisions and corrective actions. Procedures for *in situ* analysis of biodiesel in the whole production and distribution cycle are then required, including monitoring of fuel at gas-stations, screening of samples and prompt detection of anomalies.

An alternative to chromatographic methods exploits the Hantzsch's reaction and spectrophotometric detection [6]. The procedure is based on oxidation of free glycerol to formaldehyde by periodate, followed by reaction of the aldehyde with acetylacetone in ammoniacal medium, leading to the formation of 3,5-diacetyl-1,4-dihydrolutidine. This procedure is not suitable for *in situ* analysis because liquid–liquid extraction with hexane/ethanol/water and phase separation by centrifugation are necessary [6]. In addition, the chromogenic reaction requires heating at 70 °C and a strict time control. Other alternatives exploited the same reaction in flow-based systems with

* Corresponding author. Fax: +55 19 3429 4610.
E-mail address: frprocha@cena.usp.br (F.R.P. Rocha).

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spectrophotometric [7] and fluorimetric [8] detection. Despite the increasing in sample throughput and simplification of analyte extraction, this step was yet off-line performed, which hinders the *in situ* analysis. Recently, a flow-batch procedure which incorporates the extraction of glycerol, analyte derivatization and fluorimetric detection was proposed [9]. However, the low sample amount (*ca.* 15 mg) can affect sample representativeness and the time-based sampling can be affected by biodiesel viscosity. Thus, a simpler and fast procedure is still necessary, especially for small-scale production plants. Oxidation with periodate was also exploited for the indirect determination of free glycerol by capillary electrophoresis [10]. Electroanalytical methods have also been proposed, usually requiring extensive sample preparation to avoid interferences [11]. The exception is the flow-injection amperometric determination of glycerol exploiting electrocatalytic oxidation in a copper electrode [12].

The objective of this work was to develop a simple, fast and cost effective analytical procedure for the spectrophotometric determination of free glycerol in biodiesel samples without sample treatment or analyte extraction. This aimed a procedure that could be applied for *in situ* analysis of biodiesel during the production process or for inspections at gas stations.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade and the solutions were prepared using deionized water or anhydrous ethanol with a minimum purity of 99.8%.

Glycerol (99%) and ethanol were purchased from Sigma-Aldrich. Reference solutions from 4.0 to 80.0 mg L^{-1} of glycerol were prepared in ethanol by successive dilutions from 10.0 g L^{-1} stock solutions prepared in water.

A stock acetic acid solution (1.6 mol L^{-1}) was prepared by diluting 50.0 mL of the glacial acid (99.7%) in 500 mL of water. A 4.00 mol L^{-1} ammonium acetate stock solution was prepared from 77.0 g of the salt dissolved in 250.0 mL of deionized water. These solutions mixed in the volumetric ratio of 1:1 results in a buffer solution of pH 5.5. The 5.0 mmol L^{-1} potassium periodate (Fluka) solution and the 200 mmol L^{-1} acetylacetone (2,4-pentanedione, Across Organics) solutions were prepared in this buffer medium. The acetylacetone solution was prepared at least 2 h before using it.

Biodiesel samples were obtained from different sources, such as soybean, cotton or castor seeds and animal fat.

2.2. Apparatus

Absorbance measurements were carried out employing a single channel spectrophotometer (Femto, 600 plus). A multi-channel spectrophotometer (Ocean Optics, USB2000), coupled to a tungsten-halogen lamp (Ocean Optics, LS-1) by optical fibers was used in acquisition of absorption spectra and signal monitoring as a function of time. The measurements were performed in 1.0-cm optical-path cuvettes.

A pH meter (Metrohm 654) was used to measure the pH of reagents and solutions in different stages of the procedure. A water bath (Quimis) was employed for controlled heating.

2.3. Proposed procedure

The determination of free glycerol in biodiesel was carried out by the standard addition method. Biodiesel (1.000 g) was weighed directly in graduate polyethylene flasks with screw caps. Anhydrous ethanol was then added to the mark of 10.0 mL and the vessel was closed and shaken, resulting in a 10% (m/v) biodiesel solution. A volume of 400 μL of each diluted biodiesel solution was transferred to four screw-cap tubes. Anhydrous ethanol (100 μL) was added to two tubes. In the third and fourth tubes 100 μL of 10 or 20 mg L^{-1} glycerol standards prepared in anhydrous ethanol was added. The 5.0 mmol L^{-1} potassium periodate solution was added (200 μL) in all tubes, except the first that received 200 μL of a buffer solution without periodate. After 2 min at ambient temperature, the Hantzsch's reaction was initiated by addition of 500 μL of a 200 mmol L^{-1} acetylacetone solution, the flask was closed, shaken and put in a water bath at 50 °C for 10 min. After this interval the flasks were cooled with cold water until at room temperature. Anhydrous ethanol (3.5 mL) was added at the end of this process. In all experiments, pH was maintained at 5.5 in agreement with previous studies [6–8]. Spectrophotometric measurements were then carried out at 410 nm.

3. Results and discussion

3.1. General aspects and procedure optimization

Analyte extraction is the main drawback of procedures for free glycerol determination in biodiesel. This time-consuming procedure [5,6] usually requires organic solvents [6] and can result in systematic errors due to incomplete extraction and analyte losses. The liquid–liquid extraction can also be hindered by emulsion formation. In addition, the previously proposed procedures generally require analytical steps (*e.g.* mechanical shaking and centrifugation), which demand at least 25 min. On the other hand, several procedures require laborious sample clean up. These steps make the procedures unsuitable for *in situ* analysis. In this work, a single-phase procedure was proposed to overcome these drawbacks, using ethanol as a solvent for both biodiesel samples (organic phase) and chromogenic reagents (aqueous solutions). Fig. 1 shows the analytical response obtained for biodiesel by using different ethanol:water ratios in the extractor. Addition of anhydrous ethanol yielded a single-phase system and the absorbance value agreed with that obtained with extraction (25 or 50% ethanol) after correction for dilution.

In the presence of periodate, free glycerol was oxidized to formaldehyde, which reacted with acetylacetone to form 3,5-diacetil-1,4-dihidrolutidine, whose absorption maximum agreed with the described in literature (410 nm). The effects of reagent concentrations on analytical and blank signals are shown in Fig. 2. The analytical signal increases with periodate concentration up to 2 mmol L^{-1} without affecting the blank values. This concentration yields an 11-fold reagent excess in relation to the maximum recommended glycerol amount in biodiesel [1–3]. However, a 5 mmol L^{-1} concentration was selected to increase the procedure robustness and allow application to samples with higher glycerol contents. Under

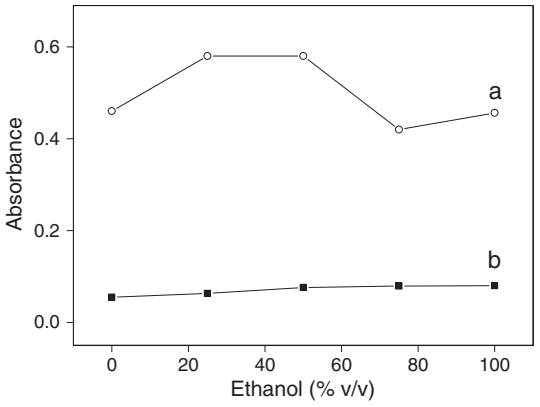

Fig. 1. Effect of ethanol concentration on extraction of glycerol from a biodiesel sample. Extractor volume = 4.00 mL and sample mass = 1.000 g. For a solution 99.8% ethanol a single-phase system was formed.