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Spot test exploiting smartphone-based digital images for determination of biodiesel in diesel blends

Samara Soares[a], Lidiane C. Nunes[a], Wanessa R. Melchert[b], Fábio R.P. Rocha[a,*]

[a] Center for Nuclear Energy in Agriculture, University of São Paulo, Av. Centenário, 303, Piracicaba, SP 13416-000, Brazil
[b] College of Agriculture "Luiz de Queiroz", University of São Paulo, PO Box 9, Piracicaba, SP 13418-970, Brazil

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ABSTRACT

A simple, inexpensive, and fast colorimetric spot test is proposed for quantification of biodiesel in diesel blends. The procedure was based on formation of a violet complex between Fe(III) and the hydroxamate ion generated by the reaction of the alkyl esters characteristic of biodiesel with hydroxylamine. The analytical measurements were based on digital images acquired using a smartphone camera and converted to RGB values using a free application software (PhotoMetrix® 1.6). Values of the G channel corresponding to the intensities of the reflected radiation were taken as analytical parameter because of the complementarity with the absorption spectrum of the Fe(III)/hydroxamate complex. The effects of the reagents concentrations were evaluated by a Box-Bhenken experimental design and the optimized condition was 0.20 mol L^{-1} hydroxylamine, 0.30 mol L^{-1} NaOH, and 5.0 mmol L^{-1} $Fe_2(SO_4)_3$. A linear response range was achieved from 3.0 to 30.0% (v/v) of biodiesel, described by equation $G = 59.4 + 1.72$ C ($r = 0.999$). The coefficient of variation and the limit of detection (99.7% confidence level) were estimated at 0.9% ($n = 10$) and 1.0% (v/v) of biodiesel, respectively. The proposed procedure consumed only 10 µL of sample, 830 µg $NH_2OH.HCl$, and 110 µg Fe(III) and generated *ca.* 1.0 mL of residue per determination. The results for determination of biodiesel in diesel blends agreed with those obtained by the official method EN14078 at the 95% confidence level. The proposed procedure also allows monitoring formation of fatty acid methyl esters during the transesterification reaction.

1. Introduction

Biodiesel is an alternative to fossil fuels because of the production from renewable sources (vegetable oils or animal fats) and the lower emission of polluting gases in its combustion. The biofuel is generally produced by transesterification of triglycerides with ethanol or methanol, under either alkaline or acidic catalysis [1]. Quality control is required during the production, transport, and storage to assure the biodiesel properties as well as to avoid changes in the physicochemical characteristics and adulterations [1,2]. The main quality parameter to be monitored in biodiesel is the content of fatty acid methyl esters (FAME) [1,2], which is related to the yield of the transesterification reaction. According to European and Brazilian Standards, the FAME content in biodiesel needs to be at least 96.5% (v/v) [1,2]. The American regulations do not establish the threshold limit, but indicate that this parameter should be measured and registered [1,2].

Biodiesel has been used directly as a fuel or in blends with diesel [3]. The official method for biodiesel determination in diesel blends (EN 14078/2009) is based on infrared spectroscopy [4]. Although it is a reliable method, as evaluated by interlaboratory studies, it requires specific operational conditions difficult to reproduce for the analysis directly at the production plants and fuel stations [5], and chemometric data processing is required [6,7]. Alternative methods based on gas chromatography coupled to a mass spectrometry [8], high performance liquid chromatography [9], and fluorescence spectroscopy [10] have been proposed, but expensive equipment and skilled analysts are required. A spectrophotometric method for measurement of FAME based on formation of ferric hydroxamate was exploited as a more cost-effective approach [11,12]. However, these procedures require several analytical steps in addition to the use of toxic solvents, such as 10 mL of heptane [11] or 5 mL of hexane [12] per determination, thus generating significant amounts of toxic wastes [11,12]. These drawbacks were circumvented in a flow-based procedure based on the lab-in-syringe approach and the same colorimetric reactions [13]. The toxic solvents were replaced by ethanol and the reagent consumption was reduced in comparison to the batchwise procedures. However, aiming at *in situ* monitoring, a more practical and inexpensive procedure is needed.

Spot tests are simple and inexpensive analytical tools, which require

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* Corresponding author.
E-mail address: frprocha@cena.usp.br (F.R.P. Rocha).

