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CROMATOGRAFIA EM CAMADA FINA COMO UMA TÉCNICA INOVADORA PARA A CARACTERIZAÇÃO DA PRODUÇÃO DE BIODIESEL POR ESTERIFICAÇÃO

THIN LAYER CHROMATOGRAPHY AS INNOVATIVE TECHNIQUE FOR QUALITATIVE CHARACTERIZATION OF BIODIESEL PRODUCED BY ESTERIFICATION

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Resumo

O objetivo deste trabalho foi avaliar o uso da técnica de cromatografia em camada fina (thin layer chromatography -TLC), utilizando o método de visualização do vapor de iodo, para identificação e caracterização preliminar do biodiesel produzido por esterificação. A reação de esterificação foi realizada a uma temperatura constante de 65 °C durante 2 horas, utilizando dimetilsulfato como

agente de alquilação e ácido dodecanoico como matéria-prima oleaginosa. A placa cromatográfica foi submetida ao método de revelação depois de adicionar 5 µL de uma amostra de ácido graxo e biodiesel na cuba cromatográfica. A indicação da formação de biodiesel foi obtida calculando o fator de retenção (Rf) e validada por ressonância magnética nuclear de hidrogênio (H^1NMR). Os valores de Rf e da região de picos de funções químicas foram idênticos aos encontrados em outros estudos, o que permitiu confirmar a formação de biodiesel. Pode-se concluir que a TLC pode ser utilizada como técnica inovadora para a caracterização qualitativa preliminar da formação de biodiesel por esterificação.

Palavras-chave: inovação, ácido dodecanóico, dimetilsulfato, biodiesel, esterificação, cromatografia camada fina.

Abstract

The objective of this work was to evaluate the use of the thin layer chromatography technique (TLC), using the iodine vapor visualization method, for identification and preliminary characterization of biodiesel produced by esterification. The esterification reaction was performed at constant temperature of 65 °C for 2 hours, using dimethyl sulfate as alkylating agent and dodecanoic acid as oleaginous raw material. The chromatographic plate was subjected to the method of revelation after adding 5 µL of a sample of fatty acid and biodiesel on the chromatographic vessel. The indication of biodiesel formation was obtained by calculating the retention factor (Rf) and validated by hydrogen nuclear magnetic resonance (H^1NMR). The Rf values and of the region of peaks of chemical functions were identical to those found in other studies, which allowed to confirm the formation of biodiesel. It can be concluded that TLC can be used as an innovative technique for preliminary qualitative characterization of biodiesel formation by esterification.

Keywords: inovation, dodecanoic acid, dimethyl sulfate, biodiesel, esterification, thin layer chromatography.

1. Introduction

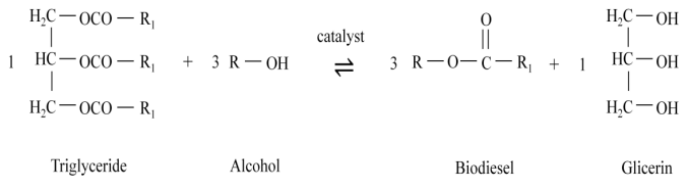
Diesel is one of the most consumed car fuels in the world (BASKAR e AISWARYA 2016), however, it is obtained from a nonrenewable fossil energy source (oil). Several reasons encourage research on alternative fuels, particularly renewable ones: the nonrenewable nature of diesel, climate change, the harmful effects caused to the environment by the use of diesel as higher emissions of sulfur, monoxide and carbon dioxide and particulate matter, as well as the trend of gradual depletion of fossil fuels and the current political-economic-social actions for sustainable development (CANESIN et al. 2014; HALEK, DELAVARI e KAVOUSI-Rahim, 2013; OLLIS, LIU e Stevenson 2012).

