



A new spectrophotometric method for determination of biodiesel content in biodiesel/diesel blends



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HIGHLIGHTS

- The new method allows the determination of biodiesel content in diesel blends.
- It is applied in a large range of biodiesel content in biodiesel/diesel blends.
- It is applicable to colorimetric device operating at 420–440 nm wavelength range.

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ABSTRACT

In this paper a new quantitative analytical method is described for determining the biodiesel content in biodiesel/diesel mixture through of the fatty acid methyl ester reaction with hydroxylamine hydrochloride and iron(III) nitrate. The ferric hydroxamate complex diluted in n-heptane was analyzed by UV–visible spectroscopy in a range of 420–440 nm wavelength to determine the biodiesel content in biodiesel/diesel blends. The method has shown excellent repeatability and linearity for determining biodiesel content in biodiesel/diesel blends in the 0.5–5.0% quantification range and in small intervals of biodiesel content in diesel oil (0.1%). For levels over 5% of biodiesel in biodiesel/diesel blends, the linearity and repeatability is also excellent but it is necessary to increase the dilution of n-heptane of the ferric hydroxamate complex to still obtain a linear relationship between concentration and absorbance. The results obtained for biodiesel produced from different feedstocks are very similar, except for biodiesel produced from castor oil, what means that the method purposed has low influence of the feedstock used in biodiesel production. The parameters evaluated indicate that the method proposed is analytically reliable for determining biodiesel content in biodiesel/diesel blends.

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1. Introduction

Fatty acid alkyl esters, known as biodiesel, may be obtained from vegetable or animal fat and oils. The acyl group chain of the esters produced normally ranges from 12 to 24 carbon atoms and the number of unsaturated bonds normally ranges from 0 to 3, both chain length and unsaturation varies significantly depending on the feedstock used. Biodiesel can be blended with petroleum-derived diesel fuel. For instance, currently Brazil determines a 5% biodiesel rate by volume in the biodiesel/diesel blend (BDB) but, in the initial stage of the Brazilian Program of Production and Usage of Biodiesel (PNPB) 2%, 3% and 4% BDB were also used, such as in many countries which are starting the utilization of BDB as a fuel [1].

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In view of the wide range of feedstock available for production biodiesel [2], in which the biodiesel have been produced with varying characteristics, the quality assessment of biodiesel and BDB is a crucial stage to ensure production reliability as well as the fuel's use in diesel engines. To achieve for the required quality parameters, considerable technical–scientific researches have been carried out in the new methods development to ensure the reliability of results [3,4]. Several studies have reported the determination of biodiesel content in diesel, including the use of techniques such as infrared (IR) spectroscopy, ¹H NMR and chromatography [5–10]. However, many of them do not show selectivity [5], whereas others may be applicable only in restricted BDB concentration intervals or employ costly [7], slow techniques that require high reagent consumption [8–10].

Determining biodiesel content in diesel may be performed by IR spectroscopy, as states Brazilian technical standard NBR 15568 [10], but in association with multivariate analysis. Data generated

by Fourier Transform Infrared Spectroscopy (FTIR) is analyzed using chemometric methods, and specialized human resources are required in view of the considerable amount of data produced by these methods [11]. In addition, IR spectroscopy is usually more costly than techniques such as ultraviolet–visible (UV–Vis) spectroscopy [12].

Nuclear Magnetic Resonance (NMR) is an excellent technique for determining biodiesel content with high correlation coefficients in a broad quantification range. However, this is technique that requires employ costly [6].

UV–Vis spectroscopy has been used to determine the content of large, heptane-diluted BDB concentration intervals. For biodiesel, UV spectra show two intense absorption bands along the 230–260 nm wavelength. Variation in the composition of diesel aromatics may falsify results, given the fact that they show intense absorbances in these spectral regions. This method proved to be non-applicable to short BDB concentration intervals [7]. Nevertheless, UV–Vis spectroscopy provides easier and faster results in routine analyses, due to reduced analysis time and lower reagent consumption. Despite all these methods, research studies continually attempt to develop alternative methods, combining low cost and fast and accurate results. Amid efforts to develop a methodology that is capable of meeting all necessary requirements, UV–Vis spectroscopy may be a potential tool to quantify biodiesel content in BDB.