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a few steps of execution and consume low amounts of reagents and solvents [14–17]. Quantitative spot tests have been developed by exploiting reflectance measurements and, more recently, by photometric measurements with a smartphone camera. This approach is attractive because smartphones with high quality cameras and free application softwares to convert the digital images into the analytical data (*e.g.* RGB values) are widely available. Advantages of this approach include practicality, portability, and cost-effectiveness as successfully demonstrated for a diversity of applications, such as the determination of furfural in sugarcane spirits [18], salicylate in biological samples [19], and surfactants as milk adulterants [20]. Aiming at biodiesel quality control, smartphone-based spot tests were developed for determination of iodine value [14] and methanol [21,22]. In this work, this analytical approach was exploited for the development of an analytical procedure for determination of biodiesel in diesel blends.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with analytical grade chemicals, ultrapure water (resistivity > 18.2 MΩ cm) and anhydrous ethanol. All reagents were supplied from Merck, except when mentioned. Biodiesel:diesel blends were obtained directly from fuel stations with expected biodiesel concentrations of 7% (v/v), established by the Brazilian legislation when the samples were collected and analyzed.

Solutions of 0.20 mol L^{-1} hydroxylamine and 0.30 mol L^{-1} sodium hydroxide were prepared by dissolution of 0.70 g of $NH_2OH.HCl$ or 0.60 g NaOH in 5 mL of water; the final volume was made up with ethanol to 50 mL. The solution 5.0 mmol L^{-1} $Fe_2(SO_4)_3$ was prepared by dissolution of 0.10 g of $Fe_2(SO_4).H_2O$ in 20 mL of 1.60 mol L^{-1} HNO_3 and making the volume to 50 mL with ethanol, obtaining a final concentration of 0.65 mol L^{-1} HNO_3.

Reference solutions were prepared within 3.0 – 30.0% (v/v) by dilution of 99% (v/v) methyl linoleate (Sigma) in anhydrous ethanol.

2.2. Procedure

Samples or reference solutions were diluted 1:50 (v/v) in anhydrous ethanol and 500 µL of each was added to 1.5 mL Eppendorf® tubes, jointly with 60 µL of 0.20 mol L^{-1} NH_2OH and 300 µL of 0.30 mol L^{-1} NaOH solutions. After 15 min, 200 µL of 5.0 mmol L^{-1} $Fe_2(SO_4)_3$ solution prepared in 0.65 mol L^{-1} HNO_3 was added to form the violet complex.

The optimization was carried out by a Box–Behnken design involving the NH_2OH, NaOH, and $Fe_2(SO_4)_3$ concentrations as variables. The experimental data were processed using the STATISTICA 7.0 software. Analysis of variance (ANOVA) was applied to evaluate the lack of fit of the regression model.

The transesterification reaction was carried out from 100 g soybean oil, 40.0 mL of methanol, and 1.00 g NaOH, under stirring and heating at 60 °C. For monitoring the formation of FAME, aliquots of the reaction mixture were taken each 10 min, 250-fold diluted in ethanol, and processed as previously described.

2.3. Acquisition of digital images

A smartphone (iPhone SE) equipped with a 12 megapixels camera and the free application software PhotoMetrix® 1.6 [23] was used to obtain the digital images and to convert them to RBG values. Images were acquired under controlled illumination in a box (190 × 210 × 125 mm height, length, and width, respectively) internally covered with a white paper and with a LED lamp (luminous intensity of 50 lx) attached at the bottom.

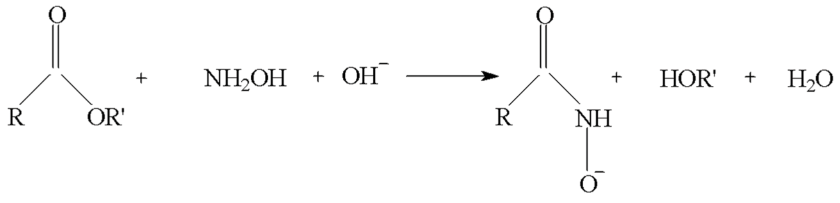
Photometric measurements were carried out directly in the Eppendorf® tubes fitted at the upper part of the box to assure measurements near the central part of the vessels, with a region of interest of 32 × 32 pixels. The cell phone was positioned 5.0 cm far from the measurement tubes.

The analytical signals were obtained by subtracting the values of the G channel (measured as the intensities of the reflected radiation) from the value corresponding to the total reflection (255), thus yielding values proportional to the color intensities.

3. Results and discussions

3.1. General aspects

The determination of the biodiesel amounts in diesel blends was based on quantification of FAME, characteristic of biodiesel. To this aim, the reaction of the alkyl esters with hydroxylamine was exploited to generate the hydroxamate ions (Eq. (1)). This species reacted with Fe(III) to yield the violet ferric hydroxamate complex (Eq. (2)) [11–13]. The first reaction occurs in alkaline medium, whereas the complexation reaction requires acid medium to avoid Fe(III) hydrolysis. In this way, the acid concentration in the iron (III) solution depends on the sodium hydroxide amount in the first step.



