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# Quantitative analysis of biodiesel in diesel fuel by ultraviolet-visible spectroscopy

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ABSTRACT
A preliminary methodology for the determination of biodiesel content in commercial diesel fuel by ultraviolet and visible absorption spectroscopy (UV-Vis) was developed, with the aim of exploring its potential application in optical sensors for monitoring the diesel/biodiesel mixtures in heavy vehicles. Absorbance of visible light between 400 nm and 500 nm showed a linear response of the optical signal as a function of the biodiesel content for the investigated volume concentration range of biodiesel (varying from 0 % to 20 %). Multivariate analysis was used to validate the construction of different calibration curves, differentiated by the origin of feedstocks for the starting pure biodiesel and diesel components, and to determine the optimal wavenumber for performing ordinary linear regression. A narrow range of the visible spectrum around 450 nm provided the best coefficient of correlation for all calibration curves. The effects of the supplier of base biodiesel and diesel components used to obtain the calibration curves on the predictions of the univariate model were also assessed.

**Section:** RESEARCH PAPER

**Keywords:** biodiesel; optical spectroscopy; multivariate analysis; sensor; measurement

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## 1. INTRODUCTION

Given a wide variety of feedstocks for biodiesel production, Brazil has the natural tendency to produce biodiesels with different specifications, which are controlled by methods defined in legislation approved by the National Agency of Petroleum, Natural Gas and Biofuels (ANP) [1]. In order to address the specific characteristics of biodiesels produced in the country, it is essential to conduct technical-scientific research aimed at developing new control methods to guarantee the quality of biodiesel and its mixtures with diesel. The identification of the oil-based diesel fuels obeys the following nomenclature: diesel BX denotes a blend of biodiesel with diesel oil, where X is the volume fraction (in %) of biodiesel in the mixture (blend level); and diesel A denotes the diesel oil with no biodiesel in its composition (B0). Currently, there are two types of commercial diesel sold in Brazil: BX S500 and BX S10, which nominally contain 500 ppm and 10 ppm of sulphur, respectively. In order to distinguish the type of BX sold at gas stations, the ANP determines that S500 diesel oil must differ from S10 diesel by colour, which is achieved through the addition of a dye. The specification for the BX mixture is regulated by ANP Resolution nº 50/2013 [2].

In this work, we developed a methodology for the determination of biodiesel content in diesel using a simple and accessible analytical technique, which is ultraviolet-visible spectroscopy (UV-Vis). The main goal of this work was to evaluate the potential of the developed methodology for its application in optical sensors for online monitoring of the diesel/biodiesel mixtures in heavy vehicles. In distinction to other similar studies ([3], [4]), we investigated the absorbance properties of biodiesel in a visible segment of the electromagnetic spectrum, between 400 nm and 500 nm. This spectral range for diesel fuel sensoring offers some advantages for the envisaged practical application: for example, there is no need for the fuel to be diluted, unlike in the case of UV region

[5], and it does not depend on the varying number of aromatic compounds in diesel fuels, unlike in the case of the near-infrared part of the spectrum [6].

## 2. EXPERIMENTAL PROCEDURE

### 2.1. Samples

For the construction of calibration curves, 60 blends of BX samples were used, which were prepared by mixing pure diesel and biodiesel components from specific suppliers. One of the base components of pure biodiesel was the certified reference material (CRM) for water content, produced at the National Institute of Metrology, Quality and Technology (Inmetro). Mixtures of pure diesel and pure biodiesel (blends of respective pure samples from various sources) were also used as base components to construct the calibration curves.

The samples were prepared by adding biodiesel to diesel in volumetric percentages ranging from 2 % (B2) to 20 % (B20) with a nominal biodiesel concentration step of 2 % in the mixtures. The construction and implementation of the analytical curves for determining the biodiesel content in diesel through UV-Vis spectroscopy were guided by the European standard EN 14078 [7].