In this sense, an experimental procedure known as hydroxamic acid test is traditionally used to confirm the ester functional group [13]. Hydroxamic acids form basic compounds for the qualitative determination in colorimetric analyses of metal ions [14]. They are produced by a nucleophilic reaction between a carboxylic acid derivative and hydroxylamine in an alkaline medium [15]. Among the features of hydroxamic acids is their ability to form stable complexes with transition metals, particularly iron(III) ions [16]. All hydroxamic acids from *N*-hydroxylamine react with iron(III), resulting in mononuclear complexes with *O*'*O*-hydroxamate bidentate ligands in octahedral geometry (Fig. 1) [17,18].

Towards developing a technique that is low-cost, easy to perform, and which requires only standard instruments and reagents, this article proposes a modified version of the hydroxamic acid test to determine biodiesel content in BDB via the reaction of esters with hydroxylamine chloride in an alkaline medium. According to this version of the method, esters are subsequently complexed

with iron(III) ions and heptane-extracted, prior to UV–Vis spectrophotometric analysis [19].

2. Material and methods

2.1. Reagents

All analytical reagents used were manufactured by Tedia Brazil®. Deionized ultra-pure water was used to prepare NaOH, HCl, NaCl, and Fe(NO₃)₃ reagent solutions. Ethanol was used to prepare an NH₂OH·HCl solution. Diesel oil A S500, according to Resolution n° 50 of the ANP is a distillate fuel for use in diesel engine applications requiring a fuel with 500 mg kg^{−1} sulfur (maximum) and without previous addition of biodiesel was provided by ALESAT, Goiânia, Goiás State, Brazil. Biodiesel was produced from commercial vegetable oils made of canola, sunflower, corn and soy. Biodiesel made of peanut, castor oil, and jatropha were produced with vegetable oils extracted from the seeds.

The biodiesel samples were prepared by transesterification reaction, and the percentage of each individual fatty acid present in each feedstock (Table 1) was determined by Hartman and Lago method [20] followed by gas chromatographic analysis determined by Menezes et al. [21]. The quality control of each biodiesel produced from such feedstocks was determined according described by Prados et al. [22].

2.2. Calibration curve

Volumetric blends of methyl biodiesel and diesel were prepared in 100 mL calibrated volumetric flasks. Different BDB of soybean biodiesel in diesel were prepared and assessed in 0.0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5% and 5.0% (v v^{−1}) concentrations of biodiesel to determine the calibration curve. Assays involved the preparation of volumetric blends in BDB contents over of 5.0% (v v^{−1}), to verify whether the proposed method shows linearity at levels over 5.0%, in which, the first calibration curve ranged in 5.0%, 6.0%, 7.0%, 8.0%, 9.0% and 10.0% (v v^{−1}), the second in 10.0%, 11.0%, 12.0%, 13.0%, 14.0%, 15.0% and 16.0% (v v^{−1}), and the third in 16.0%, 17.0%, 18.0%, 19.0% and 20.0% (v v^{−1}), following criteria related to the amount of BDB used and to the dilution in heptane. Volumetric blends were also prepared in short