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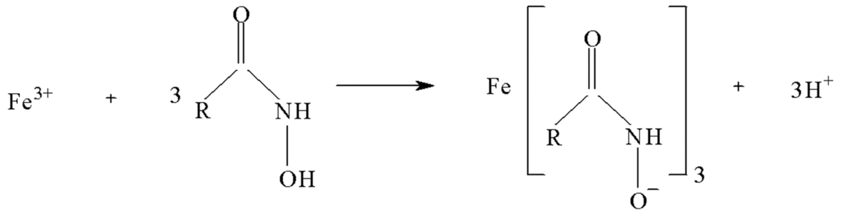

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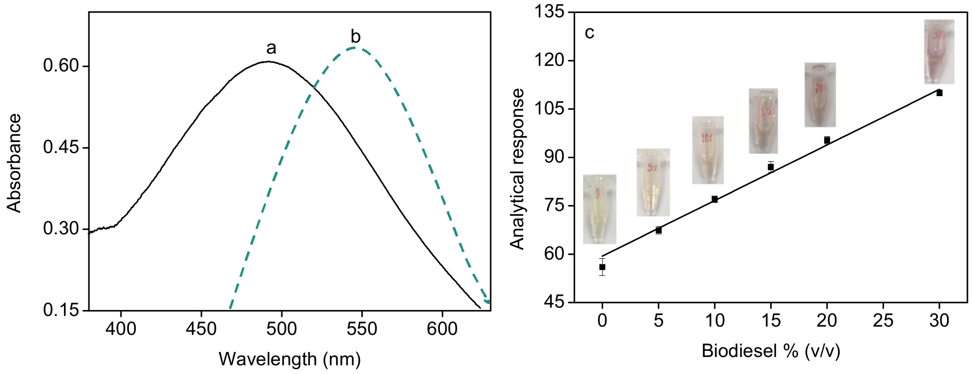

Fig. 1. (a) Absorption spectrum of the ferric hydroxamate, (b) the spectral region corresponding to the G channel (dashed lines) and (c) calibration curve 3–30%(v/v) biodiesel in the diesel blends.

The type of acid was evaluated by preparing the Fe(III) reagent in equivalent amounts of HNO_3, $HClO_4$, HCl or H_2SO_4. The highest analytical signals were obtained with HNO_3 and $HClO_4$, whereas the analytical response obtained with HCl was 15% lower because of formation of Fe(III) cloro-complexes. With H_2SO_4, the solutions became turbid and the ferric hydroxamate complex was not formed. Based on these results and on safety aspects, HNO_3 was selected for further experiments.

Because of the hydrophobic characteristics of biodiesel and diesel, and the hydrophilic properties of the reagents, ethanol was used as mediator solvent to form a single phase in sample analysis. Thus, reagents were prepared in ethanolic media containing the minimal amount of water and samples were previously diluted in ethanol. It was evaluated that a 50-fold dilution of the samples sufficed for both the determination of FAME in the diesel blends and to assure formation of a single phase with the reagent solutions. This dilution factor also avoided interferences by background absorption due to the dye used as marker in some diesel blends. The reaction rates were not significantly affected in ethanolic medium.

The absorption spectrum of the ferric hydroxamate complex (Fig. 1a) partially overlaps with the spectral region covered by the G channel of the RGB system (Fig. 1b), which corresponds to the complementary color of the absorption product. In this way, the intensities of the reflected radiation, measured as G values, were taken as the analytical signal and were directly correlated to the percentage of biodiesel in the diesel blends (Fig. 1c). The analytical response on this channel corresponds to *ca.* 40% of the total signal variation. A significant variation in channel B was also observed (*ca.* 33%), but the response was not proportional to the ester content.

Methyl linoleate (main ester produced by transesterification of soybean oil with methanol) was selected as the model species for calibration, because of the predominance of soybean oil as feedstock for biodiesel production. Alternatively, the reference solutions can be prepared from a reference biodiesel sample, whose amount of FAME is known.

Table 1
Box-Bhenken design with coded, real values, and analytical responses (G values) for optimization of the procedure for biodiesel determination in biodiesel:diesel blends.

Experiment	NH_2OH (mol L^{-1})	NaOH (mol L^{-1})	$Fe_2(SO_4)_3$ (mmol L^{-1})	G value[a]
1	−1 (0.15)	−1 (0.10)	0 (10)	67.0
2	1 (0.25)	−1 (0.10)	0 (10)	54.7
3	−1 (0.15)	1 (0.30)	0 (10)	98.7
4	1 (0.25)	1 (0.30)	0 (10)	64.7
5	−1 (0.15)	0 (0.20)	−1 (5)	69.5
6	1 (0.25)	0 (0.20)	−1 (5)	83.0
7	−1 (0.15)	0 (0.20)	1 (15)	68.0
8	1 (0.25)	0 (0.20)	1 (15)	73.3
9	0 (0.20)	−1 (0.10)	−1 (5)	69.0
10	0 (0.20)	1 (0.30)	−1 (5)	126.3
11	0 (0.20)	−1 (0.10)	1 (15)	70.7
12	0 (0.20)	1 (0.30)	1 (15)	127.0
13 - 17[b]	0 (0.20)	0 (0.20)	0 (10)	111.0, 110.0, 109.0, 108.0, 109.0

[a] Mean values of triplicate measurements; b. Central point.