Research conducted in recent years have shown biodiesel as a potential substitute for petroleum diesel fuel, because it has similar physical and chemical characteristics. However, biodiesel is more advantageous because it is biodegradable and non-toxic, and has a low-emission

profile compared with diesel (ÖZENER et al, 2012; Qi et al,. 2009; F. XUE et al., 2006). The main environmental benefits of using biodiesel as an alternative vehicle biofuel compared with diesel is the significant reduction of greenhouse gases (such as carbon monoxide and carbon dioxide), particulate materials, hydrocarbons, aromatic compounds and sulphates, which result from emissions of diesel cycle engines (BUYUKKAYA, 2010; COGGON, VASUDEVAN e SANCHEZ 2007; DEMIRBAS, 2009; J. XUE, GRIFT, e HANSEN 2011). In addition, biodiesel has high combustion efficiency, high cetane number, ability to mix with diesel under various percentages without need for modification in diesel engines and absence of aromatic and sulfur compounds, the latter of which are responsible for corrosion of engines (ATADASHI, AROUA e AZIZ 2010; DATTA e MANDAL, 2016; GAUTAM, GUPTA e SHARMA, 2014; HALEK, DELAVARI e KAVOUSI-Rahim 2013).

The global appeal to renewable and "eco-friendly" technologies and fuels favors the use of biodiesel, thus making it a consolidated alternative to compose the global energy matrix (ARANSIOLA et al.,2014). Also, the production of biodiesel is favored, by the instability of oil prices, the ease of transport and by knowledge of highly efficient technologies for industrial production (Ng, Hoon, and Gan 2010). Biodiesel consists of fatty acid alkyl esters (FAAE), obtained mainly by the transesterification reaction of an oleaginous raw material or animal fat with a short-chain alcohol (usually methanol), catalyzed by chemical or biological reagents, generating glycerol as a byproduct (BELTRÁN-PRIETO, KOLOMAZNÍK e PECHA 2013; BHARATHIRAJA et al,. 2014; ELMS e EL-HALWAGI 2010; HALEK, DELAVARI e KAVOUSI-RAHIM 2013; MEIRA et al., 2015; OLLIS, LIU E STEVENSON, 2012). The transesterification reaction is shown in Figure 1, where R represents the alkyl group of the alcohol and R1 the carbonic chain of the triglyceride or fatty acid.

Figure 1: Transesterification reaction scheme.

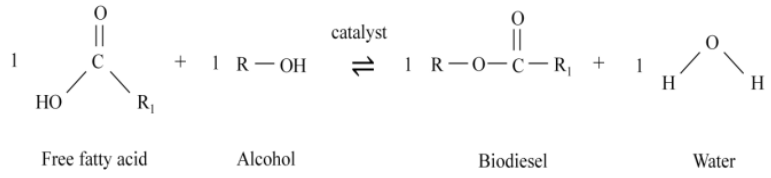


The transesterification reaction via basic catalysis is considered highly efficient and can achieve high purity and high yield of biodiesel. However, the transesterification reaction requires a high degree of purity of the raw material, mainly in terms of acidity (free fatty acids) and moisture, to prevent undesirable reactions in the process, such as saponification and hydrolysis, respectively

(CAI et al., 2015; B FREEDMAN, PRYDE e Mounts, 1984; BERNARD FREEDMAN, BUTTERFIELD e PRYDE, 1986; LEUNG, WU e LEUNG, 2010; MA e HANNA, 1999; MOREIRA, BADENES e CABRAL, 2013; TIWARI e GARG, 2016).

Thus, in situations where there is a necessary to use raw materials with a high content of free fatty acids, such as the production of biodiesel using waste frying oil, the esterification reaction becomes an alternative for biodiesel production (ALEGRÍA e CUELLAR, 2015; REZENDE e PINTO, 2016; SARAVANAN et al., 2015; YU et al., 2016). The use of waste oils can reduce biodiesel production costs, as it is a low-cost raw material when compared to vegetable oils (NEUMANN et al., 2016), it may cost up to one third of the value of straight vegetable oil (PHAN e PHAN 2008). In addition, the esterification reaction can be a step for pre-treatment of the raw material for further processing in a transesterification reaction (ADEWUYI, ODERINDE e OJO, 2012; CAI et al., 2015; MAZUMDAR et al., 2013; NEUMANN et al., 2016; URRUTIA et al., 2016). The esterification reaction scheme is shown in Figure 2, where R represents the alkyl group of the alcohol and R_1 the carbonic chain of the free fatty acid.

Figure 2: Esterification reaction scheme.



