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Fluorescence as an Analytical Tool for Assessing the Conversion of Oil into Biodiesel

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Abstract In this work fluorescence–based method to assess the biodiesel production from different refined vegetable oils is presented. Four different refined oils (soybean, sunflower, canola, and corn) and their respective biodiesel were used and the fluorescence of the compounds contained in their compositions was taken as a probe. The results show that the fluorescence intensity of the biodiesel is lower than one verified in the vegetable oil. The data achieved point out that the ratio between the fluorescence intensity of biodiesel and oil is about 0.6 regardless of the vegetable oil feedstock investigated. Reduced content of fluorophores as well as low viscosity of the biodiesel regarding the oil have been raised as hypotheses to explain the low fluorescence intensity of the biodiesel. The results obtained may provide the basis for the development of an alternative method able to give fast and accurate information about the conversion of oil into biodiesel without the requirement of dilution or pretreatment of the biodiesel.

Keywords Biodiesel · Vegetable Oil · Fluorescence · Transesterification

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Introduction

The need for developing alternative fuels with reduced environmental impact has become gradually more necessary. In this worldwide scenario, biodiesel emerges as a viable alternative fuel to be used in diesel cycle engines because its use during the operation of compression ignition engines produces less emission of carbon dioxide than petroleum diesel [1] and almost no gases containing sulfur compounds are released into the environment. Additionally, biodiesel has a high cetane number and good stability against storage and transportation [2–4].

Biodiesel is composed of alkyl esters that can be obtained from edible and non edible oils as well as animal fats [5] by means of transesterification of fatty alcohol in the presence or absence of catalyst [6]. More than 95 % of the oils and fats are made up of triglycerides, which are esters formed from one glycerol molecule and three fatty acids. Oils are insoluble in water and may be either solid or liquid at room temperature depending on the feedstock. Besides triglycerides, fats and oils may contain small amounts of mono and diglycerides (emulsifiers), free fatty acids, tocopherols (antioxidants), sterols, and fat soluble vitamins [7].

The production of biodiesel on an industrial scale has been based on the transesterification of triglycerides with methanol in the presence of an alkaline catalyst. During the transesterification process, one molecule of triglyceride reacts with three molecules of alcohol to produce one molecule of glycerin and three molecules of alkyl esters from the consecutive reactions that convert triglyceride into diglyceride, then into monoglyceride and finally into the last alkyl ester molecule and glycerin [8]. The transesterification is a reversible reaction so that the alcohol has to be added in excess in order to shift the balance of the reaction, compelling the formation of the alkyl esters and glycerin.

The chemical composition and concentration of fatty acids in oils play an important role in the biodiesel production. Structural differences such as unsaturation level and chain length lead to different viscosity and density values as well as distinct rates of conversion of vegetable oil into alkyl esters. Further, high levels of acidity and water in the reaction medium containing alkaline catalyst lead to soap formation during the transesterification process [9]. The soap formation is undesirable because it consumes a fraction of the catalyst, decreases the conversion yield of the oil into biodiesel and makes difficult the separation and purification of the biodiesel [10].

The final product of the transesterification may contain, besides alkyl esters and glycerin contaminants such as mono−, di− and triglycerides as well as catalyst and residual alcohol. Such impurities can affect the operation of the diesel engine and have an unwanted environmental impact, thus also compromising the application of biodiesel as an efficient and environmentally friendly energy source [11]. Aiming to assess the fuel production and quality, spectroscopic and chromatographic methods have been used to evaluate chemical and physical properties of biodiesel [12]. For instance, the alkyl esters formation is evaluated following the EN14103 protocol which is based on Gas Chromatography with Flame Ionization Detector (GC−FID). Viscosity measurements have also been used to verify the formation of biodiesel because as higher the content of alkyl esters lower is the viscosity of the biodiesel [13].

In the last years, a variety of oil plants has been used for biodiesel production due their high productivity and/or low requirement with respect to soil and climate. Hence there is a need for developing robust analytical methodologies capable of assessing the quality standards of the biodiesel obtained from different raw materials. Furthermore, such analytical methods need to combine low cost, fast, and accurate results as well as portability allowing biodiesel analysis either in the biodiesel plants or gas stations.

Spectroscopic techniques have also been used in the study of different fuels and have proven to be effective because they usually deliver results in a quick and precise way as well as they can be used easily in laboratory analysis or remote environments. Techniques based on absorption spectroscopy in the ultraviolet−visible−infrared spectral region (UV−Vis−IR), fluorescence and Raman spectroscopy have been successfully applied in the study of diesel [14–16], gasoline [17], alcohol fuel [18], biodiesel [19, 20], and fuel blends [21].