Table 1 summarises the base components of pure diesel and pure biodiesel used in the construction of calibration curves, and Table 2 identifies the constructed calibration curves by specifying the respective base components of pure diesel and biodiesel.

Table 1. Pure biodiesel and pure diesel samples used in the construction of calibration curves.

| **Biodiesel** | |
|---|---|
| B_1 | Biodiesel, CRM (Inmetro) |
| B_2 | Biodiesel, supplier 1 (undefined feedstock) |
| B_3 | Biodiesel, supplier 2 (blend of different feedstocks of biodiesel at supplier) |
| B_4 | Biodiesel, supplier 3 (soy feedstock) |
| B_5 | Biodiesel blend (equal-parts mixture, in terms of volume, of six samples of pure biodiesel: B_1, B_2, B_4, and three samples of B_3 collected at different times) |
| **Diesel A** | |
| D_1 | A S10, supplier 1 |
| D_2 | A S500, supplier 2 |
| D_3 | A S10, supplier 2 |
| D_4 | A S10 blend (equal-parts mixture, in terms of volume, of four samples of pure diesel: D1 and three samples of D_3 collected at different times) |
| D_5 | A S500 blend (equal-parts mixture, in terms of volume, of three samples of pure diesel: three samples of D_2 collected at different times) |

Table 2. Identification of calibration curves according to respective mixtures of base components.

| Curve ID | Base components |
|---|---|
| Curve 1 | B_1 + D_1 |
| Curve 2 | B_2 + D_2 |
| Curve 3 | B_3 + D_3 |
| Curve 4 | B_4 + D_3 |
| Curve 5 | B_5 + D_4 |
| Curve 6 | B_5 + D_5 |

### 2.2. Spectra acquisition

The absorption spectra of biodiesel-diesel mixtures were obtained in the spectral range between 200 nm and 800 nm, with a resolution of 1 nm, using a Perkin Elmer Lambda 950 spectrophotometer equipped with a quartz cell having a 1 mm optical path length. Data collections comprised three replicates for each sample.

### 2.3. Data analysis

Data processing was performed in MATLAB environment, using both multivariate analysis with the Partial Least Squares Regression (PLSR) method, which took into account absorption data from a specific range of a spectrum, and Ordinary Least Squares Regression (OLSR). It resulted in an absorbance versus biodiesel concentration calibration curve for a specific wavelength of a spectrum.

The PLSR method, although more accurate than OLSR (as it evaluates many more measurement points), is quite difficult to implement in the practical construction of a sensor. The use of PLSR was aimed at validating the samples used for the construction of calibration curves by evaluating the respective curves quality.

The validation of the mixtures prepared for the construction of the calibration curves was performed using the Leave-One-Group-Out Cross-Validation (LOGOCV) method. In this method, we generate the PLSR model based on all data points for concentrations, except for one that is then used as a blind sample to estimate the concentration predicted by the model. This procedure is repeated leaving out one by one all available data points, estimating how far the predicted values are from the true values, thus assessing the quality of a calibration curve.

In the next step, a simple linear regression was performed between absorbance values and concentrations to construct new calibration curves for each wavelength in the respective range of interest. The LOGOCV protocol was again employed to validate these curves, and the curve corresponding to the wavelength that produced the smallest prediction error was selected for the final tests on biodiesel concentration prediction in commercial diesel BX samples.

