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Apêndice 1: artigo aceito e publicado online:

Determination of Trace Elements in Vegetable Oils and Biodiesel by Atomic **Spectrometric Techniques-A Review**

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To cite this Article: Lepri, Fábio G., Chaves, Eduardo S., Vieira, Mariana A. , Ribeiro, Anderson S., Curtius, Adilson J., De Oliveira, Lígia C. C. and De Campos, Reinaldo C. (2011) 'Determination of Trace Elements in Vegetable Oils and Biodiesel by Atomic Spectrometric Techniques-A Review', Applied Spectroscopy Reviews, 1, doi: 10.1080/05704928.2010.529628, First posted on: 27 January 2011 (iFirst).

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Abstract

The determination of trace elements in edible oils and biodiesel using atomic spectrometric methods is reviewed. Problems related to sample pretreatment for appropriate sample introduction and calibration are addressed as well as the strategies to overcome them. Recent trends aimed at simplifying sample manipulation are presented. The applications and scope of AAS, F OES, ICP OES and ICP MS techniques for the determination of trace metals in edible

oils and biodiesel is discussed, as well as some present instrumental new developments.

Keywords: trace elements, vegetable oil, biodiesel, atomic spectrometry techniques

1. Introduction

The possibility of using vegetable oils as motor fuel has been recognized since the beginning of diesel engines (1). Vegetable oil is a potentially inexhaustible source of energy with an energetic content close to diesel fuel and can be used when mixed with diesel fuels. Pure vegetable oil, however, cannot be used in direct-injection diesel engines. The vegetable oils which shows adequate calorific power as engine fuels are all extremely viscous (about 11-17 times more than diesel fuel), turning their direct use in fuel engines problematic. This high viscosity causes poor nebulization of the fuel in the engine's combustion chambers and ultimately results in operational problems, such as engine deposits (2). In order to avoid the problems due to this high viscosity, some chemical or physical modifications have been tested: (i) the pyrolysis or cracking of the vegetable oil, (ii) the microemulsification, i.e., the blending of different oils (cottonseed, corn, sunflower, etc.) with conventional diesel fuel, (iii) dilution with petrodiesel and (iv) transesterification (2,3). Dilution of oils with solvents and microemulsions of vegetable oils lowers their viscosity, but some engine performance problems still exist. Among all these alternatives, the transesterification reaction leads to the product commonly known as biodiesel, and the physical characteristics of this product are very close to those of diesel fuel and the process is relatively simple (4).

Transesterification of vegetable oils and animal fats is performed with an excess of a primary alcohol (most commonly methanol) in the presence of a homogeneous or heterogeneous catalyst. A variety of vegetable oils can be used for the synthesis of biodiesel. Among the most studied are soybean, sunflower, palm, and almonds oils and a diversity of fatty acids of animal origin, as well. In addition to vegetable oils and animal fats, other materials such as used frying oils can also be suitable for biodiesel production; however, changes in the reaction procedure frequently have to be made due to the presence of water or free fatty acids (FFA) in these materials. Biodiesel represents a real alternative to diesel for internal combustion engines. Alternative fuels have the potential to solve many of the current social problems and concerns, from air pollution and global warming to sustainability issues (5-7).

1.1. Trace elements in vegetable oils

Trace elements can be naturally present in vegetable oils, absorbed by the vegetable mainly from the soil where the plant was grown. They can be also incorporated during its extraction and refining process to which the oil is submitted or due to environmental contamination. Some trace elements, such as Ca, Co, Fe, Mg, Mn and Ni, can promote the oxidative degradation of the oil, while other elements, like As, Cd, Hg and Pb might present toxic effects in humans, depending on their concentration in the oil (1,4). Also, the oil

characterization in relation to their trace element composition is the basis for further nutritional and technological investigations such as adulteration detection (8). These aspects, among others, turn trace element determination in edible oils important from both economic and health point of view and their concentration is an important criterion for the assessment of their quality in regard to freshness, keeping properties and storage.

However, the determination of trace elements in vegetal oils is hampered due to its low concentration in the sample, requiring sensitive instrumental methods. Moreover, their high viscosity turns it difficult to introduce the sample in the instrument, and the high organic content of the oil matrix, increases the possibility of interference during analysis. To overcome these difficulties different sample preparation procedures (such as digestion, emulsification, extraction and dilution) are applied in oils analysis (7).

1.2. Trace elements in biodiesel

The metal ions are introduced into biodiesel fuel during the production process or may originally come from the vegetable oil used in the synthesis. Whereas alkali metals stem from catalyst residues, alkaline-earth metals may originate from hard washing water. Sodium and potassium are associated with the formation of ash within the engine, while calcium soaps are responsible for injection pump sticking and/or to deposits on the parts. The determination of Na and K in biodiesel is especially important since alkaline hydroxides are used as catalysts in the transesterification process, and they can appear as contaminants in the final product (6, 7). Metals such as Cu, Pb and Zn may catalyze oxidation in contact with biodiesel, thereby creating residues (sediments). Transitioning from conventional diesel fuel to biodiesel blends may lead to large increase in sediments that may plug fuel filters. Thus, fuel system parts must be specially chosen for their compatibility with biodiesel (9). The evaluation of sulfur is also importance due to emission legislations and low sulfur requirements of modern diesel engines. Many elements such as Pb and V are highly active as catalyst poisons, even at low concentrations, and with the increasing use of advanced catalysts in diesel engines, the need to monitor them will gain greater importance. Organometallic compounds are also added to fuels in order to improve their physical or burn characteristics, such as Si (an antifoaming agent), Mn (a burn improver) and as additives for marine engine (Cr, Fe, Ni products), and they need to be monitored to ensure that the correct dosage is used (10,11). Phosphorus has a strongly negative impact on the long term activity of exhaust emission catalytic systems. It poisons the catalytic converters, increasing the emission of gases (CO, CO_2 , etc.) and particulate materials, besides affecting the fuel behavior (12).

1.3. Atomic spectrometric techniques and elemental determinations

Three main atomic spectrometry techniques are reviewed in this work for trace element determination in edible oils and biodiesel: atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS). Few works on flame optical emission spectrometry (F OES) will also be discusses. In the literature, there are other methods for analysis of trace elements in vegetable oil and biodiesel employing different analytical techniques such as molecular

absorciometry (13-15), potentiometry as well as voltammetry (16-19), neutral activation analysis (20) and chromatography (21). Although these techniques provide good results, the methods of atomic spectrometry are the most popular and recommended by the norms.

As has been mentioned, samples such as vegetal oils and biodiesel are of complex matrix and can present different viscosities. So, the determination of trace metals and non-metals using atomic spectrometric techniques requires special attention in relation to the procedures of sample preparation and the mode of sample introduction (22). In this context, the authors decided to organize this work according to the sample preparation procedure, following the classification presented above, since this step will be determinant for the success of the instrumental determination. Due to the lack of certified reference materials (CRM) for trace elements in edible oils and biodiesel, in general, the published works usually check the accuracy of the proposed method by recovery tests, in which the analyte is added to the samples and the recovery is evaluated using the optimum method conditions. The National Institute of Standards and technology is offering two new certified reference materials for biodiesel, however, there are only informed and not certified concentration values and only for very few elements in the certificates. A recovery value between 80 and 120% is considered acceptable for the accuracy of the proposed method.

2. Determination of trace elements in vegetal oils and biodiesel

2.1. Sample decomposition by dry and wet ashing

The decomposition procedures for edible oils and biodiesel analysis by spectrometric techniques are applied to eliminate totally or partially the organic content of the sample before its analysis. Simpler matrices decrease the chances of interferences during the instrumental measurements, and facilitate the sample introduction. Matrix decomposition usually results in aqueous solutions that can be, in principle, analyzed by any spectrometric technique using aqueous calibration solutions (23,24).

Sample decomposition for trace element analysis may be performed by dry ashing, assisted or not by an oxygen enriched atmosphere, in open or closed Recently, a microwave initiated dry ashing system has been atmosphere. introduced (25). Wet ashing makes use of liquid substances or mixtures for the sample decomposition, in open or closed systems, assisted by convective or microwave heating, or UV radiation as well. Closed systems are less prone to contamination or losses, although open systems are simpler and more accessible. Sample throughput must be critically considered, since closed systems are faster but usually limited to batches with low number of samples (26). In the literature, there is only a few works that propose the use of dry ashing decomposition for vegetable oil samples in order to determine the concentration of trace elements in these samples (11, 27-32). Different methods of sample decomposition by dry and wet ashing are resumed in Table 1. For instance, Saleh et al. developed an interesting method to determine Fe and Cu in palm oil by F AAS (29). The samples (50g) were dry ashed in platinum crucibles, and three different procedures were investigated: (i) flaming with a Bunsen burner until a black char

was attained, then ashing at 580 °C in a furnace for 1 h; (ii) flaming and ashing directly in a furnace at 350 °C for 1 h followed by 480 °C for 3 h; flaming and smothering of the flame with the crucible lid at 1 min intervals, followed by ashing in the furnace for 2 h at 480 °C. The resultant ash from the foregoing methods was dissolved in 10 mL of concentrate HNO3, which then was evaporated in a water bath. The residue was dissolved in 10 mL of a solution containing 0.01 mol L⁻¹ NaNO₃ and 0.01 mol L⁻¹ HNO₃, and this final solution was then analyzed by F AAS. A wet ashing method using glass vessels and concentrate HNO₃ under reflux was also investigated. The results obtained by the different procedures were not significantly different and recoveries were about 98%. However, all ashing procedures are time-consuming, labor intensive and present risks of contamination and/or analyte losses due to the extensively sample manipulation. Sun (30) used dry ashing with a low temperature oxygen plasma (LTA) to determine Cu and Fe in edible salad oil. Extraction with concentrate HNO₃ and H₂O₂ and direct dilution with methyl isobutyl ketone (MIBK) prior to GF AAS analysis were also investigated. For the LTA method, 3 - 4 g of oil was used, and under an oxygen flow rate of 250 mL min⁻¹ and power set between 90 and 110 W, 40 - 48 h was required to ash 4 g of sample. The sample was then dissolved in 10 mL of 0.1 mol L⁻¹ HNO₃ and its metallic content determined by GF AAS. The authors concluded that the best results were obtained, by the analysis after MIBK dilution.

An interesting study to evaluate the trace amounts of Cu that have migrated from foodstuff-packaging plastics according to the Directive 85/572/EEC was reported by Kolasa et al. (31) using F AAS. The sunflower simulant was decomposed by dry ashing at 550 °C and wet oxidation with concentrated HNO₃ and H₂SO₄ was investigated as well. Results have shown that the migration of Cu to the food simulants remained always within the permissible level, 30 mg/kg food simulant.

Microwave radiation has been described as a successful assistant for sample pretreatment in analytical chemistry. It has been used to accelerate certain organic reactions, such as hydrolysis, and also to improve the dissolution of environmental, biological, industrial and other samples (33, 34). Reagents like HNO₃, H₂SO₄, HCl or H₂O₂, as well as their mixtures, are usually applied to the wet ashing of the organic-carbon rich samples which is, generally, microwave assisted to speed up the procedure using closed system to avoid volatile compound losses and also reduce the amount of reagents necessary for sample preparation and the chances sample contamination. These procedures provide light-colored and transparent final solutions in contrast to the open vessel procedures, indicating that organic-carbon has been more efficiently removed. Thus, several authors have proposed the use of microwave assisted digestion as sample pre-treatment for the determination of trace elements in vegetable oil (35-46) and biodiesel (47).