3.2. Procedure optimization

The optimization by Box-Behnken design [24] involved the concentrations of NH_2OH, NaOH and $Fe_2(SO_4)_3$ as variables. Based on preliminary experiments, volumes of sample diluted in anhydrous ethanol (1:50 v/v), NH_2OH, NaOH, and $Fe_2(SO_4)_3$ solutions were fixed at 500, 60, 300, and 200 µL, respectively. A biodiesel sample with 30% biodiesel concentration was used as test sample for the multivariate optimization. The experimental levels (−1, 0, and +1) for the three factors NH_2OH (X1), NaOH (X2), and $Fe_2(SO_4)_3$ (X3), as well as the analytical responses of the experimental design are presented in Table 1. Upper and lower limits for each variable were chosen according to the reactions stoichiometries Eqs (1) and (2).

The Box-Behnken design matrix consisted of a set of 17 experiments and the central point (variables coded as zero) was evaluated in five replicates (Table 1, experiments 13–17). The replicates at the central point allowed estimating the experimental, pure and lack of fit errors. In addition, it is also possible to identify the most significant coefficients of the proposed model.

Fig. 2 shows the response surface obtained for the optimization of the parameters for biodiesel determination in diesel blends. The proposed model was statistically significant; by comparing the Mean Square of Regression (MSR = 878) and Mean Square of Residual (MSr = 172), the F value ($F = 5.10$) is higher than the critical value ($F = 3.68$) at the 95% confidence level. On the other hand, by comparing the Mean Square of lack of fit and Mean Square of pure error, the proposed model presented lack of fit because the calculated F value ($F = 308$) was higher than the critical F value ($F = 6.59$). This aspect means that the random error obtained during the data acquisition (pure error) and the error related to the inability of the model to adjust to the experimental points (lack of fit) are statistically different. However, as the MSR was higher than MSr, the model can be used for prediction. The final regression model is described by Eq. (3), where the standard errors are in parentheses. The b3 and b23 coefficients were not significant at 95% confidence level.

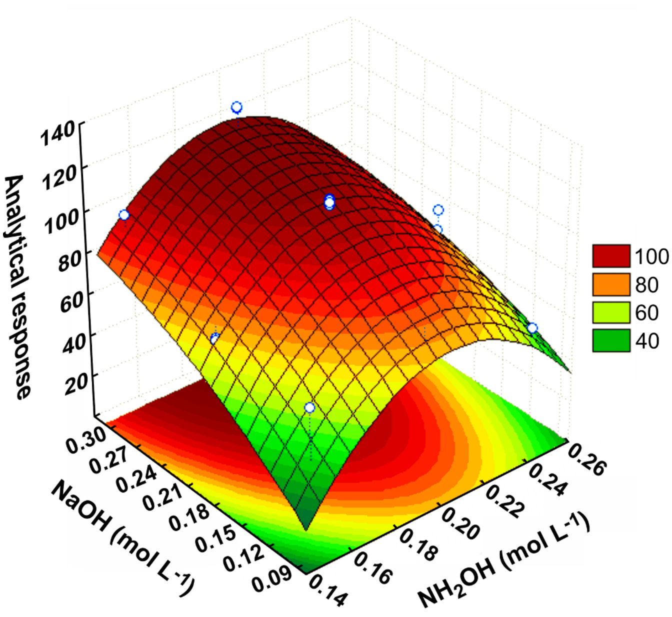

Fig. 2. Response surface plot of the G values as a function of NH_2OH and NaOH concentrations.

$$G = 109.4(\pm 0.51) - 3.44X1(\pm 0.40) + 19.42X2(\pm 0.40) - 31.47X11(\pm 0.55) - 6.68X22(\pm 0.55) - 4.47X33(\pm 0.55) - 5.42X12(\pm 0.57) - 2.04X13(\pm 0.57) \quad (3)$$

Fig. 2 shows the response surface for G values as a function of the significant factors (NaOH and NH_2OH). In order to maximize the analytical response, the NH_2OH (X1) and NaOH (X2) concentrations should be at the low (negative coefficient) and high values (positive coefficient), respectively, because the excess of hydroxylamine causes reduction of Fe(III) to Fe(II) in the second step, and the lack of NaOH hinders formation of the hydroxamate ions in the first step. By considering these aspects, interactions between NH_2OH (X1) and NaOH (X2), and NH_2OH (X1) and $Fe_2(SO_4)_3$ (X3) were observed. As these coefficients present a negative signal (−5.42 and −2.04), the best results should be obtained when hidroxylamine and NaOH concentrations and hidroxylamine and Fe(III) concentrations are in opposite levels.