## 3. RESULTS

### 3.1. Analysis of commercial B diesel samples

Figure 1 shows the raw spectra of the seven commercial diesel B samples used to evaluate the calibration curves. The most prominent differences in the diesel B spectra of the used samples appeared in the range between 400 nm and 600 nm. The absorbance of B S500 samples shows an increase in values below

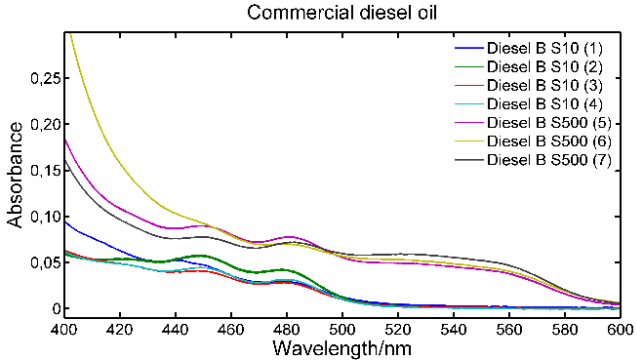


Figure 1. Spectra of commercial diesel B S10 and diesel B S500 samples.

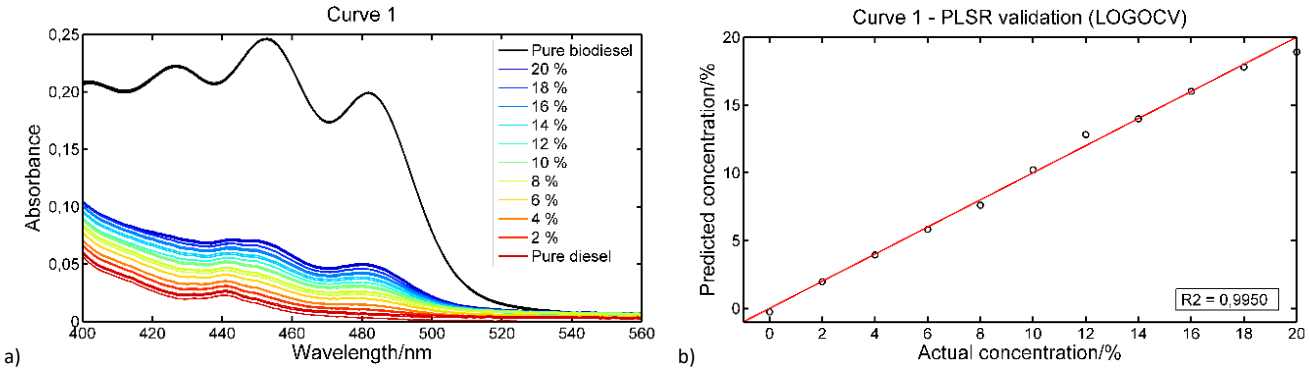


Figure 2. a) Absorbance spectra and b) PLSR validation of calibration curve 1.

580 nm, which causes a systematic displacement of their spectra compared to those of the B S10 samples. This displacement can be attributed to the presence of a dye [7] added to diesel oil A S500 as a compulsory marker [8], [9].

Figure 2a shows the absorbance spectra of the calibration samples and the validation results for calibration curve 1 (see Table 1 and Table 2). A linear trend in band intensities variation was observed in the absorbance graph as the concentration of biodiesel in the calibration mixtures increased from 0 % to 20 %. The validation process for the curve demonstrates a strong linear relationship between the predicted and actual concentrations, with a correlation coefficient of 0.995 (Figure 2b).

Figure 3a displays the predicted concentration values as a function of wavelength for the different mixtures (coloured lines), along with their corresponding reference values (black lines). The vertical line marks the wavelength for which the prediction error is minimum, determined by the sum of squared residuals from the regression model. Figure 3b presents the correlation curve corresponding to this selected wavelength.

Finally, the prediction of biodiesel concentrations in the samples of commercial blends (diesel B) was made using the calibration curve for the wavelength selected in the previous step. In order to assess the accuracy of the constructed calibration curves, the predicted biodiesel concentrations were compared with the respective concentrations determined by other standardised methods from external certified laboratories. Figure 4a shows the prediction curves for the diesel B samples, together with the prediction obtained at the specific wavelength of a spectral response (vertical red line in the figure) that results in the smallest overall error in the calculated concentrations of biodiesel in the tested samples (Figure 4b).