Allen et al. developed an atmospheric pressure microwave assisted digestion procedure for Cu, Pb and Ni determination in soybean, corn and canola oil by ICP OES and GF AAS (35). A mass of 4.55 g of each sample was pre-reacted with concentrate H_2SO_4 for 15 min, followed by two 2 mL additions of concentrate HNO₃ at 10 min intervals, and let to stand for 20 min prior to place in the microwave cavity. After the microwave heating, the samples were diluted with 1% (v/v) HNO₃, and analyzed by GF AAS using 0.1% (m/v) (NH₄)₂HPO₄ as modifier. Recovery tests led to recoveries between 90 and 117%. Cindric et al.

developed a method for the inorganic profile determination in edible oils, including olive, pumpkin seed, sunflower and soya using microwave assisted digestion (36). A mass of 0.5 g of oil sample was digested with 4.0 mL of HNO₃ and 2.0 mL H₂O₂. Seventeen elements were surveyed by ICP OES but only the major elements like Ca, Fe, Mg, Na and Zn presented concentrations above LODs obtained. Thus, for the trace elements (Al, Co, Cu, K, Mn, Ni, Cr and Pb) GF AAS using Zeeman background correction was employed. However, Mn, Cr and Pb were still below the respective LODs. The authors concluded that microwave assisted digestion is a reliable and simple sample preparation method for edible oil analysis, and also that Ca and Mg or Fe concentrations can be used for oil adulteration evaluation. A similar procedure was described by Gonzálvez et al (37) to determine the trace element composition in argan oil samples from Morocco by ICP OES. The digestion was performed by adding 6 mL of concentrated HNO₃ and 2 mL of concentrated H_2O_2 to the samples (0.5 g) and submitting them to the microwave program. The accuracy of the procedure was evaluated by recovery studies, carried out on argan oil samples spiked at different concentration levels from 10 to 200 mg/L. Quantitative average recovery values were obtained for all elements evaluated.

Juranovic et al. determined trace elements in pumpkin seed oil and pumpkin seeds by ICP OES after wet digestion using a mixture of HNO_3 and H_2O_2 and compared three different digestion procedures: open and closed vessel in a steel bomb as well as microwave assisted digestion in a closed system (38). The authors used aqueous calibration solutions, and concluded that for most elements of interest the measured concentration using open system was up to 50% lower than using closed ones due to losses by volatilization. No statistically significant difference was found between the two evaluated closed systems, for most of the elements.

Benincasa et al. proposed a method to determine eighteen elements in Italian virgin olive oils to characterize them according to their geographical origin using ICP-MS with a dynamic reaction cell (DRC) (39). Samples were prepared by microwave assisted digestion with HNO_3 , and external calibration was performed with aqueous calibration solutions. For tracing the origin of the olive oils, the data were processed by linear discriminant analysis (LDA), which allowed classifying unknown samples after checking the method with samples of known origin.

Mendil et al. used microwave assisted digestion to analyze olive, hazelnut, sunflower and corn oil, margarine and butter by AAS (40). Approximately 1 g of sample was digested with 6 mL of HNO₃ and 2 mL of H_2O_2 , both concentrated, in a microwave oven and diluted to 10 mL with double deionized water. Flame AAS was used to determine Fe, Mn, Zn, Cu, Na, K, Ca and Mg, while GF AAS to determine Cd, Co and Pb. High metal accumulation levels were found for Cu, Pb and Co in olive oil, Fe and K in margarine, Zn and Mn in corn oil, Na and Mg in butter, Ca in sunflower oil and Cd in hazenut oil.

A study to ascertain the different trace elemental patterns in oils from different origin was developed by Zeiner et al (41). The elements screened were Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn. The samples were digested in triplicate in a microwave digestion unit using a mixture of HNO_3 and H_2O_2 as reagent. The determination of trace elements in olive oil was performed by ICP OES and GF AAS. The low achievable LODs enable the determination by ICP OES of even very low concentrations of most elements of interest, such as Ca, Fe,

Mg, Na, and Zn in olive oils. Elements present in small amounts (Al, Co, Cu, K, Mn and Ni) were measured by GF AAS in the same sample digest.

Bakkali et al. used a similar procedure to determine Cd, Cr, Cu, Mn and Pb in various types of vegetable oils by GF AAS (42) and Ansari et al. also used the microwave-assisted acid digestion method to determine Cd, Pb and Zn in different varieties of sunflower seed oil by GF AAS (43). Llorent-Martínez et al. developed a method for the quantitative analysis of the legislated metals (As, Cu, Fe and Pb) in oils (olive and olive pomace oils fit for consumption) by ICP-MS, following microwave digestion with HNO₃ (44). The method has been validated using recovery experiments, having obtained satisfactory results in both cases. An analytical procedure by ICP-MS was developed by Joebstl et al. for the determination of rare earth elements (REE) to assure the geographic origin of Styrian pumpkin seed oil, a high priced local product which is protected by the European Union (45). The oil samples were digested in duplicate in a high pressure asher (HPA-S) using concentrated subboiled HNO₃. Reyes et al. developed a method to determine Cu and Ni in vegetable oils (corn and soybean) by GF AAS and used the EPA 3051 for comparison of results (46). The method described by EPA 3051 consists in a HNO₃ digestion assisted by microwave heating.

In relation to biodiesel, a dry decomposition was the procedure used by Oliveira et al. for determination of Na by F OES in samples from different sources: Soybean, sunflower, cotton and cow fat (47). For the sample preparation, about 0.5 g was weighed and heated in a muffle furnace at 250 $^{\circ}$ C for 1 h and after that the temperature was increased up to 600 $^{\circ}$ C and kept so for 4 h. The residues were dissolved and made up to 100 mL with 1.0% v/v HNO₃. External calibration with aqueous calibration solutions and analyte addition were compared, and as no significant difference was observed, external calibration was chosen.

2.2. Direct analysis

The direct introduction of vegetal oil samples for the determination of trace metals by atomic spectrometric methods presents general problems, related to their large organic content and viscosity, for instance the (i) production of very fuel rich and unstable flames in the case of F AAS or F OES (ii) difficulty of sample introduction if pneumatic aspiration is used, (iii)) difficulties of sample introduction in GF AAS; (iii) excessive spreading of the sample during thermal pretreatment in GF AAS if wall atomization is used; (iv) deposition of carbon residues on the components of the torch and de-stabilization or extinction of plasma in ICP methods; (v) interferences due to carbon-based polyatomic species in ICP-MS (26). Such problems could be at least partially overcome by strategies such as (i) reducing the acetylene supply or increasing the flow rate of air in flame methods, (ii) use of flow injection systems for sample introduction, (iii) use of transversally heated graphite atomizers which avoids wall atomization in most cases, and use of oxygen as auxiliary gas (iv, v). Despite these strategies only few works propose the analysis of vegetal oil or biodiesel without any previous treatment. Thus, there are only a few works in the literature, summarized in Table 2, on vegetal oil analysis without any previous treatment.

In this sense, Cheng et al. used derivative F AAS combined with flowinjection technique to determine trace Cu and Zn in vegetable oils (48). The sensitivity and selectivity were enhanced when derivative technique was also used resulting in characteristic concentration of 0.0040 mg L^{-1} for Cu and 0.0012 mg L^{-1} ¹ for Zn, with relative standard deviation between 1.1 - 5.1%. While the results of Cu and Zn determination in vegetable oils were satisfactory by the proposed method, it was not possible to obtain satisfactory results by conventional F AAS or even using flow-injection F AAS. Carbonell et al. developed a method for Cu and Fe determination in edible oils by flow injection F AAS (32). Calibration was performed by analyte addition method using an organometallic standard, added to the sample using a reverse single line manifold. A similar approach was used by Zalts et al. (49) in the determination of microamounts of Cu and Fe in edible oils by F AAS. Sun et al. described a method for Pb and Zn determination in oil plant seeds by F AAS with micro injection and derivative signal processing (50). The characteristic concentration, LOD (n=10, 3s) and RSD were 0.052, 0.242 mg L^{-1} and 3.5% for Pb and 0.013 mg L^{-1} , 0.452 µg L^{-1} and 2.9% for Zn, respectively. Fischer and Rademeyer (51) described a method using a heated sample introduction tube, a Babington V-groove nebulizer and a heated spray chamber to nebulize edible and lubricating oils directly introduced into ICP to determine Ag, Al, Cr, Cu, Fe, Mg, Na, Ni and Ti by ICP OES. For the analysis of sunflower oil the optimum conditions were oil temperature between 90 - 105 °C, sample introduction tube temperature of 109 °C, nebulizer pressure of 7.0 kPa, spray chamber temperature of 190 °C and plasma power of 1200 W. As the viscosity of the aircraft lubricating oil samples were very similar to the sunflower oil ones, the same optimized conditions were used for its analysis. Calibration was performed by analyte addition for sunflower oil samples, due to the lack of blank oil presenting characteristics similar to the samples.

Martin-Polvillo et al described a method for the direct determination of Al, Cr, Cu, Fe, Ni and Pb in sunflower oil and olive oils by GF AAS (52). The authors suggested the use of L'vov platform since atomization from the tube wall resulted in interference in the determination of Al and Fe. Contamination with Fe was only detected in the olive oil that had been in contact with carbon steel, in this case, Fe concentration increased from 120 ± 12 to $3520 \pm 157 \ \mu g \ kg^{-1}$. Reves and Campos (46) proposed a method for the direct determination of Cu and Ni in vegetable oils by GF AAS using a solid sampling strategy (SS-GF AAS). A mass of approximately 0.5 mg of sample was directly weighted in the solid sampling boat and introduced in the graphite tube. Pyrolysis and atomization temperatures were 1200 and 2300 or 2400 °C for Ni, 1000 and 2000 °C for Cu, respectively, and the calibration as performed against aqueous calibration solutions. Accuracy of the proposed method was assessed by the analysis of the same samples by two comparative independent procedures, named EPA 3031 and EPA 3051, and good concordance was obtained between the proposed and reference methods. Limits of detection were 0.001 and 0.002 $\mu g g^{-1}$ for Cu and Ni, respectively, in the original samples considering the average mass of 0.5 mg. Another work involving the direct analysis without dilution was developed by Zakharov et al. (53) for the determination of phosphorus in vegetable oils by electrothermal atomic absorption spectrometry with automated sample injection. Atomization from an L'vov platform resulted in lower background signal and higher P atomic absorption signal, comparing to the atomization from the graphite wall. The developed method can be applied to monitor P concentration in different types of oil within the range from 10 up to 790 mg kg⁻¹. Results, performed at three concentration levels, were in good agreement with those obtained by photocolorimetry.