As can be seen in Table 1, the lower NH_2OH concentration (0.15 mol L^{-1}) was used in experiments 1, 3, 5, and 7. The lack of hydroxylamine to react with the alkyl esters hindered the formation of hydroxamate ion, thus resulting in low analytical responses. In fact, under this experimental condition, the FAME concentration is approximately 12.5% above the stoichiometric amount of hydroxylamine. On the other hand, the higher hydroxylamine concentration evaluated (0.25 mol L^{-1}) hindered the complex formation due to the reduction of Fe(III) to Fe(II), as observed in experiments 2, 4, 6 and 8. Experiments 9 and 11 were carried out with enough hydroxylamine and Fe(III), but lack of NaOH (0.10 mol L^{-1}), which affected directly the formation of the hydroxamate ions.

The working condition predicted for a maximum response corresponds to 0.20 mol L^{-1} NH_2OH, 0.35 mol L^{-1} NaOH, and 5.0 mmol L^{-1} $Fe_2(SO_4)_3$, and the predicted G value for this combination of variables was 125. However, under this experimental condition, the obtained G-value was 86. This difference represents a relative error of *ca.* 30% because the model presents lack of fit. In addition, the excess hydroxide causes the formation of $Fe(OH)_3$ and diminished the amount of the metal ion available for complexation with hydroxamate. Therefore, the working conditions of experiment 10 (0.20 mol L^{-1} NH_2OH, 0.30 mol L^{-1} NaOH, and 5.0 mmol L^{-1} $Fe_2(SO_4)_3$) were selected for further experiments.

Additional tests were carried out to evaluate the effect of different smartphone cameras and the controlled illumination on the analytical measurements. Figs. 3a and 3b show the results obtained under controlled illumination, by using cameras with lens apertures of f/2.2 (camera used for the development of the method) and f/1.8 (iPhone 8 Plus, 12 MP camera). The corresponding calibration curves ($G = 57.3 + 1.7C$, $r = 0.991$ and $G = 104.4 + 1.5C$, $r = 0.982$, respectively) indicate that both cameras can be used, although the device with lens aperture of f/2.2 yielded better linearity and sensitivity *ca.*

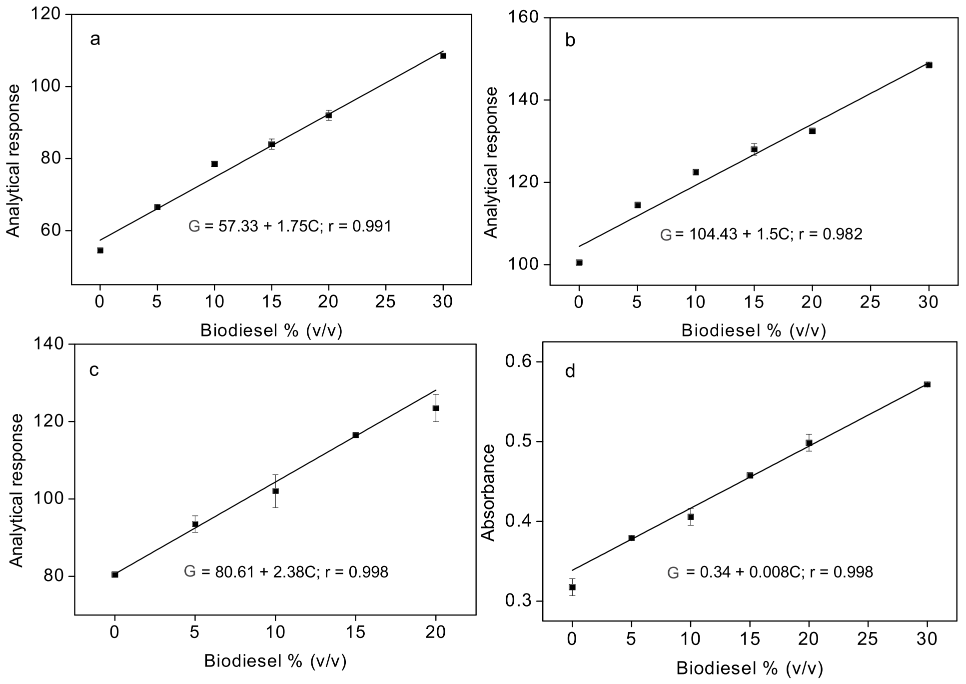

Fig. 3. Calibration curves obtained under controlled illumination, 12 megapixels camera and lens aperture of (a) f/2.2 and (b) f/1.8; (c) measurements without controlled illumination with a 12 megapixels camera and lens aperture of f/2.2, and (d) UV–vis spectrophotometry (measurements at 510 nm).