The circles in Figure 4b are visual depictions of the expected accuracy of the developed measurement method. We can observe in Figure 4b that the predicted results for diesel samples

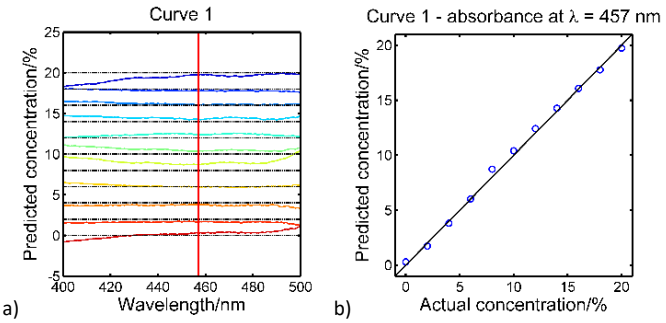


Figure 3. a) Graphical representation of wavelength selection using OLSR for b) the calibration curve 1 with the best accuracy.

of type S500 show a significant systematic shift to greater values of concentrations. This result was expected, since calibration curve 1 was constructed using S10 type of diesel in the reference samples, whereas the absorbance spectra of the B S500 diesel samples show a systematic shift to higher absorbance values over the entire wavelength range of interest (see Figure 1). Nevertheless, the relative dispersions of the predicted values of biodiesel concentrations for both B S10 and B S500 samples (red and blue circles, respectively) were nearly the same, that is, approximately 3 %. The effect of the separation of B S10 and B S500 results can be attributed to the presence of a dye in the B S500 samples. The quality of calibration curve 1 can be assessed from the residual parameters shown in Figure 4b (defined as a square root of residual sum of squares). The value of total residual of 16.1 % (all commercial diesel samples are considered) is mainly defined by the residual related to B S500 samples (15.9 %), whereas the residual corresponding to B S10 samples was significantly smaller (2.8 %). These residual parameters can be used to compare the calibration curves constructed using different suppliers for the base pure diesel and biodiesel components.

### 3.2. The effect of diesel and biodiesel supplier on the results of biodiesel concentration measurements by UV-Vis spectrometry

Figure 5 shows the correlations between the predicted and the actual values of commercial diesel samples B obtained for all types of calibration curves constructed in this study (see Table 2). It is noteworthy that, in all cases, the wavelength that provides the best accuracy in the prediction is around 450 nm, which corresponds to the centre of the absorption band of the

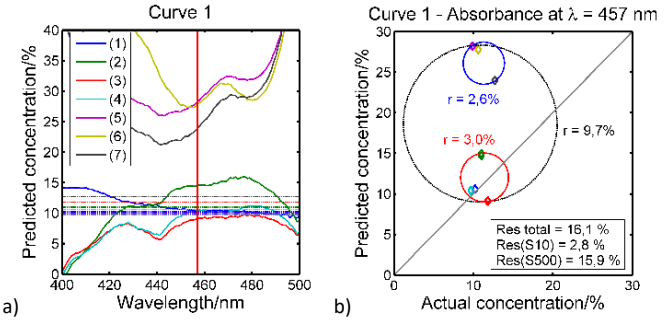


Figure 4. a) Prediction of concentrations for commercial diesel B samples in the overall wavelength range of interest, and b) for the specific wavelength using calibration curve 1. Consecutive numbers in the legend denote the samples' feedstock. The radius of the circles and the residual parameters are given in the figure (see the text for details).