Lyra et al. performed the direct determination of P in biodiesel samples by GF AAS (54). The analysis was also made using an automatic solid sampling accessory. The pyrolysis and atomization temperatures and the mass of modifier were optimized by multivariate optimization. The best condition achieved were 1300°C and 2700°C for pyrolysis and atomization temperature respectively, and 30 μ g of Pd as modifier. The accuracy was confirmed by the analysis of reference materials (ASTM BIOD0804 and ASTM LU0801) and by comparison with the EN 14107 procedure (55). No statistically significant difference was observed between obtained and expected values. The determinations were performed using aqueous calibration solutions.

2.3. Sample dilution

Sample dilution with an appropriate solvent (xylene, kerosene, toluene, etc.) may overcome problems associated to the high viscosity of oily samples, facilitating sample introduction. The procedure is attractive, since it is simple and fast, although interferences in the further instrumental process must be considered (26). Also, there is a price to pay in terms of the limits of detection of the whole analytical procedure, due to the dilution itself. Calibrations are frequently performed with available organic soluble organo-salts, but the influence of the analyte speciation must be considered, since dilution does not lead the analyte in the sample and calibration solutions to the same form. Table 3 summarizes some published papers involving the dilution with solvents for analysis of vegetable oils and biodiesel.

Karadjova et al. developed a simple and fast procedure for the direct determination of Al, Cd, Cr, Cu, Fe, Mn, Ni and Pb in olive oil by GF AAS (56). The samples were diluted with 1,4-dioxane, which was found to be the most suitable solvent for oil dilution prior to GF AAS analysis, as it improves the decomposition of triglycerides during the pre-treatment step and permits aqueous calibration solutions to be used. Uncoated graphite tubes with platforms were used for the determination of Cd, Cu, Fe, Mn and Fe, and pyrolytic coated graphite tubes with grooves were used for Al, Cr and Ni determination.

Bozhanov and Karadjova (57) determined Bi, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Sb in ten Bulgarian lavender oils by ET AAS. Samples were prepared by simple dilution with isopropanol or 1,4-dioxane. Matrix matching calibration was performed by dilution of aqueous standard in the respective organic solvent, and LOD were well below the maximum permissible concentrations for the studied elements in cosmetic or food products. Chen et al. developed a method for determination of As in edible oil by GF AAS (58). Samples were prepared by dilution with n-heptane, and an organic-As standard solution was used for calibration. The same authors used a lecithin-cyclohexane to dilute edible oil for Cu determination by GF AAS (59). The samples were analyzed using the standard addition method.

Canario and Katskov employed a transversely heated filter atomizer (THFA) for the direct determination of Cd and Pb in ill edible oils (olive, avocado, grape seed, sunflower, salad and cooking) using a simultaneous atomic absorption spectrometer (60). The determination was performed after sample dilution with n-heptane. A metallorganic solution in oil, Conostan S-21, similarly diluted was employed for calibration. Results were verified using both external and analyte addition calibration methods. Omission of chemical modification, a

shorter temperature program and stability of analytical signals during 600-700 temperature cycles within 15%, with the use of single filter represented additional advantages of the THFA atomizer.

Marfil et al determined the metal content and physicochemical parameters in virgin argan oil to investigate the influence of the extraction method in the quality of the oil (61). For this purpose, the samples were previously filtered and diluted with MIBK prior to the analysis. Chromium, Cu, Fe, Mn and Pb were determined in the oil samples by GF AAS. The accuracy of the proposed method was checked by recovery experiments using a vegetable oil sample with low content of metals. Analyte addition and external calibration were employed to perform the analysis. The results showed that the use of the L'vov platform avoided dispersion of the sample inside the tube, and improved the reproducibility of measurements, and led to a complete mineralization of samples with low thermal conductivity, such as oils, thus creating a more uniform temperature.

Sun et al diluted edible salad oil with MIBK for the determination of Cu and Fe by GF AAS (50). Dijkstra and Meert determined P and Cu in edible oils by direct current plasma optical emission spectroscopy, using Conostan organometallic standards (62). Oil samples for analysis were diluted with kerosene. Kassa (63) and Piechowski et al. (64) also determined P and other trace metals in edible oils after dilution with formic acid and dioxane. For calibration, they used lecithin dissolved in refined peanut oil for their solutions.

There are few works dealing with the determination of trace metals in biodiesel. Indeed, for the determination of Na, K, Mg, Ca and P in biodiesel by ICP OES and AAS sample dilution with xylene is the recommended pre-treatment in the official American, European and Brazilian norms (55, 65-71). ICP OES is recommended all these elements, (depending on the norm), while F AAS is recommended only for Na and K in the American and European norms. However, Brazilian norm 15556:2008 (71) recommends the use of AAS for Mg and Ca too. Calibration is performed with organometallic standards dissolved in base oil, diluted in xylene, and care must be taken in relation to the viscosity matching between calibration and sample solutions, what is done by adding an adequate concentration of mineral oil in the calibration solutions . These norms take advantage of their simplicity and fastness, low cost and sufficient LODs, in accordance with the current specifications for biodiesel.

Woods et al. performed the direct determination of 29 elements in biodiesel samples diluted with kerosene by reaction cell ICP-MS (10). In order to decrease the impact of the organic matrix in the plasma an auxiliary gas (oxygen) was used. According to the authors, the octopole reaction cell was effective to remove spectral interferences and to enable the measurement of all important analytes. The calibration was performed with calibration solutions prepared with organometallic standards and using internal standardization. The LODs obtained were in $\mu g k g^{-1}$ order for almost all the analytes, unless for S, where in the LOD was at the mg kg⁻¹ range.

Edlund et al. determined Ca, Cl, K, Mg, Na and P in biodiesel samples by ICP OES (73), after samples dilution with kerosene in the proportion of 1:4 m/m. Oxygen addition to the intermediated gas, outer gas and nebulizer were evaluated. The argon-oxygen mixture showed significant effect over the background reduction for Na and K only, and was used for the determination of these elements only. Calibration was performed using base oil diluted in kerosene and organometallic standards. The method accuracy was investigated with three

round-robin biodiesel samples, and good concordance was observed between found and recommended values for all elements and samples. Santos et al. reported a simultaneous determination of Ca, K, Mg, Na and P in biodiesel samples by ICP OES, after a simple 10% (v/v) dilution with ethanol (74). Oxygen was added as auxiliary gas to promote carbon elimination in the plasma. Calibration was performed in ethanol medium and Y was used as internal standard in order to minimize non-spectral interferences.

2.4. Emulsification and microemulsification

Emulsification can be defined as the dispersion of drops of a liquid into an immiscible one, forming a two-phase system which is not thermodynamically stable, but can be homogeneous during a short period of time if some mechanical energy (agitation) is provided. This dispersion is usually facilitated by the use of surfactants that diminish the superficial tension of water, improving the interaction between the water and oil phases. When two immiscible liquids are stirred, a macro emulsion is obtained, either oil-in-water (O/W, droplets of oil in water) or water-in-oil (W/O, droplets of water in oil). In O/W emulsions the oil or fuel is dispersed in the aqueous phase as micro-drops stabilized by micelles or vesicles generated by the addition of a detergent. When properly stabilized, the emulsified oil sample is compatible with most analytical instrumentation, allowing the use of simple calibration procedures due to the minimization of interferences. The kind of emulsion that is formed is mainly related to the formulation and to a lesser degree to the O/W ratio. Microemulsions, on the other hand, are spontaneously formed and thermodynamically stable, that is, they are stable for an indefinite period of time. Just a small agitation is enough to form the microemulsions if the right proportion of components are mixed. In the case of micro-emulsions without detergent a co-solvent allows the formation of a homogeneous and long-term stable system containing the aqueous and organic phase (75). In the case of O/W systems, aqueous calibration solutions can be eventually used. Table 4 summarizes some published papers utilizing the emulsification or microemulsification in order to prepare the samples of vegetable oil and biodiesel.

Benzo et al. developed an emulsion based method for the determination of Ni and Mn traces in neem oil from Venezuela by GF AAS (76). The stability of the emulsion as a function of time was evaluated and the optimal concentration of the neem oil in the emulsion was 30 and 4% (v/v) for Ni and Mn, respectively, based on the analytes concentration in the samples. Calibration was performed with aqueous calibration solutions. Recovery tests led to recoveries between 97 and 101% for samples presenting 1.39 and 0.21 mg kg⁻¹ of Mn and Ni, respectively, and these values were in agreement with the results obtained with a comparative wet digestion procedure.

Jimenez et al. used a simple method to determine Fe in several virgin olive oils samples from Spain by GF AAS, using automatic emulsion formation for sample introduction (77). The samples were analyzed without any other pretreatment but the formation of the emulsion in the autosampler vessel using 10 s of ultrasonic agitation and 2% (v/v) of Triton X-100 as emulsifying agent. The accuracy of the method was verified by recovery tests, and recoveries ranging from 89.7-100.6% were obtained for the different samples investigated. A certified sample of lubricating oil (LO-010698) was also analyzed and the results obtained were in good agreement with the indicated value for Fe. De Souza et al. determined Cd, Co, Cr, Cu, Ni and Mn in olive oil, soya oil, margarine and butter by ICP OES (78). Olive and soya oil samples were stabilized by an emulsification procedure using a 70:30 m/m propan-1-ol/water solution, forming a long term stable dispersion. A 70:25:5 m/m/m propan-1-ol/water/xylene solution was used for margarine and butter dispersion stabilization. The samples only kept their homogeneity and stability for a few hours when the aqueous phase was acidified with HNO₃. Calibration was performed using inorganic standards prepared in the same medium as the samples with recoveries ranging from 91.7 to 105.5%.

Murillo et al. developed a method for Cu, Fe an Ni determination in edible oils by ICP OES (79). Emulsion composition was optimized using surface response methodology and three different surfactants, ethoxynonylphenol, Triton X-100 and Tween 80 were investigated. Varying the surfactant concentration in the emulsion between 0.5 and 9%, the optimum amount of oil in the emulsion was in the range of 2 - 35% for ethoxynonylphenol and Tween 80, while Triton X-100 showed a maximum response at 35% in oil. Oil samples emulsified with ethoxynonylphenol showed fairly good stability after manual shaking, while the emulsions formed with Triton X-100 were unstable. Good agreement was found for calibration curves prepared with calibration solutions consisting of emulsified aqueous standards using any of these surfactants. However, the best results were obtained when Tween 80 was used. In a similar work, Benzo et al. determined P in edible oils by ICP OES using oil-in-water (o/w) emulsion for sample preparation (80). The optima amount of oil and surfactant (ethoxynonylphenol) in the emulsion ranged from 8.0 to 37% (m/m) for oil and 1.0 to 10% (m/m) for ethoxynonylphenol for the P emission line at 213.620 nm. For the emission line at 214.911 nm, the optimum range became 18 to 32 and 3 to 8 % (m/m), respectively. Recoveries ranged from 98 to 105% for the 213.620 nm line and from 102 to 116% for the 214.911 nm line.

Ibrahim et al. investigated two methods for Pb determination in used frying oil collected from different parts of Cairo (81). In a first procedure an o/w emulsion was formed with Emulsogen MS-12 mixed with lauryl ether and distilled water. After vigorously shaking, the emulsion was directly aspirated into the plasma. The second method involved Pb extraction from oil into an aqueous ethylenediaminetetraacetic acid (EDTA) phase before measurement. Relatively higher standard deviation values were obtained from the emulsion method due the low stability of the plasma in organic matrices because organic vapors tend to cool the plasma, which results in analyte signal depression.