To enable the use of biodiesel in the supply of diesel-powered vehicles, high conversion is required of oleaginous or fatty raw material into fatty acid alkyl esters. In Brazil, for example, the regulatory agency is the National Agency for Petroleum, Natural Gas and Biofuel. Through Resolution nº. 07/2008, it requires a minimum percentage of 96.5 % (w/w) of esters in the sample of biodiesel (ANP 2008). The use of biodiesel fuel with a low percentage of esters may cause loss of efficiency and problems to engines, because triglycerides that are not converted can polymerize and accumulate in the lines of the engine (FERNANDO et al., 2007). Therefore, a quality control is necessary for biodiesel to meet business requirements and gain acceptance in the energy market.

The analytical techniques used more often to characterize biodiesel quantitatively and qualitatively are chromatography and spectroscopy, respectively (TARIQ, ALI R KHALID, 2012). Because of its high accuracy, chromatography has been the most widely used technique to quantify major and minor components in biodiesel samples (MONTEIRO et al., 2008). Qualitatively, infrared spectroscopy is an analytical technique that allows the determination of various properties of biodiesel samples, mainly to check the progress of reaction (ZHANG, 2012). However, although

gas chromatography and infrared spectroscopy are highly reliable, they both have large operational costs and require long times of analysis for each sample. In addition, the equipment and inputs involved in the analysis are expensive (FEDOSOV, BRASK E XU 2011). Thus, the use of less expensive techniques for the quality control of biodiesel helps to reduce the total cost of production, which is one of the major obstacles to the viability of this type of biofuel (MONTEIRO et al., 2008).

Within this context, thin layer chromatography (TLC) is considered an interesting alternative for use in preliminary analyses of qualitative characterization in biodiesel production. It allows the analysis of esters as well as tri-, di- and monoglycerides (TARIQ, ALI e KHALID, 2012). Moreover, TLC allows the determination of the purity of a compound, identification of the components in a mixture, isolation of pure components of a solution and monitoring of the progress of the reaction by the appearance of products and the disappearance of reagents (ILBEIGI et al., 2016; MARTELANC, VOVK e SIMONOVSKA 2009). The main advantages of applying TLC include ease comparison and utilization, separation of components within a short period of time, versatility and, especially, low operational costs involved in the process because it works at milder pressures and temperatures (MROCZEK et al., 2006; RISTIVOJEVIC´ et al., 2017). The process of separation of the sample components by the TLC technique is accomplished by adsorption, whereby the chromatographic plate is placed vertically in a chromatographic tank or an elution chamber, containing a small amount of eluent, which will elute the plate (layer of adsorbent) (DAWAN et al,. 2017). Various methods can be used for visualizing the revelation of the results of the analysis on the chromatographic plates. The most common use iodine vapor or ultraviolet light (UV) bulbs (COLLINS, BRAGA e BONATO, 2006). In the method of iodine vapor visualization, the iodine in the form of dust reacts with the compounds of the sample, forming brown (product) or yellowish (raw material) complexes. The advantage of the method is that the metal complexes with unsaturated compounds, while through UV, compounds appear as bright spots when they are under the action of UV light (ILBEIGI et al., 2016; MROCZEK et al., 2006). The disadvantage of the iodine vapor visualization method is the fact that the sample cannot be recovered, because a chemical reaction has occurred. The UV light visualization technique allows the sample to be regenerated, because there is no chemical reaction. However, it is even more robust than the use of iodine vapors.

Given the above, the objective of this work was to evaluate the use of thin layer chromatography, using the method of iodine vapor visualization, as a simplified qualitative assessment technique for identification and characterization of biodiesel produced by esterification.