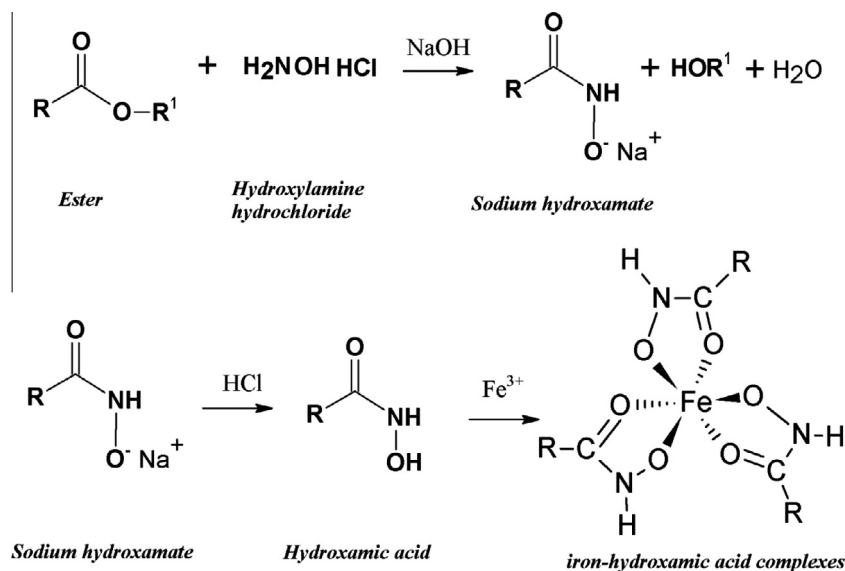


Fig. 1. Chemical reactions involved in the hydroxamic acid test.

Table 1
Fatty acid methyl esters (FAME) composition of biodiesel from different feedstock.

FAME	Representation	Biodiesel						
		Peanut	Canola	Sunflower	Castor	Corn	Jatropha	Soybean
Myristic	C14:0	0	0.1	0.1	0	0	0.1	0.1
Palmitic	C16:0	11.1	5.3	6.7	1.6	12.8	15.1	11.2
Palmitoleic	C16:1 <i>cis</i> 9	0.1	0.4	0.1	0.3	1.1	0.8	0.2
Margaric	C 17:0	0.6	0	0.1	0	0.1	0	0
Stearic	C18:0	2.9	2.4	3.1	1.2	2.1	5.9	3.4
Oleic	C18:1 <i>cis</i> 9	37.9	61	29.9	5.9	35.2	41.7	22
Cis-vaccenic	C18:1 <i>cis</i> 11	0.6	3.3	0.8	0	0.9	1.4	1.6
Ricinoleic	12-OH C18:1 <i>cis</i> 9	0	0	0	84.5	0	0	0
Linoleic	C18:2, <i>cis</i> 9, <i>cis</i> 12	39.7	19.6	57.8	6.5	46.2	34.4	53.9
Linolenic	C18:3 <i>cis</i> 9, <i>cis</i> 12, <i>cis</i> 15	1.3	7.3	0.5	0	1.4	0.3	7.1
Arachidic	C20:0	3.6	0.2	0.6	0	0.2	0.1	0.4
Gadoleic	C20:1 <i>cis</i> 9	1.8	0.1	0.3	0	0	0.1	0.1
Behenic	C22:0	0.2	0.1	0	0	0	0.1	0
Lignoceric	C24:0	0.2	0.2	0	0	0	0	0

intervals of biodiesel content such as 0.0%, 2.8%, 2.9%, 3.0%, 3.1% and 3.2% (v v⁻¹).

2.3. Influences of various biodiesel feedstock

Methyl biodiesel derived from peanut, canola, sunflower, corn, jatropha and soy oils at a 3% level in petroleum diesel were prepared to determine whether the type of oil seeds influences the absorbance of the end product of complexation reaction.

2.4. Assay

The method was modified based on hydroxamic acid test results [19], and the following quantitative-analytical methodology was proposed:

In autoclavable test tubes, 0.4 mL of the BDB, 1.0 mL of NH₂OH·HCl solution (0.5 mol L⁻¹), and 0.2 mL of NaOH solution (0.5 mol L⁻¹) were mixed together, and the mixture was submitted to vortex agitation for 40 s. 2.0 mL of HCl solution (1.0 mol L⁻¹) was added and submitted to vortex agitation for 20 s. 3.0 mL of NaCl solution (5% m v⁻¹) was added and later submitted to vortex agitation for 10 s. 0.5 mL of Fe(NO₃)₃ solution (5% m v⁻¹) was added and submitted to vortex agitation for 30 s. Lastly, 10.0 mL heptane was added and submitted to vortex agitation for 20 s. Time was given for the complete separation of phases to occur. The upper phase, of a reddish hue, was analyzed by UV–Vis spectrophotometry.

A diode array UV–Vis absorption spectrophotometer (Biochrom Ultraspec) was used to perform absorbance readings of each biodiesel blend, and the B0.0 sample was used in blank cell.