Anthemidis et al. developed an on-line emulsion formation system with Triton X-100 using a specially designed stirring chamber for the introduction of vegetable oil samples (olive, sunflower and corn) directly into the ICP OES (82). The optimum concentration of oil in the emulsion for maximum sensitivity was 50% (v/v). In all investigated conditions, the resulting emulsions remained stable for at least 30 min, showing that the stirring chamber is very effective for on-line emulsification.

Chang and Jiang (83) developed a method for determination of As, Cd and Hg in emulsified soybean and peanut oil samples by flow injection chemical vapor generation ICP-MS (FI-CVG-ICP-MS). An oil-in-water emulsion containing vegetable oil, Triton X-100 and HCl was prepared and 200 μ L were injected into the FI-CVG-ICP-MS to perform the analysis. The emulsion was stable for at least 20 min. The results for various vegetable oil samples obtained

by analyte addition and isotope dilution calibration were in good agreement with those arose from digested samples analyzed by pneumatic nebulization ICP-MS.

Jimenez et al. developed a method involving on-line emulsion formation for the determination of Al, Ba, Bi, Ca, Cu, Mg, Mn, Na, Pb and Sn in virgin olive oil samples by flow injection analysis and determination by ICP-MS and F AAS (84). However, the on-line preparation of emulsions in FI systems that make use of ultra-sonic bath presents serious drawbacks in the preparation of stable emulsions, as reported by others authors. (85,86)

Jimenez et al. determined Al, Ba, Bi, Cd, Co, Cu, Mn, Ni, Pb, Sn and V in olive oil by ICP-MS using on-line emulsion formation with Triton X-100 as emulsifier (85). The method is less time consuming than other procedures but various experimental parameters should be optimized: emulsifier concentration at the mixing point and in the carrier solutions, injected sample and emulsifier volumes, emulsion formation flow rate, design of the FIA manifold and the radiofrequency power in the plasma. The emulsions formed had a uniform, milky appearance and did not have oil drops on the total volume or show signs of coagulation. RSD (%) values were between 1.38 and 6.22 for an oil sample spiked with 10 μ g kg⁻¹ of each analyte. Recovery values were satisfactory with good agreement between the results obtained with external and analyte addition calibrations.

Castillo *et al.* used o/w emulsion sample preparation for the semiquantitative determination of several metals in olive oil by ICP-MS (86). Span 20, Tween 20 and Triton X-100 non-ionic emulsifiers were tested, and Triton X-100 showed the best stabilization performance. Sample emulsions were prepared by mixing 2 g of olive oil with 1.5 mL of Triton X-100. The mixture was mechanically stirred until the mixture became homogeneous and water was added to a final mass of 50 g. The solution was then stirred again for 5 min and put in an ultrasonic bath for another 5 min, resulting in total preparation time of around 13 min. The obtained emulsion was stable up to 24 h. Recoveries were in the range of 90 – 120% for most of the determined elements, and the LODs were in the level of ng g⁻¹.

Huang and Chiang developed a method for Zn, Cd and Pb determination in vegetable oil by electrothermal vaporization ICP-MS (87). Samples were prepared as an o/w emulsion containing vegetable oil, Triton X-100, H_2O_2 and HNO_3 and were sonicated in an ultrasonic bath. Since the sensitivity for the o/w emulsion samples were quite different from that observed for aqueous solutions, the authors carried out the calibration by analyte addition and isotope dilution. The prepared oil emulsion was stable for at least 20 min. The method was applied to the analysis of various sweet corn plumule and canola oil samples and the results using both analyte addition and isotope dilutions agreed satisfactory.

Several works have shown that solutions formed by trace metals dissolved in organic liquids do not have long term stability, and the signal is lost in minutes or hours, probably due to adsorption onto the container wall. However, it has also been shown that long-term stabilization can be achieved by mixing these solutions samples (pure organic solvents and mixtures) with propan-1-ol and nitric acid solution, forming microemulsions (46). In this sense, Vieira *et al.* proposed a procedure for the determination of As in vegetable oils and biodiesel of different origins by GF AAS (88). The samples were prepared by mixing them with appropriate volumes of propan-1-ol and nitric acid. Using a dilution factor of ten times the authors observed that the slopes of the analyte addition curves were significantly different and related to the difference in viscosity of the samples. However, with a dilution factor of hundred times, no significant difference was found in the slopes of the analyte addition curves of samples from different origins, neither between them and that of external calibration curves using inorganic or organic standard solutions. The analysis were then performed using palladium as chemical modifier, pyrolysis and atomization temperatures of 1200 °C and 2300 °C, respectively, and calibration with aqueous calibration solutions. The As signal of this microemulsion spiked with 100 ng mL⁻¹ of inorganic or organometallic As was stable up to at least 300 min. Recoveries, using both organic and inorganic As ranged from 95 to 110%. The limit of detection in the original sample was 0.3 mg kg⁻¹, which is adequate for the analysis of vegetable oils and biodiesel, according to the Brazilian legislation.

Jesus et al. proposed a method to determine Na and K in biodiesel and vegetable oil samples by F AAS after the formation of a water-in-oil microemulsion with butan-1-ol and Triton X-100 as surfactant (89). They reported that calibration solutions prepared as recommended by the European norms, that is, by dilution of organic standard solutions in xylene in the presence of base oil, are not stable after some hours, and the authors suggested the same microemulsion formation treatment to the calibration solutions. In this way, signal stability was observed up to at least 3 days. Accuracy was evaluated by comparison with the results obtained by the European norms (EN 14108 (68) and EN 14109 (67)) and no significant difference was observed considering 95% confidence level. In another work (90), the same authors carried out the determination of Ca and Mg in biodiesel and vegetable oils samples using a similar procedure: biodiesel samples were prepared using a water-in-oil microemulsion, obtained by mixing the biodiesel or vegetable oil with Triton X-100 and 1.4 mol L^{-1} HNO₃ and the final volume was completed with the cosurfactants n-butanol or n-pentanol. Stability was observed for up to five days. Calibration was performed using emulsified aqueous inorganic standards with base oil as matrix simulator and the accuracy were evaluated by recovery tests and comparison with the Brazilian norm 15556:2008 (71), showing good concordance.

Chaves et al. developed a method for determination of Na and K in biodiesel samples by F OES preparing the sample as a microemulsion with propan-1-ol and aqueous acid solution, without surfactant (91). The calibration solutions were prepared also in microemulsion medium using inorganic standards and base oil for simulating the matrix. The signal was stable during the evaluated period of time of 250 min. The method accuracy was assessed by recovery tests and comparison with results obtained by ICP OES. Recoveries of each analyte in the five samples analyzed ranged from 83 to 120% and the RSD values were below than 7%. Continuous aspiration led to LOD of 0.08 (Na) and 0.09 μ g g⁻¹ (K) while discrete aspiration to 0.10 and 0.06 μ g g⁻¹ for Na and K respectively.

De Souza et al. (92) performed the sequential determination of Ca, Cu, Fe, Mg, Mn, Na, and P in biodiesel by axial and radial ICP OES. The emulsions were prepared by mixing the biodiesel samples with concentrated HNO_3 and Triton X-100. The samples were mixed in vortex during 2 min and the mass completed with water. The calibration was performed using aqueous calibration solutions containing Triton X-100 and Y was used as internal standard.

Sample emulsification was also used by Chaves et al. (93) for the determination of Co, Cu, Fe, Mn, Ni and V in diesel and biodiesel samples by ETV-ICP MS. In this case the authors obtained the emulsions by mixing 1.0 g of

the sample (diesel or biodiesel) with 2.0 ml of 5% (m/v) Triton X-100 and 0.5 mL of concentrated HNO₃. This mixture was submitted to ultrasonic bath during 5 min, made up to the final volume with water and sonicated again for more 15 min. In the case of the reference material, residual fuel oil, a mass between 10-15 mg was diluted with 1 mL of xylene for viscosity reduction, and 2.0 ml of 5% (m/v) Triton X-100, 0.5 mL of concentrated HNO₃ and water up to 10 mL were added. Palladium (1.0 μ g) was used as carrier/modifier and the calibration was performed with aqueous calibration solutions; Rh, 10 μ g L⁻¹ was used as internal standard. The optimized conditions for ETV were 800 and 2500 °C for pyrolysis and vaporization temperatures, respectively. The method accuracy was evaluated by the analysis of the reference material (NIST 1634c – Residual fuel oil), comparison with the results by GF AAS and also recovery tests. According to the t-test at 95% of confidence level the results were in agreement and the recoveries ranged from 80 to 120%. The RSD values were lower than 20%, the LODs were 0.5, 1.5, 3, 0.3, 0.5 and 1.0 ng g⁻¹ for Co, Cu, Fe, Mn, Ni and V, respectively.

Silva et al. used samples prepared as a microemulsion to evaluate different calibrations techniques and modifiers for the determination of Cd, Pb and Tl in biodiesel samples by GF AAS (94). Microemulsions were prepared by mixing 2 g of biodiesel, 1 mL of a 10% (v/v) HNO₃ aqueous solution and n-propanol to a 10 mL final volume. The results showed that calibration with aqueous calibration solutions did not compensate the non-spectral interferences but matrix matching calibration led to accurate results. The results obtained using the analyte addition or by matrix matched calibration were in agreement, confirming the accuracy of the proposed procedure. Organometallic standards were not required and different samples were analyzed.

Amais et al. performed the determination of Cd, Co, Cu, Mn, Ni, Pb, Ti, and Zn in biodiesel by ICP-MS and microemulsion formation as sample preparation (95). The microemulsions were obtained by mixing the biodiesel samples with Triton X-100, 20 % v/v HNO₃, and n-propanol. An argon-oxygen mixture was used due to the high-carbon content in the microemulsions. The calibration was performed with inorganic standards in microemulsion medium, using light mineral oil to simulate the samples, for viscosity matching between the calibration and sample microemulsions.

In a rare case of speciation analysis in this kind of sample, Aranda et al (96) determined total and inorganic mercury in biodiesel samples prepared as oil-inwater emulsion by flow injection cold vapor atomic fluorescence spectrometry (FI-CV-AFS). UV irradiation was used for decompose organic mercury species (e.g. MeHg⁺ and PhHg⁺ among others) for the total mercury content determination. The inorganic mercury content was determined without the use of UV radiation. Analyte addition was the calibration technique used and the method accuracy was evaluated by comparison with total mercury results obtained after sample microwave assisted digestion and recovery tests.