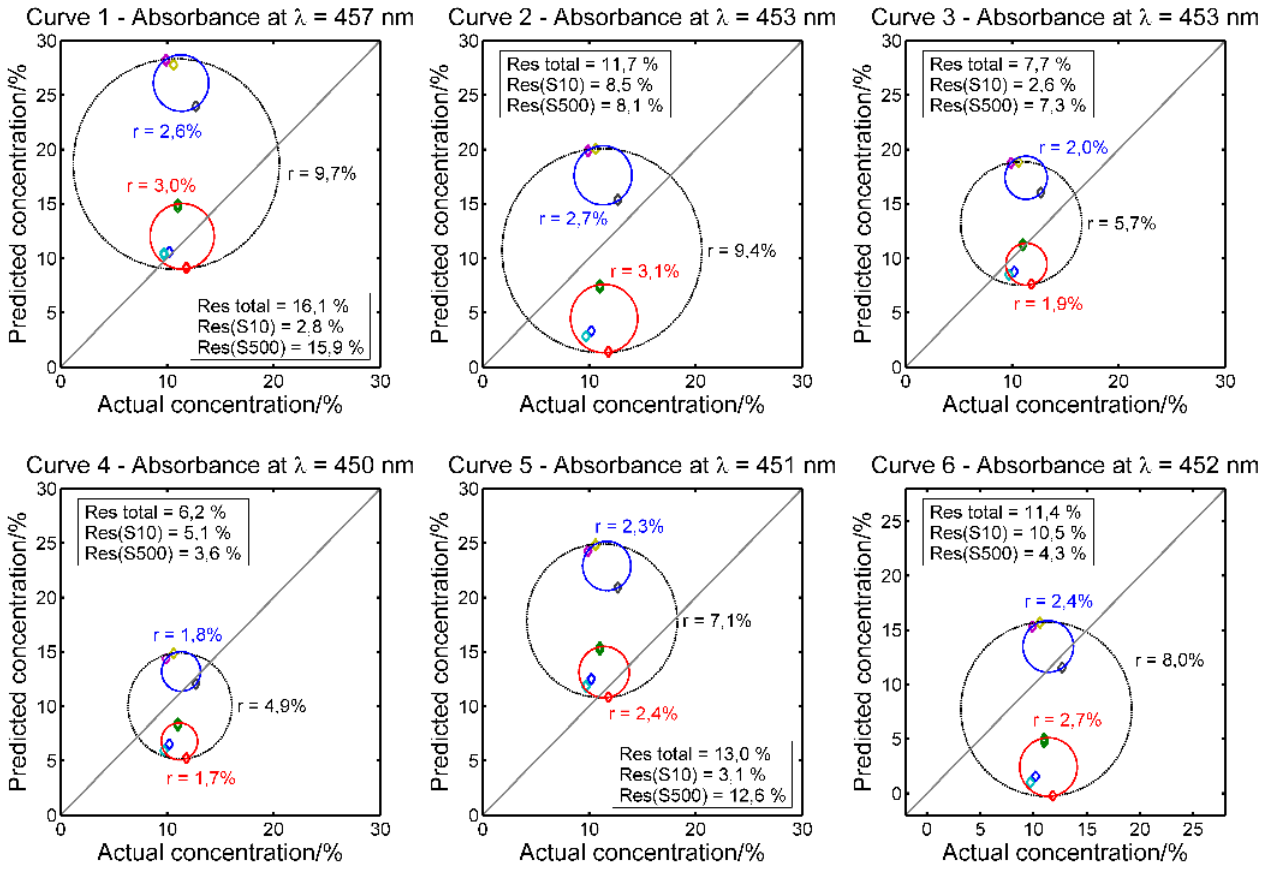


Figure 5. The effect of the calibration curve model on the predicted versus actual results.

conjugated double bond systems, characteristic, for example, of carotenoids [8] naturally present in biodiesel.

As explained in Section 3.1, the circles in the prediction figures illustrate the scale of relative scattering of the results upon the determination of biodiesel concentrations in commercial BX blends using the respective calibration curve. The black circles represent the relative accuracy scale expected when a specific calibration curve is used for measurements of both S10 and S500 types of diesels. Blue and red circles outline the relative spread of measured concentrations for S500 and S10 type mixtures, respectively.