Analyses were performed in the 300–600 nm wavelength range, equipped with cells of optical path length 1 cm. A single 420–450 nm wavelength ($R > 0.999$) may be used in the calibration curve calculation to determine methyl biodiesel content in biodiesel/diesel blends via UV–Vis spectrophotometry. When using UV–Vis spectrophotometers, whether diode array-based or standard, monochromatic light-based, the 435 nm wavelength is recommended for the calibration curve calculation, given that it showed the maximum absorbance wavelength. For filter colorimeter measurements, one may choose to carry out the absorbance reading at 420 nm, because this instrument normally offers filters for this particular wavelength. In addition, according to the UV–Vis spectrum reading, no other compound interferes in the wavelength region or in the absorption of the reagents used.

3. Results and discussion

The spectrophotometric analysis of upper heptane phases of BDB samples submitted to the reactional procedure generates the absorption profile shown in Fig. 2a. Based on the zero-order spectrum, determining the wavelength of maximum absorbance in the 400–460 nm band proves difficult. However, by determining the first derivative of the absorption spectrum, the wavelength of maximum absorbance of 435 nm is easily distinguished (Fig. 2b).

Fig. 3 shows that the calibration curve obtained by the method has excellent linearity ($r = 0.9994$; mean standard deviation = ± 0.0422); its application proved, therefore, to be reliable as regards determining biodiesel content in biodiesel/diesel blends in the 0.5–5.0% quantification range. The limit of detection is the value whose analyte signal is three times the noise level measured or

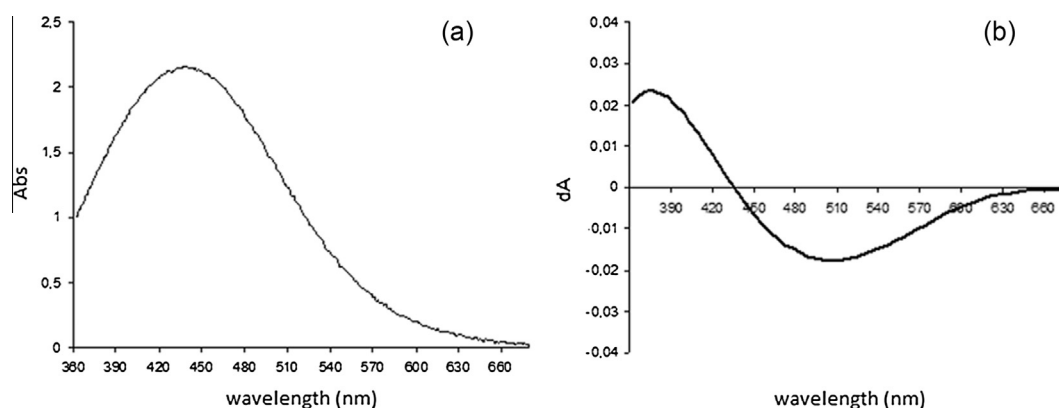


Fig. 2. UV–Vis absorbance spectrum for the Fe(III)–hydroxamate complex (a) zero order and (b) 1st derivative.

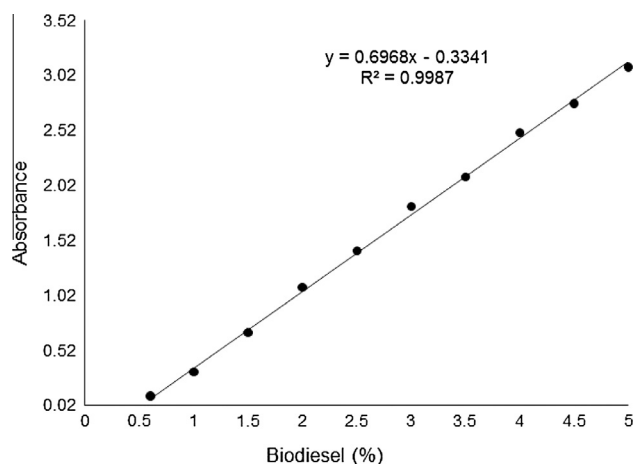


Fig. 3. Calibration curve of biodiesel in diesel (0.5–5.0% v/v) at 435 nm wavelength.

the standard deviation of the blank. After the triplicate reading of the blank, the error mean of the blank signal was calculated at the 435 nm wavelength, the mean absorbance value being 0.0043 with a standard deviation of ± 0.0087 . Multiplying the error value by three produced the absorbance value 0.0261, which amounts to a 0.04% limit of detection of BDB content (see Table 2).