Lobo et al. used two procedures of sample pretreatment prior to the analysis of biodiesel by GF AAS for the determination of Cu, Pb, Ni and Cd (97). In the first method, the microemulsions were prepared by mixing the biodiesel samples with surfactant (Triton X-100) and the volume completed with 1% (v/v) HNO₃. The second was a wet digestion procedure performed with concentrated HNO₃, H_2O_2 and V_2O_5 as catalyst. The mixture was allowed to rest for 24 h, and after that it was placed in a focused microwave system. The results for Cu and Pb indicated that variables of sample preparation for digestion by focused microwave were the

most important one for both analytes. Lyra et al. performed the determination of Na, K, Ca and Mg in biodiesel prepared as microemulsion without surfactant by F AAS (98). The microemulsion was obtained by mixing biodiesel, concentrated HNO₃, an ionization suppressor solution, and n-propanol. The analytes response stability was satisfactory during 15 days. Calibration curves were obtained using organometallic standard solutions in the microemulsion medium. The comparison with an independent Brazilian norm 15556:2008 (71) and recovery tests were used to confirm the accuracy of the proposed procedure.

2.5. Extraction

The process of extraction of the analyte from the sample is another way of sample preparation. As advantages, it is cited the separation the analyte from a more complex matrix into a simpler one, and occurring, the same time, a preconcentration. The extraction is in general, performed with inorganic acids, and followed by centrifugation. The liquid-liquid extraction efficiency depends on the extractant used as well as on the extraction conditions. Sonication (99) has been gaining increasing acceptance for assisting extraction procedures for the analysis of trace elements in vegetal oils and biodiesel as an alternative to other well established types of energy such as convective and microwave assisted heating. Table 5 summarizes some published papers involving various extraction methods.

Abe et al. reported the simultaneous multielement analysis of some foods by ICP OES (100). Phosphorus in vegetable oil, olive oil, wheat germ oil, and safflower oil was extracted with nitric acid and then measured. Sun (38) used an extraction with HNO₃ and HNO₃ plus H_2O_2 to determine Cu and Fe in edible salad oil by GF AAS. The solutions were mixed at 800 rpm for 24 h with a magnetic stirrer. After centrifugation, the lower layer of the acid solution was removed, diluted four times and then analyzed by GF AAS. The extraction with HNO₃ plus H_2O_2 shows higher measured values than with HNO₃ alone.

Ansari et al. proposed a procedure for the ultrasonic assisted extraction of Fe, Cu and Ni in 16 varieties of sunflower seed oil samples. Conventional wet acid digestion method was used for comparative purposes (101). The separation of aqueous and organic phases after extraction using centrifugation required only 3 min, as compared to the conventional equilibration method that required 90 min. Under the optimum operating conditions, the limits of detection obtained from the analyte addition curves were about 20 μ g L⁻¹. The authors also concluded that all varieties of sunflower oil which contained significant amounts of Fe, Cu, and Ni also presented indications of deterioration.

Anwar et al. developed a method wherein the samples of vegetable oils and fats were prepared using an ultrasonically assisted acid-extractive technique in order to determine Fe, Cu, Ni and Zn (102). The use of sonication followed by centrifugation for phase separation, reduced the conventional acid extraction time from 180 to 10 minutes only. The range of recovery of Fe, Cu, Ni and Zn ranged from 93.6 to 101.2 % in a soybean oil which was spiked with 0.10, 0.25, 0.50, 0.75, 1.00 μ g g⁻¹ of each of the studied metals in close agreement with those of a wet digestion method. Most of the samples of commercial oils and fats analyzed were found to be contaminated with notable amounts of iron and nickel ranging from 0.13-2.48 and 0.027-2.38 μ g g⁻¹, respectively. The contents of Cu and Zn were also high in many brands, ranging from 0.01-0.15 μ g g⁻¹ and 0.03-0.21 μ g g⁻¹, respectively.

Kowalewska et al. used the procedure of liquid-liquid extraction with inorganic acids and adsorption on activated carbon for the determination of different forms of metals such as Fe, Ni, Cu, Pb, Cd and As, present in edible oils (13). The aim of the work was to perform a liquid-liquid extraction procedure adequate to all different analyte forms as well as to enrich the final total concentration of the analytes. In the second pre-treatment metals' adsorption on activated carbon was performed, in order to differentiate the behavior of particular analyte forms during fractionation. The liquid-liquid extraction efficiency was lower for organic forms of analytes in comparison to inorganic ones. For Fe, the efficiency of the extraction procedures was close to 100% using HNO₃ as extractant; for other elements, HCl was similarly effective, independently of the forms of the investigated metals. The preconcentration resulted in low LOD, in the range of 0.001 up to 0.04 mg kg⁻¹, permitting even the use of F AAS for the determinations. The behavior of different analyte forms was very distinct toward activated carbon adsorption, showing that this sort of material can be used for fractionation of the metals' forms in different types of oils.

De Leonardis et al. determined Cu and Fe in edible vegetable oils using acid extraction and GF AAS (103): To 2-3 g of oils samples, 1 mL of 10% v/v HNO₃ was added, the mixture was shaken at 40 Hz for 30 s with a test tube mixer, and then placed in a shaking water bath at 50 °C for 2 h. After centrifugation at 5000 rpm for 5 min, the lower acid aqueous layer was withdraw with a polypropylene pipette and was loaded directly into the autosampler of the atomic absorption spectrometer. Analyte addition calibration was required for Cu, while external calibration with aqueous calibration solutions was used for Fe determination. Concentrations in the samples were in the range of $1 - 14 \ \mu g \ kg^{-1}$ for Cu and $52 - 517 \ \mu g \ kg^{-1}$ for Fe, with RSD below 15% for Cu and 10% for Fe, while recovery values (%) were 94 ± 23 for Cu and 97 ± 12 for Fe.

A procedure for the preconcentration and separation of copper in edible oil samples using a solid (powder) Pb-piperazine-dithiocarbamate complex (PbPDC) for extraction and a potassium cyanide solution for back extraction was described by Bati et al. (104) The determination was performed by F AAS, and recoveries close to 100% were observed. Asci et al. developed a solid-phase extraction method for Cd determination in commercial edible oils by F AAS based on the adsorption of Cd(II) onto zinc-piperazinedithiocarbamate (Zn-PDC) and its elution with mercury(II) nitrate (105). Calibration curve was linear up to 3.0 mg L^{-1} , and the accuracy of the method was evaluated by recovery studies. Under optimum conditions, recovery and LOD values were 99.67% and 0.028 mg L^{-1} , respectively.

Ooms et al. also used the extraction with EDTA for determination of trace metal content in corn oil by atomic absorption spectroscopy (106). Pehlivan et al. used acid extraction to determine Cu, Fe, Mn, Co, Cr, Pb, Cd, Ni and Zn in seventeen edible vegetable oils by ICP OES (107). The metals were extracted from low quantities of oil (2 - 3 g) with HNO₃ solution, which was directly injected in the ICP OES. The accuracy of the results, estimated in a percent average of the standard addition recoveries, was higher than 95% for all metal ions. When the concentration of metal ion was very low, the results obtained were influenced by noise and instrumental interference.

3. Conclusions

Atomic spectrometric methods are more popular for the determination of trace elements in vegetable and biodiesel samples. Despite inherent difficulties, such as large viscosity and organic load of these samples, that may cause interferences or even impede a confident analysis, well designed sample preparation strategies have been successful in overcome them, permitting the accurate determination of several elements. Sample decomposition by dry or wet procedures is a universal pre-treatment for further atomic spectrometric analysis frequently using external calibration with aqueous calibration solutions. However, even assisted by microwave heating, in closed systems, what accelerates the process and avoids contamination or losses, these procedures are still time consuming and labor intensive. This is a serious disadvantage especially if a large number of samples are to be analyzed. On the other hand, these procedures are important as references for comparison checking of other pre-treatments, since no certified reference material is available.

Only few works deal with the direct analysis of vegetable oils and biodiesel samples. In some of them the sample is weighed on sampling boats and direct introduced into de graphite furnace. For equipment with continuous introduction, FIA systems are used, what responds for the high viscosity, and alternative sample heating is also proposed. Despite the immediate advantages of direct analysis (lower blanks, no sample dilution, less chances of contamination or losses due to the pre-treatment, this alternative has not gained yet large acceptability: solid sampling accessories are necessary, and they are not widespread, and FIA-heating devices implies a more complex montage. Calibration may be still a challenge, and must be cautiously investigated.

Several organic solvents or solvent mixtures have been proposed for vegetable or biodiesel sample dilution prior to the atomic spectrometric determination. It is a very simple, and consequently attractive procedure, and various international biodiesel analysis norms are based in this procedure. However, dilution implies in poorer method LOD, and care must be taken in relation to the viscosity matching between sample and calibration solutions. The organic load of flames and plasmas must be also considered, and the use of oxygen as auxiliary gas in ICP methods finds application. A point to be considered is the question of the stability of the analyte in the diluted solution. This point is seldom considered in the literature, and the few works that investigate signal stability have observed only a short term stability of these solutions. This must be considered in the analysis strategy, especially in relation to the waiting time of these solutions in large autosampler trays.

Emulsion and microemulsion formation also finds application for trace elements determination in vegetable and biodiesel by atomic spectrometric methods. These procedures also imply in sample dilution and the procedures, although simple, are more complex than sample dilution with a solvent. However, the systems may be more stable in relation to the analyte signal, especially if microemulsions in acid medium are used. Also, aqueous calibration solutions, or at least inorganic standard solutions can be used for calibration.

Liquid-liquid and solid phase extractions are also proposed as sample pretreatment. Nitric acid solutions are the most used liquid extractant, while carbon and organic complexes are the solid phase used. Aqueous calibration, as well as sample pre-concentration is made possible, facilitating the instrumental determination. However, only few works are proposed in the literature, and more slid phases should be investigated.

4. Acknowledgements

The authors are thankful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for financial support and scholarships.

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Table 1. Determination of trace elements in vegetable oil and biodiesel by spectrometric techniques after sample decomposition by dry and wet ashing.