Table 2
Recoveries of fatty acid methyl esters in diesel blends. Mean values and uncertainties from triplicate measurements determined by the proposed and reference procedures ($n = 3$).

Sample[a]	Recovery (%)	CV (%)
B10	107 ± 6	5.6
	109 ± 6	5.5
B20	106 ± 2	1.9
	107 ± 4	3.7
B30	95 ± 2	2.1
	97 ± 3	3.1
B50	98 ± 2	2.0
	96 ± 2	2.1
B100	95 ± 2	2.1
	97 ± 2	2.1
	107 ± 2	1.9

[a] Mixtures of BX were prepared in duplicate (B10-B50) or triplicate (B100). X corresponds to the % (v/v) of biodiesel in the samples. B50 and B100 samples were analyzed after 4-fold dilution in ethanol.

Table 3
Biodiesel amounts in diesel blends determined by the proposed and reference procedures ($n = 3$).

Sample	Biodiesel% (v/v)		F value[a]
	Proposed	Reference [4]	
1	7.0 ± 0.4	6.6 ± 0.2	4.0
2	7.3 ± 0.1	6.6 ± 0.2	4.0
3	7.3 ± 0.2	6.9 ± 0.3	2.2
4	7.6 ± 0.3	7.7 ± 0.3	1.0
5	6.8 ± 0.1	6.8 ± 0.3	9.0
6	7.1 ± 0.3	6.7 ± 0.3	1.0
7	7.0 ± 0.2	6.9 ± 0.3	2.2

[a] F critical = 19.0 (95% confidence level).

17% higher. As the measurements are based on the intensities of the reflected radiation, it is expected that the camera with higher lens aperture provide better sensitivity.

Fig. 3c shows the results obtained with the camera with lens aperture of f/2.2, but without controlled illumination. The analytical response was also linear with the percentage of biodiesel ($r = 0.998$), but in a narrower range of concentrations (<20% v/v biodiesel). However, the absence of controlled illumination significantly hindered the precision of measurements (Fig. 3c). The possibility of measuring with different cameras and different lighting conditions demonstrates the robustness of the procedure. Alternatively, measurements can also be carried out by UV–Vis spectrophotometry (Fig. 3d), which is also an inexpensive and widely available equipment.

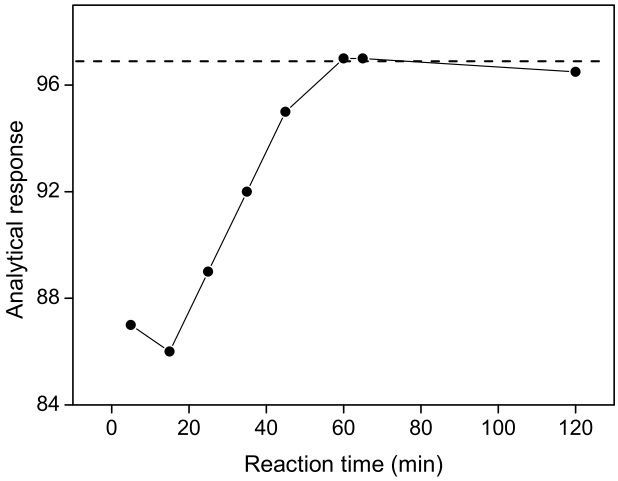

Fig. 4. Monitoring of formation of FAME in a transesterification reaction. Dashed lines indicate the threshold limit established from a 96.5% methyl linoleate solution. All aliquots of the reaction mixture were analyzed after a 250-fold dilution.

3.3. Analytical features and application

Under the optimized conditions, a linear response was obtained from 3.0 to 30.0% (v/v) biodiesel, described by equation $G = 59.4 + 1.7C$, $r = 0.999$. The coefficient of variation and the limit of detection (99.7% confidence level) were estimated at 0.9% ($n = 10$) e 1.0% (v/v) of biodiesel, respectively. The procedure consumes 830 µg of NH_2OH and 110 µg of Fe(III), and generates only *ca.* 1.0 mL of residue per determination.

Recoveries of biodiesel in diesel blends from 10 to 50% (v/v), as well as FAME in pure biodiesel were estimated within 95 and 109% (Table 2). The precision (coefficients of variation < 5.6%) did not vary significantly with the percentage of biodiesel in the samples. These results demonstrate the applicability of the proposed procedure for quality control of biodiesel and to determine FAME in mixtures with different amounts with biodiesel.