The method also proved applicable to short BDB intervals (0.1%), as well as showed an excellent correlation coefficient (0.9996) and mean standard deviation (± 0.0085), all of which confirm satisfactory linearity obtained in 2.8–3.2% BDB contents (Fig. 4).

Table 3 shows the results obtained by the repeatability study for the analysis of a nominal mixture of 3.0% BDB. Assays were carried out for 20 blend rates to verify the variability of the absorbance value and consequently of the value obtained experi-

Table 2

Variability obtained for the spectrophotometric analysis of the ferric hydroxamate complex of 20 samples of biodiesel in diesel in the estimated level of 3.00% (v/v).

Parameters	Absorbance	Specified level (%)
Min value	1.655	2.85
Max value	1.932	3.25
Average	1.759	3.00
Standard deviation	± 0.083	± 0.12
Median	1.743	2.98
Coefficient of variation CV (%)	4.751	4.01

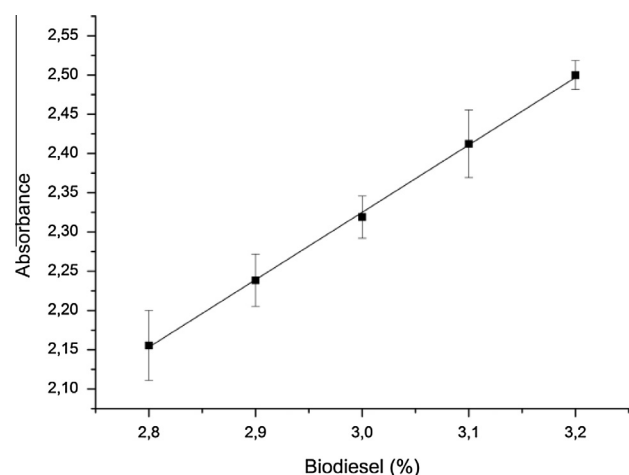


Fig. 4. Calibration curve for 2.8–3.2% v/v of biodiesel/diesel blends at 435 nm wavelength.

Table 3

Results of the statistical parameters for calibration curves of different biodiesel blends range at 435 nm wavelength.

Working range (% v/v)	Correlation coefficient (R)	Standard deviation (sd)	Slope	Intercept	Number of replicates (n)
0–5	0.9994	0.0422	0.6737	−0.3223	3
5–10	0.9992	0.0254	0.2979	−0.0601	3
10–16	0.9994	0.0100	0.1771	0.1002	3
16–20	0.9958	0.0140	0.0847	0.1622	3

mentally for the BDB content. Even though values ranged from 2.85% to 3.25%, the mean and the median of determined samples were very close to the 3.0% blend content with a $\pm 0.12\%$ standard deviation.

The linearity was verified by the assay performed on biodiesel concentrations over 5%; calibration intervals analyzed were 5–10%, 10–16%, and 16–20% ($v v^{-1}$). According to results shown in Table 3, the method may also be safely applied to all these BDB content intervals (0–5, 5–10, 10–16, and 16–20), showing excellent linearity with a correlation coefficient ranging from 0.9958 to 0.9994. However, certain criteria related to the amount of BDB used and to the dilution of the reaction product after iron(III) ion complex formation need to be met so as to prevent absorbance extrapolation. To determine the quantification band of the 5–10% BDB content, 200 μ L of BDB must be used and the end product diluted in 10 mL heptane. As for the quantification band of the 10–16% BDB content, 200 μ L of BDB must be used and the end product diluted in 20 mL heptane. For the quantification band of the 15–20% BDB content, 100 μ L of the BDB must be used and the end product diluted in 20 mL heptane.