Procedures	Analytes	Samples	Comments	Ref.
Vegetable oil				
Dry ashing with H_2SO_4	Fe, Cu, Co, Ni, Pb, Al, Zn, Cd, Cr, Mn, Mg	Vegetable oils (soy, peanut, sesame, rape, tea and blended oils)	ICP OES. LOD (μg g ⁻¹): 0.1 - 3.6. RSD (%): 1.0 - 10.6. Recoveries (%):70.4 and 113, for samples spiked 0.1 - 0.5 mg kg ⁻¹ , respectively.	(27)
Dry ashing (50 g sample dry ashed in Pt crucibles). Ashes dissolved in 10 mL of concentrate HNO ₃ . Comparison with wet ashing method using glass vessels and concentrate HNO ₃ under reflux.	Cu, Fe	Palm oil	F AAS. Recoveries (%): ~98.	(29)
Ashing with low temperature oxygen plasma. Comparison with HNO ₃ and H ₂ O ₂ extraction or direct dilution with MIBK.	Cu, Fe	Edible salad oil (soybean, corn and sunflower)	GF AAS. Best results with MIBK.	(30)
Dry ashing (4-20 g sample + HNO ₃ + muffle furnace). HCl to dissolve the ashes.	Cu, Fe	Edible oils	 FI - F AAS. Analyte addition method using organometallic standards. Characteristic concentrations (mg L⁻¹): 0.22 (Cu); 0.6 (Fe). Repeatability (%): 3 (Cu); 8 (Fe). 	(32)
Atmospheric pressure microwave assisted digestion $(H_2SO_4, HNO_3 \text{ and } H_2O_2)$	Cu, Pb, Ni	Edible oils (soybean, corn and canola)	LOD (ng g ⁻¹): \leq 30 for GF AAS and \leq 50 for ICP OES. Recovery (%): 90 – 117	(35)
Microwave assisted digestion with HNO_3 and H_2O_2	Al, Cu, Co, Cr, K, Ni, Mn, Pb (GF AAS); Ca, Fe, K, Mg, Na, Zn (ICP OES).	Edible oils (olive, sunflower, soya and pumpkin seed)	LOD (µg g ⁻¹): 0.001 – 0.005 (GF AAS) and 0.06 – 0.41 (ICP OES).	(36)
Microwave assisted digestion with HNO_3 and H_2O_2	Al, As, Ba, Be, Bi, B, Cd, Ca,Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo,	argan oil and edible oils (olive, coconut, sunflower)	ICP OES. LOD (µg/L):0.03 (Er) to 220 (Ca). Recovery (%): > 92	(37)

	Ni, K, Se, Na, Sr,Tl,			
Open digestion; closed digestion in a steel bomb; microwave assisted digestion in a closed system.	Al, Ca, Cd, Co Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Ti, V, Zn	Pumpkin seed oil	ICP OES. LOD (μg g ⁻¹): < 0.1 (Ca, Cd, Mg, Mn, Ti, Zn); between 0.1 to 0.8 (Co, Cu, Fe, K, Mo, Na, Ni, Pb,V); > 0.8 (Al, Cr, P). Recoveries: > 95%, except for S (50%).	(38)
Microwave assisted digestion with HNO ₃	As, Be, Ca, Cd, Co, Cr, Eu, Fe, Gd, Mg, Mn, Ni, Sb, Sc, Se, Sm, Sr, Y	Olive oils	DRC-ICP-MS. LOQ (ng g ⁻¹): 0.009 to 10.2. RSD (%): 0.8 – 12. Calibration with aqueous solutions. Recovery (%): 91 – 119.	(39)
Microwave assisted digestion with HNO_3 and H_2O_2 .	Fe, Mn, Zn, Cu, Na, K, Ca, Mg (F AAS): Cd, Co, Pb (GF AAS)	olive, hazelnut, sunflower and corn oil, margarine and butter	F AAS and GF AAS. Concentrations of trace element found (μ g g ⁻¹): 291.0–52.0 (Fe), 1.64–0.04 (Mn), 3.08–1.03 (Zn), 0.71–0.05 (Cu), 0.03–0.01 (Pb), 1.30–0.50 (Co), 84.0–0.90 (Na), 50.1–1.30 (K), 174.2–20.8 (Ca), 20.8–0.60 (Mg), 4.57–0.09 (Cd).	(40)
Microwave assisted digestion with HNO_3 and H_2O_2 .	Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Zn	Olive oil	ICP OES and GF AAS. Concentrations of Al, Co, Cu, K, Mn, and Ni ranged from 0.15 to 1.5 μg/g	(41)
Microwave assisted digestion with HNO_3 and H_2O_2 .	Cd, Cr, Cu, Mn, Pb	virgin olive, olive, sunflower, maize and pomace olive oils.	GF AAS. LOD (µg g ⁻¹): 0.06- 2.15. RSD (%): 2.6 – 4.2	(42)
Microwave assisted digestion with HNO_3 and H_2O_2 .	Cd, Pb, Zn	sunflower seed oil	GF AAS. LOD (ng mL ⁻¹): 0.327 (Cd), 3.38 (Pb), 10.0 (Zn)	(43)
Microwave assisted digestion with HNO_3 and H_2O_2	As, Cu, Fe, Pb	oils (olive and olive pomace oils fit for consumption)	ICP-MS. LOD (ng g ⁻¹): 0.9 (Pb), 1.5 (Cu), 3.0 (As), 40 (Fe)	(44)
Microwave assisted digestion with HNO ₃	Cu, Ni	vegetable oils (corn and soybean)	SS - GF AAS. EPA 3051 method for comparison of results. LOD ($\mu g g^{-1}$): 0.001 (Cu) and 0.002 (Ni)	(46)
Biodiesel				
Dry decomposition (0.5 g of sample) Ashing in muffle furnace (250 °C – 600°C)	Na	biodiesel	GF AAS. LOD (mg kg ⁻¹): 1.3; RSD (%): ≥ 4 Recovery: 93 - 110%	(47)

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Procedure	Analytes	Samples	Comments	Ref.
Vegetable oils				
Flow injection and derivative signal processing.	Cu, Fe	Edible oils	F AAS. Analyte addition using an organometallic standards. RSD (%): 3 (Cu) and 2 (Fe). Recoveries (%): > 98 (Cu) and > 97 (Fe).	(32)
Solid sampling strategy: approximately 0.5 mg of sample directly weighted in the solid sampling boat and introduced in the graphite tube .	Cu, Ni	vegetable oils (corn and soybean)	SS- GF AAS. LOD (µg g ⁻¹): 0.001 (Cu) and 0.002 (Ni).	(46)
Flow injection and derivative signal processing.	Cu, Zn	Vegetable oils	F AAS. Characteristic concentration (mg L ⁻¹): 0.0040 (Cu) and 0.0012 (Zn), respectively.	(48)
	Cu, Fe	Edible oils	F AAS. Analyte addition using organometallic standards.	(49)
Micro injection and derivative signal processing.	Pb, Zn	Oil plant seeds: soybean, peanut, sunflower seeds, sesame black and sesame	F AAS. Micro injection and derivative signal processing. LOD (μ g L ⁻¹): 0.052 (Pb) and 0.013 (Zn). Characteristic concentration (μ g L ⁻¹): 0.242 (Pb) and 0.452 (Zn).	(50)
Directly nebulization using a Babington V-groove nebulizer and heated spray chamber	Ag, Al, Cr, Cu, Fe, Mg, Na, Ni, Ti	edible oils (sunflower)	ICP OES. Analyte addition. LOD (μg g ⁻¹): 0.032 (Fe); 0.069 (Cu); 0.051 (Cr); 0.045 (Ti); 0.328 (Al); 0.131 (Ni); 0.003 (Mg); 0.077 (Ag)	(51)
Use of L'vov platform.	Al, Cr, Cu, Fe, Ni, Pb	Sunflower oil and olive oil	GF AAS. L'Vov platform for Al and Fe determinations eliminates the matrix effects.	(52)
Automated sample injection.	Р	Vegetable oils	GF AAS. La as modifier and calibration with organic standard.	(53)
Biodiesel				
Solid sampling coupled to AAS: ~5 mg of sample directly weighted in the solid sampling boat and introduced in the graphite tube .	Р	Biodiesel	SS- GF AAS. Aqueous calibration. LOD (ng): 7.2, using 20 mL of a chemical modifier solution	(54)

Table 2. Direct determination of trace elements in vegetable oil and biodiesel by spectrometric techniques.

Procedure	Analytes	Samples	Comments	Ref.
Vegetable oils				
Dilution with MIBK	Cu, Fe	Edible salad oil (soybean, corn and sunflower)	GF AAS. Dilution with MIBK: the organic substances can be removed at low charring temperature and the possibility of loss is reduced.	(30)
Dilution with 1, 4 – dioxane	Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb	Olive oil	GF AAS. Aqueous calibration solutions.Uncoated graphite tubes with platforms (Cd, Cu, Fe, Mn and Fe) and pyrolytic coated graphite tubes with grooves (Al, Cr and Ni).LOD (μg g ⁻¹): 0.02 (Al, Cu, Cr, Pb); 0.01 (Cd, Mn); 0.1 (Fe); 0.05 (Ni)	(56)
Dilution with isopropanol (1:1) or 1,4 – dioxane (1:2)	Bi, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sb	lavender oils	GF AAS. Matrix matching calibration. Within-run and between-run precisions (%): 25.7- 3.10	(57)
Dilution with n-heptane	As	salad oil, fish oil, palm oil	GF AAS. LOD (µg g ⁻¹): 0.01. Recovery (%): 91.2 - 96.7.	(58)
Dilution with lecithin- cyclohexane	Cu	Edible oil	GF AAS. LOD (µg L ⁻¹): 0.01. Recovery (%): 85.5 t - 93.0. Standard addition method	(59)
Dilution with n-heptane	Cd, Pb	Edible oils: olive, sunflower, avocado, grape seed, salad and cooking oil	Transversely heated filter atomizer (THFA) – GF AAS, analyte addition. LOD (μ g L ⁻¹): 0.06 (Cd) and 0.7 (Pb)	(60)
Dilution with MIBK	Cr, Cu, Fe, Mn, Pb	virgin argan oil, vegetable oil	GF AAS. LOD (ng g ⁻¹): 0.5 (Cr); 13 (Cu); 42 (Fe); 2 (Mn); 4 (Pb). External and analyte addition calibration	(61)
Dilution with kerosene	P, Cu	edible oils	ICP OES. LOD: 0.5 ppm P and 5 ppb Cu	(62)
Dilution with formic acid and dioxane	P and other elements	Vegetable oils	ICP OES. RSD (%): 1-3%	(64)
Biodiesel				
Dilution with kerosene $(1:3 \text{ m m}^{-1})$	Na, K, S, Be, B, Mg, Si, P, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr,	Biodiesel	ICP-MS with oxygen as auxiliary gas. Calibration with organometallic standards and internal standardization; 10h run repeatability: 1.3% (As) - 6.4% (B)	(10)

Table 3. Determination of trace elements in vegetable oil and biodiesel by spectrometric techniques after sample dilution.
	Mo, Ag, Cd, Sn, Sb, Ba, W, Hg, Pb			
Dilution in kerosene (1:4 m/m)	Ca, Cl, K, Mg, Na, P	Biodiesel	ICP OES. LOD (μg kg ⁻¹): 1.6 (Na,588.95 nm); 1.4 (Na, 589.592 nm); 7.1 (K, 766.490). RSD (%): 0.2 – 1.3 for elemental concentration in the range 0.1 – 10 mg kg ⁻¹	(73)
Ethanol dilution 10% m/m	Ca, K, Mg, Na, P	Biodiesel	ICP OES. Calibration in ethanol medium and Y as internal standard. LOD (μ g kg ⁻¹), considering 2.5g/ 25 mL: 0.03(Ca); 0.005 (Mg); 0.5 (P); 0.3 (K); 0.1 (Na). RSD (%): ≤ 9 . Recovery (%): 82 - 114.	(74)

Table 4. Determination of trace elements in vegetable oil and biodiesel by spectrometric techniques after sample emulsification and microemulsification.