To illustrate, Fourier transform mid-infrared (FT–IR) spectroscopy has been successfully used both to indicate the formation of alkali esters and quantify of biodiesel in diesel−biodiesel blends (DBB) [20–23]. In both cases the infrared absorption around 1746 cm^{-1} assigned to molecular vibration of C=O bonds in biodiesel is used as a probe because it is red-shifted after conversion of the oil into

biodiesel and petroleum-derived diesel does not contain C=O bonds in its composition. Despite the good results obtained by FT−IR spectroscopy, some challenges must be overcome such as the possibility of obtaining misleading results due to the presence of contaminants containing C=O bonds and the difficulty of applying this methodology directly at gas stations.

Here, an analytical tool based on fluorescence spectroscopy that allows the monitoring of the conversion of vegetable oils into biodiesel is proposed and it was applied to evaluate the transformation of four different oils into their respective biodiesel. The method takes into account the reduction of the fluorescence of biodiesel as compared to the one observed in the starting oil. The possible mechanisms that could lead to such fluorescence decrease are discussed.

Experimental

Soybean, sunflower, canola, and corn biodiesel were produced via alkaline transesterification of the respective refined oils of same brand using sodium hydroxide as alkaline catalyst (0.4 wt.% with respect to oil) and 6:1 molar ratio of methanol/oil. The sodium methoxide was first prepared by dissolving the catalyst in methanol and then poured into a beaker containing oil preheated at 60 °C. The reaction was performed under constant stirring during 60 min at 60 °C. The reaction time was recorded from the addition of the oil to the alkoxide. At the end of the reaction, the crude product was allowed to stand in separating funnels for 24 h and two phases were observed, one containing mainly biodiesel and other consisting of glycerol and residues. Both phases were separated, and the excess of methanol in the phase rich in methyl ester was removed by rotary evaporation under reduced pressure during 1 h at 70 °C. Afterward, the biodiesel was again placed in separating funnels and washed three times with distilled water (3:1, v/v) at room temperature. Finally, the biodiesel samples were filtered through anhydrous sodium sulphate to eliminate traces of water. In contrast, the glycerin samples used in this investigation were not purified so that they should contain impurities such as methanol and sodium hydroxide. Biodiesel and glycerin were produced in triplicate from the respective oils. Accordingly, fluorescence (emission and excitation) and mid−infrared absorption measurements were done in 36 samples.

Emission and excitation spectra of the oils and their respective biodiesel and glycerin samples were obtained without any dilution in solvents using a bench fluorescence spectrophotometer (Cary Eclipse, Varian). This system is based on two monochromators, a photomultiplier, and a Xenon pulsed lamp. Fluorescence was measured at a 90°−angle relative to the excitation light (right-angle geometry). In the emission measurements all samples were excited at 350 nm and the

fluorescence was collected in the 370–800 nm range. In its turn, the excitation measurements were performed monitoring the fluorescence of the samples at 430 nm and the excitation wavelength was scanned in the spectral region between 250 and 420 nm. All emission and excitation spectra were recorded at room temperature using 1–cm path length quartz cells. Mid-infrared absorption spectra were carried out using a FT−IR spectrophotometer (Nexus 670, Thermo Nicolet) along with an attenuated total reflectance accessory at room temperature. The spectrophotometer was purged with dry air to eliminate water vapor during the experiments. The spectra were obtained between 4,000 and 400 cm^{-1} with a 2–cm^{-1} resolution.

Results and Discussion

Figure 1 shows the mid-infrared absorption spectra of the oils and their respective biodiesel samples. The molecular structures of oil and biodiesel molecules are similar so that the vibrational modes of the units and their respective frequencies lead to the appearance of absorption bands spanning the same spectral ranges. The curves indicate that the presence of water molecules is negligible since it is not observed an absorption band in the 3000–4000 cm^{-1} range. Apart from the similarity of the spectra, it is possible to notice a change in the vibrational frequency of the absorption band assigned to the symmetric stretching of carbonyl groups (−C=O) from about 1743 cm^{-1} in the oil samples towards 1741 cm^{-1} in the related biodiesel. This redshift is

expected because of the replacement of the glycerol groups by −O−CH_3 units in consequence of the transesterification [20]. Therefore, the data suggest the effectiveness of the biodiesel production method used in this investigation.