The results presented in Figure 5 provide important insights about the application of the investigated method for the monitoring of biodiesel in vehicles. A systematic difference in absorbance properties, resulting in consistent variations in the measured biodiesel concentrations between S500 and S10 diesel types within the studied spectral segment, cannot be mitigated by the choice of calibration curve. The predicted versus actual concentrations of biodiesel in all graphs of Figure 5 are clearly divided into two respective groups of data. The use of A S10 diesel as a base component for calibration curves results in much lower residual parameters for B S10 diesels (see plots for calibration curves 1, 3, and 5), whereas the use of A S500 diesel provides lower residuals for B S500 diesels. These results point out that two calibration curves can be provided in the eventual monitoring system, for S10 and S500 types of diesel, and this may extend the respective capabilities of the system to distinguish between S10 and S500 types of fuel.

The influence of the pure biodiesel component employed in constructing the calibration curve is best rationalized by jointly analysing the calibration curve parameters (Figure 6) and the overall residual distribution, represented by the size of the black circles in Figure 5.

It is possible to note that calibration curves 4, 3, and 5 result in smaller overall residual parameters ($r$ = 4.9 %, 5.7 %, and 7.1 %, respectively), as compared to the respective parameters estimated in case of calibration curves 6, 2, and 1 ($r$ = 8.0 %, 9.4 %, and 9.7 %, respectively). Not only this grouping of calibration curves, but also their ranking according to the values of residuals correlates perfectly with the sensitivities of the obtained calibration curves (see Figure 6). The highest sensitivity (slope of the line) of calibration curve 4 results in the lowest residual parameter. The lowest sensitivity obtained for calibration curve 2 and calibration curve 1 produced the highest values for the respective residual parameters. As mentioned before, the absorbance of a biodiesel at approximately 450 nm is determined by the amount of unsaturated bond systems with a

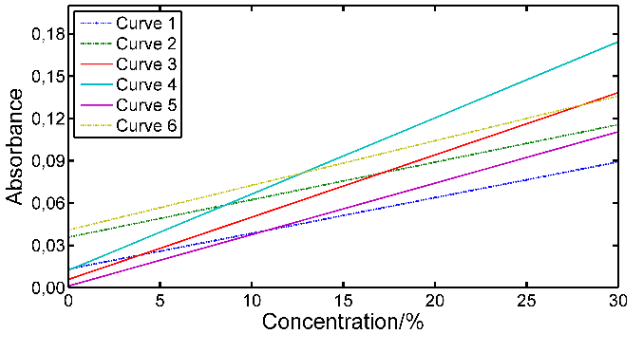


Figure 6. Calibration curves for the studied combinations of pure diesel and biodiesel components.

certain number of double bonds in conjugation (11 in the case of β-carotene) [10] present in a biodiesel. The reduction of the amount of such systems can be an indicative of the overall degradation of oxidative resistance of biodiesel by the transformation of unsaturated C=C bonds of esters in biodiesel as well [11]. The consequence of a reduced absorbance of a pure biodiesel used in the construction of calibration curves is a reduced sensitivity of the respective calibration curve.

## 4. CONCLUSIONS

The study of UV-Vis spectroscopy of diesel-biodiesel blends in the wavelength region between 400 nm and 500 nm showed that the quantification of biodiesel concentration in the blends, which is based on the absorbance at around 450 nm, strongly depends on two main factors. The first one is related to a dye, which is added compulsorily to the S500 type of diesel, and is not present in the S10 type. The second factor is defined by the origin of the pure biodiesel used for the construction of a calibration curve.

The differences in the number of systems with unsaturated bonds, such as polyenes, present in a pure biodiesel component used for the calibration curve definition, will affect the sensitivity of the quantification method and, as a consequence, will influence its accuracy. Nevertheless, a standardisation of the amount of dye in diesel and of naturally or artificially present antioxidants, such as carotenoids, in biodiesels may offer an important feature to the method. Such a standardisation may not only provide better accuracy for the quantification of biodiesel in diesel blends, but it will also make the method selective to the type of diesel blend (S500 or S10), as well as potentially sensitive to the oxidation stability of the diesel.