Biodiesel fuels obtained from canola oil, corn, jatropha fruit, peanut, soy, sunflower, and castor oil were analyzed with the present method. The resulting absorbance values at 435 nm are shown in Fig. 5. There were no significant differences in the absorbance of the end products of methyl biodiesel extracted from peanut, canola oil, sunflower, corn, jatropha, and soy in the reaction caused by the proposed method. On the other hand, methyl biodiesel from castor oil revealed greater sensitivity and high absorbance when compared to the other vegetable oils. The greater absorbance for castor oil biodiesel is probably due to the high amount of ricinoleic acid methyl ester [23], approximately 85% (Table 1). Ricinoleic acid contains a hydroxyl on carbon 12, which may have increased this compound's absorbance through the affinity of hydroxyl with iron(III) ions. Therefore, determining biodiesel content in diesel also depends on the feedstock used, as does the mid-infrared method proposed by Pimentel et al. [5] and reported in the

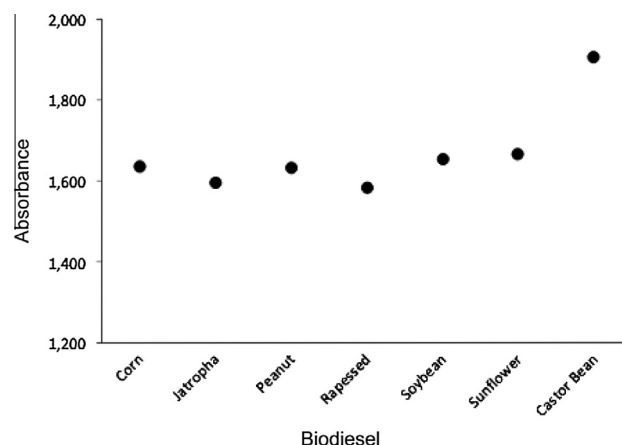


Fig. 5. Absorbance of the iron-hydroxamic acid complex for B3 from different feedstock at 435 nm wavelength.

Brazilian technical standard NBR 15568 [10]. In spite of the fact that castor oil biodiesel showed the highest absorbance value, castor oil has not been normally used as feedstock for biodiesel production mainly to some problems related to the quality control specifications of this biofuel [24].

Then, it is possible to determine the amount of biodiesel in diesel if castor oil is absence as feedstock for the production of the biodiesel used in blends with diesel oil. If the castor oil was used as feedstock for biodiesel production, the method only works if the calibration curve is prepared with the original biodiesel produced with castor oil. The utilization of the original biodiesel is not necessary if the biodiesel is produced from feedstocks other than castor oil. Moreover, any type of diesel may be used, even those that possess high sulfur levels, for none of its components interfere in its reaction with hydroxylamine. Moreover, any type of diesel may be used, even those that possess high sulfur levels, for none of its components interfere in the reaction with hydroxylamine.

4. Conclusions

The proposed method is selective as regards fatty acid alkyl esters present in biodiesel. Moreover both aliphatic and aromatic hydrocarbons present in diesel oil do not react with hydroxylamine and do not form Fe-hydroxamic acid complexes through the reaction procedure previously described.

The method proposed allows the determination of biodiesel content in diesel through the use of cheap reagents, of wide commercial availability and low toxicity. The fact that such products have significant coloring enables them to be used in the visible ultraviolet region in diode array UV–Vis spectrophotometers, monochromatic light UV–Vis spectrophotometers, and filter colorimeters.

Given the fact that diesel, regardless of sulfur content and aromatic hydrocarbons, does not include esters in its composition and that the hydroxamic acid test is ester-specific, the method may be applied to all types of diesel fuel. By using this method, it becomes possible to quantify BDB in small and large biodiesel rates as long as BDB volumes used for constructing the calibration curve and for quantification are reduced in proportion to the increase in biodiesel mass.

In summary, results regarding linearity, limit of detection and quantification, accuracy, selectivity, and specificity indicate that the method proposed is analytically reliable for determining biodiesel content in biodiesel/diesel blends.

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