The emission spectra of the different samples of refined oil, biodiesel, and glycerin under excitation light at 350 nm are presented in Fig. 2. These spectra were achieved taking the average over three spectra of the biodiesel, oil, and glycerin samples and they were not corrected for the inner filtering effects related to the optical densities [24]. Fluorescence was observed from the biodiesel and oils in the spectral range between 370 and 700 nm, while the fluorescence from the glycerin samples was detected in a slightly different and narrow spectral region (400–600 nm). Despite the fact that the oils and biodiesel samples exhibit fluorescence spectra with similar profile, the fluorescence intensity of the oils is higher than one detected in the corresponding biodiesel.

Researchers have attributed the fluorescence observed in oils to components such as tocopherols and free fatty acids which are present in vegetable oils along with triglycerides and other compounds in lower concentration [25–27]. As the fluorescence in the biodiesel and oils investigated occurs in the same spectral region and the spectral profiles are quite similar it is plausible to consider that the fluorophores present in the oil remained in the biodiesel after transesterification and consequently they could contribute for the emission observed in the biodiesel samples. Here, the contribution of free fatty acids for the fluorescence of the samples is negligible provided that there is no broad absorption band in the 3300–2500 cm^{-1} range (Fig.1), which is characteristic of the stretching of

Fig. 1 FT−IR spectra of the vegetable oils and respective biodiesel samples
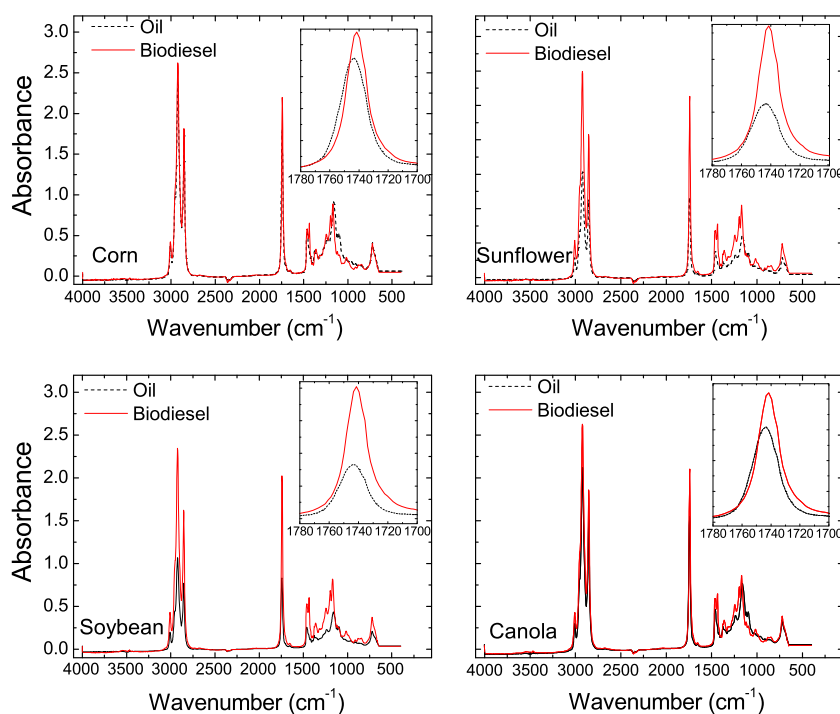
Fig. 2 Fluorescence spectra of the oil, biodiesel and glycerin obtained from the different oils under light excitation at 350 nm. The average deviation from the mean value is indicated by error bars
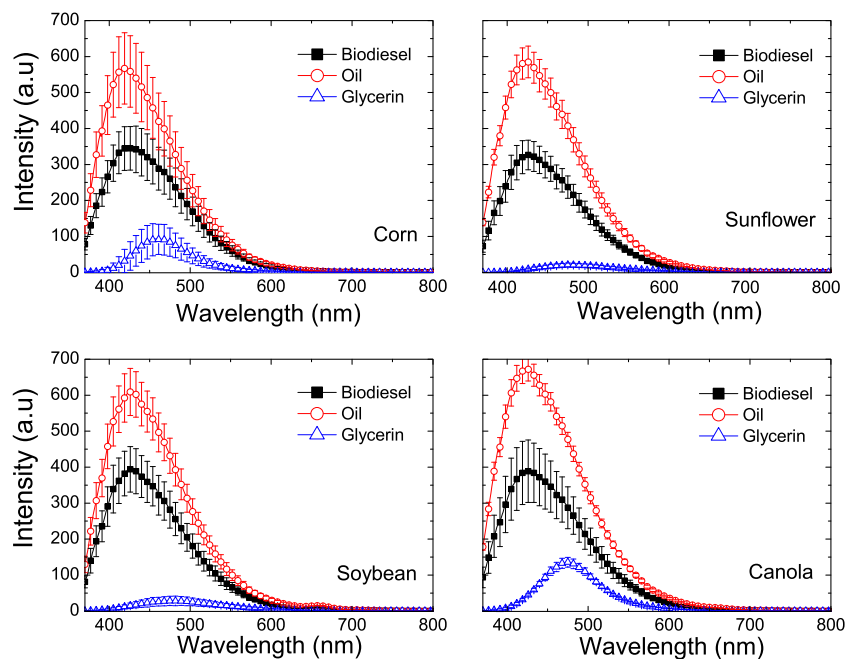