Accuracy of the procedure was assessed by comparing the results with those attained by the official method, based on infrared spectroscopy [4] (Table 3). The variances were compared by the F-test and values for all the analyzed samples were lower than the critical value for two degrees of freedom (19.0), thus indicating that the precision of the procedures are not significantly different at the 95% confidence level. Moreover, the results obtained by the proposed procedure agreed with those attained by the reference procedure at the 95% confidence level, as evaluated by a paired *t*-test.

The applicability of the proposed procedure for monitoring the yield of the transesterification reaction is demonstrated in Fig. 4. The analytical signal is measured periodically and comparison of the measured values with a threshold limit (established from a reference methyl linoleate or biodiesel solution) allows detecting the steady state of the reaction. By taking the current legislation into account, the threshold limit was established from a 96.5% FAME solution. A 250-fold dilution of the reference solution or the reaction mixture sufficed to adjust the ester content to the linear response range.

Some analytical features of the proposed procedure are shown in Table 4. The consumption of NH_2OH and the waste amount generated are 434- [11,12] and 100-fold [12] lower when compared to the spectrophotometric procedures based on the same chemical reactions [11,12]. Another advantage of the present proposal relies on the replacement of toxic solvents (hexane and heptane) by ethanol and consumption of 5- [12] and 10-fold [11] less solvent in comparison to the procedures based on formation of ferric hydroxamate. Moreover, the previous procedures are more time-consuming because additional steps are involved, such as liquid-liquid extraction [11,12] and heating [12]. The coefficient of variation was 4-fold lower when compared with the spectrophotometric procedure [11] and it was similar to the achieved in the flow-based procedure [13]. Moreover, data acquisition by smartphone-based digital images makes the procedure more practical and portable, thus more attractive for *in situ* analysis.

The proposed approach is also advantageous in comparison with procedures based on HPLC [9] and fluorescence [10]. In addition to the cost significantly lower, better precision (*e.g.* CV = 8% of the HPLC procedure) and simple sample pretreatment (involving only dilution in ethanol) can be mentioned. The proposed procedure is also more environmental friendly, as the HPLC and fluorimetric procedures require 12 mL of hexane [9] or 5 mL dichloromethane [10] per determination.

The smartphone-based photometric procedure is quite attractive considering the instrumental and operational costs, and the analytical response is obtained quickly and with minimal reagent consumption and waste generation, in agreement with green chemistry principles [25]. In addition, it is suitable for analysis directly at the fuel stations.

Table 4
Comparison of procedures for the determination of biodiesel in diesel blends based on formation of ferric hydroxamate.

Procedure	CV (%)	$NH_2OH.HCl$ (mg)	Solvent (mL)	Waste (mL)	Reference
Spectrophotometry	4.0	347	heptane (10.0)	17	11
Spectrophotometry	–	347	hexane (5.0)	102	12
Flow-based spectrophotometry	0.8	0.4	ethanol (2.2)	3.0	13
Colorimetric spot test	0.9	0.8	ethanol (1.0)	1.1	This work