Procedure	Analytes	Samples	Comments	Ref.
Vegetable oils				
Emulsion: sample $(0.4000 \text{ g}) + p$ -xylene (0.4000 g) + Triton X-100 (0.3000 g) + water. Ultrasonic bath for 20 min.	Ni, Mn	Neem oil	GF AAS. Aqueous external calibration. Recoveries (%): 97 (Mn) and 101 (Ni). Emulsion stability up to at least for 24 h.	(76)
Emulsion formation with 2% Triton X-100 in the autosampler vessel (10 s of ultrasonic probe agitation)	Fe	virgin olive oils	GF AAS. LOD (ng g ⁻¹): 4.25. Recovery (%): 89.7 – 100.6%	(77)
Emulsion with propan-1-ol/water (70:30 m/m) for olive and soya oils, and with propan-1- ol/water/xylene (70:25:5 m/m/m) for margarine and butter.	Cd, Co, Cr, Cu, Ni, Mn	olive oil, soya oil, margarine and butter	ICP OES. LOD (μg g ⁻¹): 0.06 – 0.68. Recovery (%) 91.7 – 105.5.	(78)
Emulsion formation with ethoxynonylphenol, Triton X-100 or Tween 80 as surfactants.	Cu, Fe, Ni	edible oil: sunflower	ICP OES. RSD (%): < 8%. Best results using oil in water emulsion and Tween 80 as surfactant. Recovery (%): 90 to 110.	(79)
Emulsion formation with ethoxynonylphenol as surfactant	Р	edible oil: sunflower	ICP OES. RSD (%): < 7%. Calibration with emulsified aqueous standard. Recovery (%): 98 to 105 (213.620 nm) and 102 to 116 (214.911 nm).	(80)
O/W emulsion formation with Emulsogen MS-12 solution + lauryl ether solution + distilled water	Pb	frying oil	ICP OES.LOD (μg g ⁻¹): 0.12 (emulsion method); 0.08 (extraction method) Recovery (%): 91 to 105.5.	(81)
Emulsification with Triton X-100	Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, Mg, Mn, Ni, Pb, Tl and Zn	vegetable oil (olive, sunflower and corn)	ICP OES. Recovery (%): $81 - 112$ (spiked 0.5 mg L ⁻¹). Good agreement with GF AAS after wet digestion	(82)
O/W emulsion formation (final composition) 10%	As, Cd, Hg	soybean and peanut oil	FI-CVG-ICP-MS. LOD (ng g ⁻¹): 0.01 (As); 0.04 (Cd, Hg). RSD (%): 2 – 9.7; 26% for Cd.	(83)

Al, Ba, Bi, Cd, Co, Cu, Mn, Ni, Pb, Sn, V	virgin olive oil	ICP-MS. LOD (μg g ⁻¹): 0.02 - 5.31; RSD (%): 1.38 - 6.22 (oil sample spiked with 10 μg kg ⁻¹ of each analyte).	(85)
Ba, Cd, Co, Cr, Cu, Mn, Ni, Tl, Pb, U, V, Zn	olive oil	ICP-MS. LOD (ng g ⁻¹): $0.25 - 0.50$. Recovery (%): 90 - 120%.	(86)
Zn, Cd, Pb	Vegetable oils	ETV-ICP-MS. LOD (µg L ⁻¹): 20 (Zn); 1 (Cd); 2 (Pb) Analyte addition calibration and isotope dilution.	(87)
As	biodiesel and vegetable oils	GF AAS. LOD (mg kg ⁻¹): 0.3. RSD: \leq 3 for sample solutions spiked with 150 ng mL ⁻¹ of both inorganic and organometallic As. Stability of the response: at least 5h	(88)
Na, K	biodiesel and vegetable oils	 F AAS. Stability of the response: 3 days. LOD (μg L⁻¹): 0.1 (Na); 0.06 (K). Characteristic concentrations (μg L⁻¹): 25 (Na); 28 (K). RSD (%): 0.4-1.0. Recoveries ranged from 95% to 115% for biodiesel and 90% to 115% for vegetable oil samples 	(89)
Ca, Mg	biodiesel and vegetable oils	 F AAS. Stability of the response: 5 days. LOD (μg g⁻¹): 0.04 (Ca); 0.1(Mg). Characteristic concentrations (μg L⁻¹): 30 (Ca); 6 (Mg). Recoveries (%): 92 - 117% 	(90)
Na, K	Biodiesel.	F AES. LOD (μ g g ⁻¹): 0.01(Na and K(Mg). RSD (%): \leq 7. Recovery (%): 83 to 120.	(91)
Ca, Cu, Fe, Mg, Mn, Na, P	Biodiesel.	Axial and radial ICP OES. LOQ (μg g ⁻¹): 0.165 (Ca); 0.099 (Cu); 0.033 (Fe); 0.007 (Mg); 0.016 (Mn); 0.132 (Na); 0.660 (P). RSD (%): 1 - 8. Recovery (%): 90 - 109	(92)
Co, Cu, Fe, Mn, Ni, V	Biodiesel and diesel	ETV-ICP-MS. LOD (ng g ⁻¹): 0.3 – 3.0	(93)
	Al, Ba, Bi, Cd, Co, Cu, Mn, Ni, Pb, Sn, V Ba, Cd, Co, Cr, Cu, Mn, Ni, Tl, Pb, U, V, Zn Zn, Cd, Pb As Na, K Ca, Mg Na, K Ca, Mg Na, K Ca, Cu, Fe, Mg, Mn, Na, P Co, Cu, Fe, Mn, Ni, V	Al, Ba, Bi, Cd, Co, Cu, Mn, Ni, Pb, Sn, Vvirgin olive oilBa, Cd, Co, Cr, Cu, Mn, Ni, Tl, Pb, U, V, Znolive oilZn, Cd, PbVegetable oilsAsbiodiesel and vegetable oilsAsbiodiesel and vegetable oilsNa, Kbiodiesel and vegetable oilsCa, Mgbiodiesel and vegetable oilsNa, KBiodiesel and vegetable oilsCa, Cu, Fe, Mg, Mn, Na, PBiodiesel.Co, Cu, Fe, Mn, Ni, VBiodiesel and diesel	Al, Ba, Bi, Cd, Co, Cu, Mn, Ni, Pb, Sn, Vvirgin olive oilICP-MS. LOD ($\mu g g^{-1}$): 0.02 - 5.31; RSD (%): 1.38 - 6.22 (oil sample spiked with 10 $\mu g kg^{-1}$ of each analyte).Ba, Cd, Co, Cr, Cu, Mn, Ni, Tl, Pb, U, V, Znolive oilICP-MS. LOD ($n g g^{-1}$): 0.25 - 0.50. Recovery (%): 90 - 120% .Zn, Cd, PbVegetable oilsETV-ICP-MS. LOD ($\mu g L^{-1}$): 20 (Zn); 1 (Cd); 2 (Pb) Analyte addition calibration and isotope dilution.Asbiodiesel and vegetable oilsGF AAS. LOD ($m g kg^{-1}$): 0.3. RSD: \leq 3 for sample solutions spiked with 150 ng mL $^{-1}$ of both inorganic and organometallic As. Stability of the response: at least 5hNa, Kbiodiesel and vegetable oilsF AAS. Stability of the response: 3 days. LOD ($\mu g L^{-1}$): 25 (Na); 28 (K). RSD (%): 0.4-1.0. Recoveries ranged from 95% to 115% for biodiesel and 90% to 115% for iodiesel and vegetable oilsCa, Mgbiodiesel and vegetable oilsF AAS. Stability of the response: 5 days. LOD ($\mu g g^{-1}$): 30 (Ca); 6 (Mg). Recoveries (%): 92 - 117%Na, KBiodiesel.F AES. LOD ($\mu g g^{-1}$): 0.01(Na and K(Mg). RSD (%): \leq 7. Recovery (%): 83 to 120.Na, KBiodiesel.F AES. LOD ($\mu g g^{-1}$): 0.015 (Ca); 0.099 (Cu); 0.033 (Fe); 0.007 (Mg); 0.016 (Mn); 0.132 (Na); 0.660 (P). RSD (%): 1 - 8. Recovery (%): 90 - 109Co, Cu, Fe, Mn, Ni, VBiodiesel and dieselETV-ICP-MS. LOD ($n g g^{-1}$): 0.3 - 3.0

m/v) Triton X-100 + 0.5 mL HNO ₃ and deionized		Recovery (%): 80 to 120.			
water up to a 10 mL					
Microemulsion formation without surfactant: (2 g	Cd Db Tl		GF AAS. Matrix matching calibration using base oil		
biodiesel + 1 mL 10% (v/v) HNO ₃ + n-propanol)	Cu, F0, 11	Biodiesel	recoveries (%): 80 - 116.	(94)	
Microemulsion formation: 0.25 mL Triton X-100 +	Cd Co Cu Mn Ni		ICP-MS with oxygen as auxiliary gas. LOD (μ g L ⁻¹):		
$0.25 \text{ mL } 20\% \text{ (v/v) } \text{HNO}_3 + 0.5 \text{ mL biodiesel} + 4$	$5 \text{ mL } 20\% \text{ (v/v) } \text{HNO}_3 + 0.5 \text{ mL biodiesel} + 4$		9.63x10 ⁻³ - 19.5 Recoveries (%): 76.5 - 116.2% (Zn, 65.0 -		
mL n-propanol	r 0, 11, Zii		76.2%).		
Emulsion formation with Triton X-100: biodiesel			ELCV_AES I OD: 0.2 ug kg ⁻¹ (0.03 ug L ⁻¹ for emulsions)		
(0.88g) + 65% (v/v) HNO ₃ (3.0 mL): sonication	Hσ	Biodiesel	RSD(%) < 8 (at levels of 0 3ug L ⁻¹ in the emulsion) IV		
$(5.0 \text{ min}) + \text{Triton X-100} (1.5 \text{ mL}) \text{ and } H_2O (2.56)$	ng		radiation Total and inorganic Hg determination		
mL).			radiation rotar and morganic rig determination.		
Microemulsions formation with surfactant (Triton	Cu Ph Ni Cd	Biodiesel	GEAAS	(97)	
X-100) and 1% (v/v) HNO ₃ .	Cu, 10, 14, Cu	Diodicsei	OI AAS.	(\mathcal{I})	
Microemulsion formation without surfactant (final					
composition): 10% (m/v) biodiesel, 75% (v/v) n-			F AAS. LOD (µg kg ⁻¹): 0.1(Na); 0.01 (K); 0.04 (Ca);		
propanol, 1% (v/v) concentrated HNO_3 and 14%	Na, K, Ca, Mg	Biodiesel	0.004 (Mg). Recovery (%): 89 to 103. Good concordance	(98)	
(v/v) of aqueous solution (0.2% (v/v) of HNO ₃ and			with NBR 15556:2008.		
0.5% (v/v) ionization suppressor).					