the R-COOH group in free fatty acids [28]. Besides, fluorescence due to fatty-acid methyl esters may not be ruled out so that investigations are underway in order to identify the species responsible for the fluorescence in biodiesel.

The excitation spectra measured at the emission wavelength of 430 nm for oil, biodiesel, and glycerin samples are shown in Fig. 3. Fluorescence at this wavelength was detected when the oil and biodiesel samples were excited from 300 to 420 nm, whereas the maximum fluorescence

intensity was induced by excitation at about 358 nm. The spectral similarities corroborate the statement that the same fluorophores are present in both the oils and biodiesel. At the same time, it is not possible to rule out that such emitters also contribute for the fluorescence observed in the glycerin samples. The unpurified glycerin is darker than the related oil and biodiesel indicating that the absorbance at the excitation wavelength is higher than one verified in oils and biodiesel. As a result, lower emission intensity and different

Fig. 3 Excitation spectra of the oil, biodiesel and glycerin measured at the emission wavelength of 430 nm. The error bars indicate the average deviation from the mean value
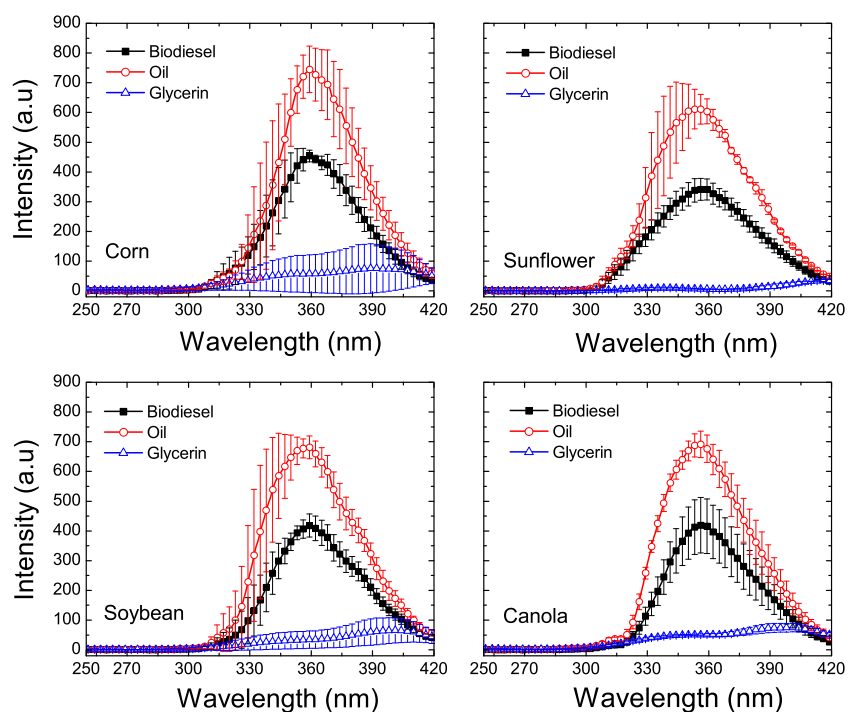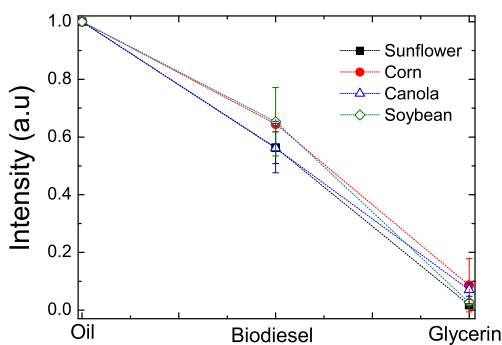
Fig. 4 Fluorescence intensity of the oils, biodiesel and glycerin at 430 nm normalized by the fluorescence intensity of the respective vegetable oil. The error bars indicate the average deviation from the mean value

emission and excitation spectral profiles are expected (Figs. 2 and 3) [24].