## AUTHORS' CONTRIBUTION

Simone de Britto: investigation, data curation, methodology, analysis, validation, writing (original draft).

Erlon H. Martins Ferreira: conceptualisation, formal analysis, methodology, software, writing (review and editing).

Daniel Giacometti Amaral: conceptualisation, funding acquisition, project administration.

Oleksii Kuznetsov: conceptualisation, funding acquisition, methodology, resources, supervision, writing (review and editing).

## REFERENCES


[1] Agência Nacional de Petróleo, Gás natural e Biocombustível (ANP). "Estabelece, consoante às disposições contidas no Regulamento Técnico ANP nº 8/2009, as especificações do óleo diesel de uso rodoviário, para comercialização pelos diversos agentes econômicos em todo o território nacional", Resolução nº 42 de 16/12/2009 (DOU 14/01/2010) [In Portuguese]

[2] Agência Nacional de Petróleo, Gás natural e Biocombustível (ANP). "Regulamenta as especificações do óleo diesel de uso rodoviário, contidas no Regulamento Técnico ANP nº 4/2013, e as obrigações quanto ao controle da qualidade a serem atendidas pelos diversos agentes econômicos que comercializam o produto em todo o território nacional". Resolução nº 50, 23/12/2013 (DOU 24/12/2013). [In Portuguese]

[3] M. D. Scherer, S. L. Oliveira, S. M. Lima, L. H. Andrade, A. Caires, Determination of the Biodiesel Content in Diesel/Biodiesel Blends: A Method Based on Fluorescence Spectroscopy. J. Fluoresc 21 (2011) 3, pp. 1027–1031. DOI: 10.1007/s10895-010-0815-x

[4] L. L. N. Guarieiro, A. C. Pinto, P. A. Aguiar, N. M. Ribeiro, Determination of biodiesel percentage in biodiesel:diesel mixtures using mid-infrared spectroscopy. Quim Nova 31 (2008), pp. 421–426.

[5] A. Zawadzki, D. S. Shrestha, B. He, Biodiesel blend level detection using ultraviolet absorption spectra, Trans ASABE 50 (2007), pp. 1349–1353.

[6] F. Chen, G. M. Brown, M. Song, Overview of 3-D shape measurement using optical methods, Optical Engineering 39(1) (2000). DOI: 10.1117/1.602438.

[7] EN 14078:2014 Liquid petroleum products. Determination of fatty acid methyl ester (FAME) content in middle distillates. Infrared spectrometry method.

[8] Agência Nacional do Petróleo, Gás Natural e Biocombustíveis (ANP). "Estabelece a obrigatoriedade de adição de marcador a solventes e a derivados de petróleo eventualmente indicados pela ANP bem como a proibição da presença de marcador na gasolina". Portaria 274 de 01/11/2001 (DOU 05/11/2001). [In Portuguese]

[9] According to the regulation, the marker in biodiesel S500 must be a liquid with a density varying from 990 kg/m$^3$ to 1 020 kg/m$^3$, and an absorbance between 0,6 and 0,7 in the range from 520 nm to 540 nm. Source: Agência Nacional do Petróleo, Gás Natural e Biocombustíveis (ANP), Resolução nº 968 de 30/04/2024. [In Portuguese]

[10] S. Kumar, Spectroscopy of organic compounds, National Science Digital Library at NISCAIR, New Delhi, 2006.

[11] P. Grabowski, D. Tomkielski, P. Szajerski, H. Gwardiak, Changes of biodiesel composition after electron beam irradiation, J. Radioanal Nucl. Chem. 319 (2019), pp. 727–736. DOI: 10.1007/s10967-018-6364-5