2. Methodology

2.1 Chemicals and reagents

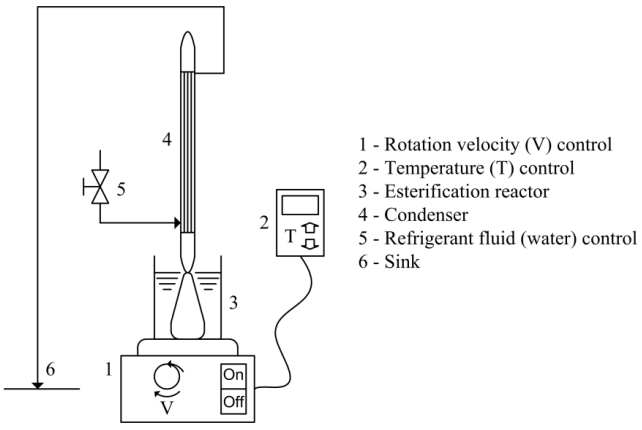
The experimental esterification reaction was performed using as raw material the dodecanoic fatty acid ($C_{12}H_{24}O_2$) (Biotechnos, Brazil). Chemical reagents used were dimethyl sulfate (analytical standard), ethanol 95% (v/v), acetic acid 99.7% (v/v) and deuterated chloroform 99.9% (w/w) (Sigma-Aldrich, USA), and iodine powder 99.5% (w/w) (Merck, Germany). The chromatographic plate was DC - Fertigfolien ALUGRAM® Xtra SIL(G) (10 cm high x 20 cm wide, 0.020 cm of silica gel 60 UV 254) (Merck, Germany).

2.2 Experiment

2.2.1 Dodecanoic acid esterification

The esterification reaction was performed based on the methodology of Machado (Machado 2013). A total of 1.03 g of the alkylating agent dimethyl sulfate ($C_2H_6O_4S$, M.W.: 126.1 $g.mol^{-1}$) was added in 10.3 g of dodecanoic acid ($C_{12}H_{24}O_2$, M.W.: 200.3 $g.mol^{-1}$) in a flat-bottomed flask kept under a mineral oil bath whose function was to keep constant the temperature of reaction. The system bath/flask was in contact with a temperature and stirring speed controller. Figure 3 describes the layout of the experimental apparatus in use.

Figure 3: Experimental apparatus used for esterification of the dodecanoic fatty acid.



The tests were performed at a constant temperature of 65 °C for 2 hours, under constant agitation of 250 rotations per minute (rpm). To avoid the loss of reagents by evaporation, a condenser was used, operating continuously with water (refrigerant). Later, the components were separated using a funnel.

2.2.2 Characterization of Thin Layer Chromatography (TLC)

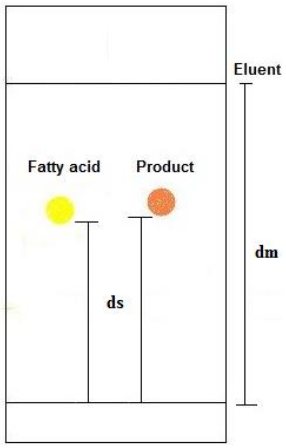
The thin layer chromatography technique was used for the characterization of the biodiesel formed, using iodine vapor for visualization and identification of biodiesel formation. The eluent was prepared as a 1:1 (v/v) solution of ethanol (C_2H_6O, M.W.: 46.07 $g.mol^{-1}$) and acetic acid (CH_3COOH, P.M: 60.05 $g.mol^{-1}$). To reveal the appearance of the product on the chromatographic plates, was used the method of iodine vapor visualization. The chromatographic plate measured 10 cm x 20 cm (height x width) with a layer of 0.020 cm of silica gel (ALUGRAM® Xtra SIL(G). The chromatographic plate was subjected to the technique of revelation by the iodine vapor method, whereby iodine powder and the chromatographic plate, after contact with the eluent, were placed in a closed container so that the iodine vapors could come into contact with the surface of the plate and reveal the run of the products of interest in the form of brown spots. The samples of fatty acid and biodiesel (5 µL per run) were deposited on the plate without any dilution, apart 1.30 cm from one another and 1 cm away from the bottom edge of the plate.

The procedure was performed in triplicate, and the average of the experimental data was calculated. The indication of biodiesel formation was obtained through a retention factor (Rf), calculated by Equation (1):

$$Rf = \frac{ds}{dm} \quad (1)$$

where *ds* is the measured value of the distance traveled by the sample and *dm* is the measured distance traveled by the solvent when adsorbed and drawn up by capillarity to come into contact with the iodine vapor, on the chromatographic plate. The displacements of the formed product and of the dodecanoic fatty acid were obtained by simply measuring the distance traveled in the chromatographic plate, revealed by the iodine vapors as showed in Figure 4.