Figure 4 displays the emission intensity of oil, biodiesel, and glycerin at 430 nm normalized by the intensity of the related oil when excited at 350 nm. A decrease of about 40 % in the fluorescence intensity of the oil is evident after transesterification and purification of the biodiesel. Another fact that also draws attention is that similar reduction factors were observed in all biodiesel samples regardless the vegetable oils used in their production.

The drop in the fluorescence, which is also reflected in the excitation spectra (Fig. 3), could not be attributed to a reduction, for instance, of the fluorophores in biodiesel as a consequence of the washing process adopted after transesterification. Indeed, the fluorescence spectra of biodiesel and oils do not exhibit any noteworthy alteration as a function of several washes (data not shown). On the other hand, the fluorescence diminution may have to do with the separation of the glycerin from the triacylglycerides. Following transesterification, the glycerin being denser settle to the bottom of the separating funnel bringing with it, apart from methanol and catalyst, mono–, di–, unreacted triglyceride, and fluorophores which were diluted in the oil. In view of that, fluorescence of the biodiesel should be lower than one detected in respective vegetable oil.

An alternative explanation for such a reduction in fluorescence can be derived taking into account the viscosity of the environment in which the emitters are diluted. It has been reported that the predominant deexcitation pathway of some fluorophores might depend on the viscosity of the host media [24, 29–31]. In highly viscous media the nonradiative relaxation of such molecules is restricted so that the radiative decay pathway plays a major role. On the other hand, an enhancement of nonradiative relaxation mechanisms has been observed in fluorophores hosted in environment with low viscosity because such molecules in the excited-state may undergo, for instance, internal rotation or collisions with other molecules (quenchers) in the medium.

Consequently, a decrease in the fluorescence quantum yield of the fluorophores should be detected. It is well known that one of the advantages of biodiesel with respect to the oils is its lower viscosity which improves its atomization in the fuel spray as well as reduces the formation of deposits, coking, and clogging of engine components such as injectors and valves. Thus, the high fluorescence intensity of the oil regarding to the biodiesel could be associated to difference of viscosity of the two media. The fluorophores contained in the oils would return to the ground state mainly by means of the radiative mechanism since nonradiative mechanisms such as internal rotation and collisional quenching would be limited due to restricted surroundings. Further investigations are in progress in order to evaluate if the hypothesis raised above are indeed responsible for the difference between the fluorescence intensities of the refined vegetable oil and respective biodiesel.

Mid-infrared spectroscopy together with multivariate analysis [20], near-infrared spectroscopy [32], and ^{1}H nuclear magnetic resonance spectroscopy [33] has been pointed out as useful methods for evaluate the conversion of oil into biodiesel. In this scenario, the finding that the fluorescence of the biodiesel is appreciably reduced with regard to the vegetable oil source may provide the basis for the developing of an alternative method able to give fast and accurate information about the conversion of vegetable oils into biodiesel without the requirement of dilution or pre-treatment of the biodiesel. Besides, a low-cost portable fluorescence device operating in the UV−Vis region and adopting right-angle geometry could be developed to be used in laboratory analysis as well as in rapid monitoring in biodiesel plants.

Conclusion

In summary, the results pointed out that the fluorescence from biodiesel is lower than one observed in the corresponding refined vegetable oil source. The fluorescence from the oils as well as biodiesel has been assigned to similar emitters inherent to their compositions. The finding reveals that the comparison of the fluorescence intensity of biodiesel and vegetable oil may be used to assess the production of biodiesel. In this work, it was determined that the ratio between the fluorescence of the biodiesel and oil is about 0.6 regardless of the vegetable oil. Decrease of the content of fluorophores in biodiesel due to transesterification and purification of the biodiesel as well as the low viscosity of the biodiesel, which would lead to activation of nonradiative decay pathways, have been raised as hypotheses to explain the low fluorescence intensity of the biodiesel regarding to the vegetable oil. Although the fluorescence-based method described here so far does not provide quantitative data about the formation of methyl esters in contrast to the NMR and CG−FID techniques,

it provides a reliable indicative parameter of ending of the biodiesel production. Therefore, the results here reported may be useful in the development of an alternative method able to delivers data about the biodiesel production in a quick and precise way and to be easily used in laboratory analysis or in biodiesel plants.

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