4. Conclusions

A spot test exploiting smartphone-based digital images was developed for the determination of biodiesel in diesel blends. The proposed procedure is fast, precise, minimizes the consumption of samples, reagents, and solvents, thus also the waste volumes. It requires only a measurement chamber, manufactured in the laboratory and a cell phone for analytical measurements, being attractive for *in situ* analysis. Ethanol was successfully used as mediator solvent for formation of a single phase among sample and reagents. The analytical features such as linear response range, limits of detection and quantification, accuracy, and selectivity indicate that the proposed procedure is a reliable alternative to the official method based on infrared spectroscopy for determining concentrations of biodiesel in diesel blends.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] G. Knothe, Analyzing biodiesel: standards and other methods. National center for agricultural utilization research, J. Am. Oil Chem. Soc. (2006) 823–833.
[2] M. Mittelbach, Diesel fuel derived from vegetable oils, VI: specifications and quality control of biodiesel, Bioresour. Technol. 56 (1996) 7–11.
[3] ANP - Agência nacional do petróleo, gás natural e biocombustíveis, <http://www.anp.gov.br/biocombustiveis/biodiesel> Acessed in 22/06/2019.
[4] European Standard - EN 14078, Liquid petroleum products – Determination of fatty methyl ester (FAME) content in middle distillates – Infrared spectrometry method.
[5] G. Knothe, Determining the blend level of mixtures of biodiesel with conventional diesel fuel by fiber-optic near-infrared spectroscopy and 1H nuclear magnetic resonance spectroscopy, J. Am. Oil Chem. Soc. 78 (2001) 1025–1028.
[6] M.F. Pimentel, G.M.G.S. Ribeiro, R.S. Cruz, L. Stragevitch, J.G.A. Pacheco Filho, L.S.G. Teixeira, Determination of biodiesel content when blended with mineral diesel fuel using infrared spectroscopy and multivariate calibration, Microchem. J. 82 (2006) 201–206.
[7] M.F. Ferrão, M.S. Viera, R.E.P. Pazos, D. Fachini, A.E. Gerbase, L. Marder, Simultaneous determination of quality parameters of biodiesel/diesel blends using HATR-FTIR spectra and PLS, iPLS or siPLS regressions, Fuel 90 (2011) 701–706.
[8] C. Besser, L. Pisarova, M. Frauscher, H. Hunger, U. Litzow, A. Orfaniotis, N. Dörr, Oxidation products of biodiesel in diesel fuel generated by artificial alteration and identified by mass spectrometry, Fuel 206 (2017) 524–533.
[9] T.A. Foglia, K.C. Jones, J.G. Phillips, Determination of biodiesel and triacylglycerols in diesel fuel by LC, Chromatographia 62 (2005) 115–119.
[10] M.D. Scherer, S.L. Oliveira, S.M. Lima, L.H.C. Andrade, A.R.L. Caires, Determination of the biodiesel content in diesel/biodiesel blends: a method based on fluorescence spectroscopy, J. Fluoresc. 21 (2011) 1027–1103.
[11] M.A.A. Silva, R.A. Correa, M.G.O. Tavares, N.R. Antoniosi Filho, A new spectrophotometric method for determination of biodiesel content in biodiesel/diesel blends, Fuel 143 (2015) 16–20.
[12] R.P.M. Costa, T.C. Khalil, A.P.F. Santos, D.F. Andrade, L.A. Avila, Determinação do teor de biodiesel em diesel empregando o ensaio colorimétrico do ácido hidroxâmico, Quim. Nova 38 (2015) 563–569.
[13] S. Soares, W.R. Melchert, F.R.P. Rocha, A flow-based procedure exploiting the lab-in-syringe approach for the determination of ester content in biodiesel and diesel/biodiesel blends, Talanta 174 (2017) 556–561.
[14] S. Soares, M.J.A. Lima, F.R.P. Rocha, A spot test for iodine value determination in biodiesel based on digital images exploiting a smartphone, Microchem. J. 133 (2017) 195–199.
[15] F. Feigl, V. Anger, Spot Tests in Organic Analysis, 7th ed., Elsevier, 1966.
[16] A.Z. Michael, M.S. Stewart, The paper spot test: a rapid method for quantitating stannous concentrations in radiopharmaceutical kits, J. Nucl. Med. 22 (1981) 465–467.
[17] A. Dar, U. Shafique, J. Anwar, W. Zaman, A. Naseer, A simple spot test quantification method to determine formaldehyde in aqueous samples, J. Saudi Chem. Soc. 20 (2016) 352–356.
[18] M. Oliveira, K. Franco, W.T. Suarez, V.B. Santos, Digital image method smartphone-based for furfural determination in sugarcane spirits, Food Anal. Methods 10 (2017) 508–515.
[19] E.M. Linares, L.T. Palermo, A.B. Moreira, M.D.P.T. Sotomayor, L.T. Kubota, A fluorescence spot test for salicylate determination, Anal. Lett. 40 (2007) 573–583.
[20] M.S.M.S.F. Acevedo, M.J.A. Lima, C.F. Nascimento, F.R.P. Rocha, A green and cost-effective procedure for determination of anionic surfactants in milk with liquid-liquid microextraction and smartphone-based photometric detection, Microchem. J. 143 (2018) 259–263.
[21] S. Soares, K.G. Torres, E.L. Pimentel, P.B. Martelli, F.R.P. Rocha, A novel spot test based on digital images for determination of methanol in biodiesel, Talanta 195 (2019) 229–235.
[22] S. Soares, F.R.P. Rocha, A simple and low-cost approach for microdistillation: application to methanol determination in biodiesel exploiting smartphone-based digital images, Talanta 199 (2019) 285–289.
[23] G.A. Helfer, V.S. Magnus, F.C. Böck, A. Teichmann, M.F. Ferrão, A.B. Costa, PhotoMetrix: an application for univariate calibration and principal components analysis using colorimetry on mobile devices, J. Braz. Chem. Soc. 28 (2017) 328–335.
[24] S.L.C. Ferreira, R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David, G.C. Brandão, E.G.P. Silva, L.A. Portugal, P.S. Reis, A.S. Souza, W.N.L. Santos, Box-Behnken design, An alternative for the optimization of analytical methods, Anal. Chim. Acta 597 (2007) 179–186.
[25] R.S. Amais, L.S.G. Teixeira, F.R.P. Rocha, Greener procedures for biodiesel quality control, Anal. Methods 7 (2015) 4396–4418.