Figure 4: Distance traveled by the reactant and product samples on the thin layer chromatographic plate.



2.2.3 Characterization of Hydrogen Nuclear Magnetic Resonance (H^1NMR)

As an alternative to validate the characterization of biodiesel produced in the experiment, the technique of hydrogen nuclear magnetic resonance (H^1NMR) was used. The H^1NMR equipment was a Varian Inova 300 system (Palo Alto, USA), using a spectrometer frequency of 300 MHz, at a temperature of 22 °C with a 2-pulse sequence and using chloroform as a solvent.

3. Results and discussion

3.1 Thin Layer Chromatography (TLC)

The distance traveled by the samples was observed on the thin layer chromatographic plate after the deposit of the sample reagent (dodecanoic fatty acid) and of the product generated by the esterification reaction.

The distance traveled by the eluent was 4.2 cm while the distances traveled by the dodecanoic fatty acid and the product were 2.5 cm and 2.6 cm, respectively. In addition, the reagent showed a yellowish color and the product, a brown color. Based on the measurement information for the distance traveled by the samples, the retention factor was calculated for each substance.

The retention factor (Rf) obtained for feedstock (fatty acid dodecanoic) and product (biodiesel) was 0.59 and 0.62, respectively. The value of the retention factor of the product must be greater than that of the raw material so that the values found by the technique can be considered satisfactory and indicative of biodiesel formation (FROEHNER, LEITHOLD e LIMA, 2007). Ferrari et al. (2005) used the thin layer chromatography technique to qualitatively assess the transesterification reaction of pre-treated soybean oil and the qualitative validation of the results was performed using the chromatography technique. Other authors have used thin layer

chromatographic as a way to qualitatively characterize biodiesel production processes, as shown in Table 1.

Table 1: Retention factors using the TLC technique.

Feedstock	Reation	Retention Factor (Rf)		Reference
		Feedstock	Product	
Refined soybean oil	Transesterification	0.40	0.82	Froehner et al. (Froehner, Leithold, and Lima 2007)
Pretreated soybean oil	Transesterification	0.44	0.70	Ferrari et al. (Ferrari, Oliveira, and Scabio 2005)
Crude sunflower oil	Transesterification	0.80	0.90	Souza et al. (Souza, Ferrari, and Scabio 2006)
Waste cooking oil	Transesterification	-	0.82	Cubas et al. (Cubas et al. 2016)
Fatty acid dodecanoic	Esterification	0.59	0.62	This study

Table 1 show that all studies used TLC to characterize the formation of biodiesel produced by transesterification, while the present study used the esterification reaction. The results showed that the Rf values presented similar behavior be both the esterification and the transesterification reactions, i.e., the Rf for product must be higher than for feedstock to evidence biodiesel formation. In addition, the results of this study were satisfactorily similar to the results obtained by Ferrari et al. (Ferrari, Oliveira, and Scabio 2005). This is an evidence of the applicability of TLC for qualitative characterization of the biodiesel produced by esterification.

3.2 Hydrogen Nuclear Magnetic Resonance (H^1NMR)

The application of the hydrogen nuclear magnetic resonance of (H^1NMR) technique was performed as a form of complementary characterization of biodiesel formed as a product of the esterification of dodecanoic acid. In addition, it validates the results found by applying TLC techniques. The spectrum generated by H^1NMR is shown in Figure 5.

Figure 5: H^1NMR spectrum of esterification for the dodecanoic acid.

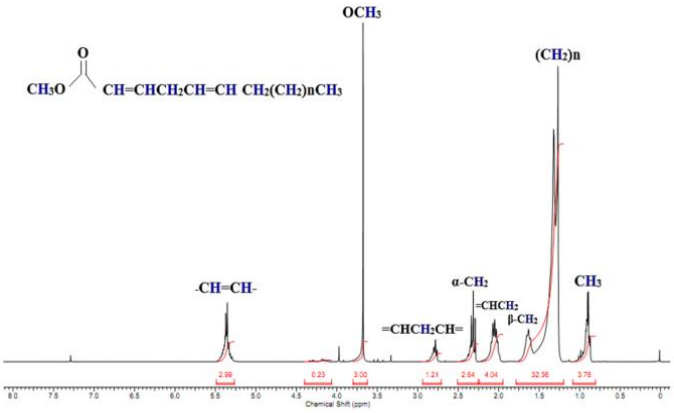


Figure 5 indicates the allocation of signals to the hydrogen nuclei, where the peak at the triplet at 0.9 ppm refers to three hydrogens of methyl ester. The peak between 1.3 and 1.5 ppm is assigned to hydrogens of the methyl groups (CH_2) from the aliphatic chains and the peak between 1.5 and 1.7 ppm is assigned to hydrogens bound to the β-carbon of the carbonyl group. The peak between 1.9 and 2.1 ppm is assigned to hydrogens of the CH_2 group neighboring the aliphatic chains, and the peak between 2.2 and 2.4 ppm is assigned to hydrogens of the α-carbon of the carbonyl group. The peak at 3.6 ppm refers to OCH_3 group and peak at 5.4 ppm corresponds to hydrogens of the CH=CH group of biodiesel. Based on the characterization of the peaks attributed to the hydrogen signals, the biodiesel molecule was formed by the reactive system.

The studies of Satyarthi et al. (Satyarthi, Srinivas, and Ratnasamy 2009), Kollar (Kollar 2012) and Shimamoto and Tubino (Shimamoto and Tubino 2016) used the H^1NMR technique for characterization of biodiesel and the H^1NMR spectra they found were similar to the one found in the present study. The spectrum obtained by Shimamoto and Tubino (Shimamoto and Tubino 2016), for the biodiesel produced from the transesterification of soybean oil, it highlights the typical signal of the methyl group represented at the peak of 3.49 ppm which is satisfactorily similar to the one found in the spectrum of the present study, represented at the peak of 3.76 ppm in Figure 5.

Kollar (Kollar 2012) used the H^1RMN analysis to characterize the biodiesel produced by the esterification of fatty acids catalyzed by commercial alumina, where signs of aliphatic chains were found between 2.78-2.67 ppm for the =CH-CH_2-CH= group, 2.30 ppm for α-CH_2, 2.12-1.96 for =CH-CH_2, 1.42-1.22 ppm for $(CH_2)_n$. and 3.67 ppm for the OCH_3 group, near the regions of peaks obtained in this study.

Based on the comparison of the signals δ (ppm) of the spectra of Shimamoto and Tubino (Shimamoto and Tubino 2016) with those of Figure 5, it can be concluded that biodiesel formation

occurred through the reaction of esterification of dodecanoic acid performed in this study. The results obtained for the factors (Rf) of raw material and product were validated through the H^1RMN spectrum, confirming the possibility of using TLC as a simple technique for qualitative characterization of biodiesel produced by esterification.

Since the TLC is a technique for qualitative characterization, it is cannot be claimed that there has been total conversion of dodecanoic fatty acid into biodiesel. Also, the peaks situated between 3.0-3.5 ppm, at 4.0 ppm and between 7.0-7.5 (Figure 5) are not intrinsic to the hydrogens the biodiesel molecule, this can be indicative of possible traces of fatty acid from the reaction system. The lack of full conversion into biodiesel may be related to possible variations of the experimental system and, especially, the purity of the raw material.

4 Conclusions

The fact that biodiesel is an alternative fuel to diesel for use in diesel cycle engines requires that the biodiesel should meet the quality standards for its physical and chemical properties. Also, characterizing biodiesel formation by using qualitative techniques, such as infrared spectroscopy, requires high technical knowledge, long time of development of experimental analysis and financial investment. Thin layer chromatography is an alternative technique, because it is cheaper, simpler and faster, and it can result in preliminary characterization of biodiesel formation in order to monitor the development of the industrial process.

The results found for the retention factor (Rf) of the raw material and of the formed product were 0.59 and 0.62, respectively, and follows the pattern of other studies that use the transesterification reaction. This study results can be considered satisfactory when compared to the values found by other studies cited. In addition, the peaks of the resulting chemical functions in the H^1RMN technique are in regions close to the regions identified by other authors, which allowed to confirm biodiesel formation.

Finally, it can be said that TLC can be used as an innovative technique for preliminary qualitative characterization of biodiesel formation by esterification.

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