Extraction procedure	Analytes Samples		Comments	Ref.
Vegetable oil				
extraction with HNO_3 and H_2O_2	Cu, Fe	Edible salad oil (soybean, corn and sunflower)	GF AAS. Low extraction: 60-70%. Best results using dilution with MIBK.	(30)
Extraction with HNO ₃	Р	vegetable oil (olive, wheat germ, safflower)	ICP OES.	(100)
Extraction with HNO ₃ assisted by sonication; phases separation by centrifugation	Cu, Fe, Ni	Sunflower oil	GF AAS (Cu, Fe) and F AAS (Zn). LOD (μg L ⁻¹): 20.4 (Cu); 21.7 (Fe); 35.6 (Ni). Recovery (%): 95.6 - 98.2.	(101)
Extraction with HNO ₃ assisted by sonication phases separation by centrifigation.	Fe, Cu, Ni, Zn	vegetable oils and fats	GF AAS (Cu, Fe, Ni) and F AAS (for Zn). Recovery test (%): 94.6-98.0 (Fe); 93.6-100.4 (Cu); 95.0-97.3 (Ni) and 96.0-101.2 (Zn)	(102)
Extraction with HNO ₃ (Fe) and HCl (As, Cu, Ni, Pb) and adsorption on activated carbon for the determination of different forms of metals	Fe, Ni, Cu, Pb, Cd, As	Edible oils	F AAS. LOD (mg kg ⁻¹): 0.001 - 0.04. Analyte addition and wet digestion for comparison	(13)
Extraction with HNO ₃ , shaking in a test tube mixer. Phases separation by centrifugation.	Cu, Fe	Edible vegetable oils	GF AAS. RSD (%): 15 (Cu); 10 (Fe). Recovery (%): 94 ± 23 (Cu); 97 ± 12 (Fe)	(103)
Solid (powder) Pb-piperazine-dithiocarbamate complex (PbPDC) for extraction and a potassium cyanide solution for back extraction.	Cu	edible oils: (sunflower, corn and soy)	F AAS. LOD (ng mL ⁻¹): 2.4; Recovery (%): > 93.	(104)
Adsorption of Cd(II) on to Zn-PDC and elution with mercury(II) nitrate.	Cd	edible oil	F AAS. LOD (mg L ⁻¹): 0.028. Recovery (%): 99.7	(105)
Extraction with HNO ₃	Cu, Fe, Mn, Co, Cr, Pb, Cd, Ni, Zn	soybean, hazelnut, almond, natural olive, riviera olive, virgin olive, olive (frying), sunflower and corn oils	ICP OES. RSD (%): 5 (Fe, Cd); 20 (Pb)	(106)

Table 5. Determination of trace elements in vegetable oil and biodiesel by spectrometric techniques after sample extraction.

Energy Fuels 2009, 23, 5942-5946 · DOI:10.1021/ef900709q Published on Web 10/07/2009

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Determination of As in Vegetable Oil and Biodiesel by Graphite Furnace Atomic Absorption Spectrometry

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Received July 10, 2009. Revised Manuscript Received September 2, 2009

In this work, an alternative procedure for the determination of As in vegetable oils and biodiesel of different origins by graphite furnace atomic absorption spectrometry (GFAAS) is proposed. The sample stabilization was achieved by the formation of three component solutions prepared mixing the samples with appropriate volumes of propan-1-ol and nitric acid. Palladium was used as a chemical modifier. Pyrolysis and atomization temperatures as well as the sample dilution factor were investigated and optimized. Because no adequate certified reference material was available, accuracy was assessed by recovery tests, using both organic and inorganic species. Recoveries ranged from 95 to 110%. The limit of detection in the original sample was 0.3 mg kg^{-1} , which is adequate for the analysis of vegetable oils and biodiesel, according to the expected Brazilian legislation.

1. Introduction

Biodiesel represents a real alternative to diesel for internal combustion engines. It is obtained by transesterification of vegetable oils and animal fats with an excess of a primary alcohol (most commonly methanol) in the presence of a homo- or heterogeneous catalyst. A variety of vegetable oils can be used for the synthesis of biodiesel. Among the most studied are soybean, sunflower, palm, and almonds oils and a diversity of fatty acids of animal origin, as well. Used vegetable oils are also considered a source of biodiesel, because they can be obtained at a low cost and permitting waste recycling. The feedstock composition will influence the properties of the produced biodiesel. The use of biodiesel lowers the environmental impact of automobile transport, with a significant reduction of the amount of emitted gaseous pollutants and a decrease in the emission of greenhouse gases.1-4

The determination of trace elements in vegetable oils and biodiesel is of importance, because their presence above certain levels can change the characteristics of use of these products. The determination of Na and K in biodiesel is especially important because alkaline hydroxides are used as catalysts in the transesterification process, and they can appear as contaminants in the final product. Na and K are associated with the formation of ash within the engine, while calcium soaps are responsible for injection pump sticking and/ or deposits on the parts. Whereas alkali metals stem from catalyst residues, alkaline-earth metals may originate from hard washing water.2,3 There are many approaches for the

measurement of these contaminants: European norms recommend sample dilution with xylene and direct determination by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Na, K, Ca, and Mg) and flame atomic absorption spectrometry (FAAS) (Na and K); calibration is performed with organic standards, diluted in xylene, and care must be taken match the viscosity between the calibration and sample solutions. A Brazilian norm also proposes FAAS and ICP-OES for the determination of Na, K, Ca, and Mg.5-10

Recently, Santos et al.11 reported a simultaneous determination of Ca, P, Mg, K, and Na in biodiesel samples by ICP-OES, after a simple dilution sample with ethanol. Chaves et al.¹² developed a method for determination of Na and K in biodiesel by flame atomic emission spectrometry (FAES) that involves preparing the sample as a microemulsion with propan-1-ol and aqueous acid solution. Jesus et al.13

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developed a method for the determination of Na and K in the biodiesel sample using water-in-oil microemulsions during the sample preparation. Other metals, such as Cu, Ni, Zn, Fe, and Cd, have also been investigated in biodiesel and vegetable oil samples. In vegetable oils, Cu and Fe usually come from the metallic surfaces of the processing equipment or storage vessels (tanks, barrels, etc.) and other elements, such as Pb, Cd, Hg, and As, are also important because of their toxicity.14-17

The presence of As in vegetable oils or biodiesel samples has not yet been reported. However, this element may be present because of its absorption from the soil by the plant, or it may be incorporated during production and storage processes. Fertilizers or insecticides are also a potential As source. The current specifications for biodiesel imposed by the Brazilian fuel agency (ANP)18 defines a limit of 5 mg kg-1 as the maximum allowed concentration for Na + K or Ca + Mg and 10 mg kg⁻¹ for P. For As, a similar specification is still under discussion, but the expected limit will probably be at about the 5 mg kg⁻¹ threshold.¹⁹ Thus, analytical procedures that can detect As in these samples at this level are necessary, and no recommended methodology is still described in the literature for biodiesel, very likely because of its recent introduction into widespread use.

In the analysis of such samples, difficulties in the calibration step may also be experienced, because of the matrix complexity. If a dilution step with an adequate organic solvent is proposed, the use of organic standards is required. These are more expensive, and the organic solutions obtained are not stable in the long term.^{20,21} Several works have shown that trace metals dissolved in organic liquids do not have the desired stability, and thus, the signal is lost in minutes or hours, probably because of adsorption onto the container wall. However, it has also been shown that long-term stabilization can be achieved by mixing the samples (pure organic solvents and mixtures, such as gasoline, diesel, and naphtha) with propan-1-ol and nitric acid solution. Concerning the determination of metal traces in biodiesel, Jesus et al.13 have recently reported that calibration solutions prepared as recommended by the norms, that is, by dilution of the organic standard solution in xylene in the presence of a base oil, are not stable after a period of some hours. Those authors suggested the emulsification of the calibration and sample solutions with Triton X-100 and butan-1-ol to solve this problem.

Sample mineralization by acid digestion may also be used, but this leads to the risk of contamination or losses and increased analysis time. Thus, the analysis of organic liquid samples using the formation of emulsions or microemulsions appears to be the best compromise among simplicity, sample stability, calibration easiness (because it is possible to use aqueous standards for preparing the calibration solutions),

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and analysis time. The emulsions differ from microemulsions by the size of the drops and the physical and chemical characteristics: the microemulsions are spontaneously formed, thermodynamically stable, and optically transparent, while emulsions are opaque and tend to undergo phase separation after some time.²²⁻²⁴ In this sense, several works propose the use of microemulsions for the determination of elements in viscous and complex organic liquids, mainly oil, fuel oil, and oil derivatives.^{17,20-26} Castillo et al.²⁴ developed a method for the semi-quantitative determination of metals in olive oil using direct emulsion nebulization. The samples were emulsified using Triton X-100. Using a microemulsion with propan-1-ol and nitric acid, arsenic and manganese were determined in diesel, gasoline, and naphtha samples by gra-phite furnace atomic absorption spectrometry (GFAAS).^{25,26}

Among the several spectrometric techniques available for the quantification of As traces, the GFAAS offers appropriate limits of detection (LODs; at the order of ng g-1 or less) at relatively lower costs and sample introduction of nonmineralized samples is facilitated, permitting faster analysis and reduced risks of contamination or analyte loss. Thus, the objective of the present study is to combine the advantages of the GFAAS with the sample preparation by microemulsion formation for the determination of As in vegetable oils and biodiesel.

2. Experimental Section

2.1. Instrumentation. The measurements of As were carried out with a model ZEEnit 60 atomic absorption spectrometer (Analytik Jena, Jena, Germany), equipped with a transversally heated graphite atomizer and a transverse Zeeman-effect based background correction system. A hollow cathode lamp (Analytik Jena) operating at 5 mA was used as the line source. The spectral band-pass was fixed at 0.2 nm, and the line used was 193.7 nm. All measurements were made in integrated absorbance, using 4 s for the integration time. Argon (99.99%, AGA, Rio de Janeiro, Brazil) was used as the protective and carrier gas. The samples were weighed in a microbalance Ohaus Adventurer AR 2140 (Pine Brook, NJ) with a precision of ±0.0001 g.

2.2. Materials, Reagents, Solutions, and Samples. All reagents were of analytical reagent grade. Ultrapure water, obtained from a Gehaka Master System apparatus (Gehaka, São Paulo, Brazil) was used throughout the experiments. Analyticalgrade HNO3 (Vetec, Rio de Janeiro, Brazil) was purified by distillation using a Teflon subboiling still (Kurner Analysentechnik, Rosenheim, Germany). Propan-1-ol (Vetec, Rio de Janeiro, Brazil) was also used. The 1000 μ g mL⁻¹ As stock solution was prepared by adequate dilution of a Titrisol concentrate (Merck, Darmstadt, Germany) with 0.2% (v/v) HNO3. Aqueous calibration solutions were prepared by further dilution of the stock solution with 0.2% (v/v) HNO3. An As organometallic stock solution in oil (Conostan, Houston, TX) was also used. The organometallic calibration solutions were prepared by adequate dilution from stock solutions with propan-1-ol.

The conventional modifier solution was prepared from a $10\,000 \text{ mg L}^{-1} \text{ Pd}(\text{NO}_3)_2$ stock solution (Merck, Darmstadt, Germany). For measurements, 5 µL (10 µg) of a solution

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Energy Fuels 2009, 23, 5942-5946 · DOI:10.1021/ef900709q



Figure 1. Pyrolysis and a tomization temperature curves for As using Pd as the modifier and samples as microemulsions (10× dilution factor): 50 ng mL⁻¹ inorganic As spiked in (\Box) soybean oil and (\bigcirc) soybean biodiesel and 50 ng mL⁻¹ organometallic As spiked in (\triangle) soybean oil and (∇) soybean biodiesel.

containing 2000 mg L^{-1} Pd in 0.2% (v/v) HNO₃ were dispensed onto the sample platform together with the sample (or calibration solution).²⁶

All plastic and glassware were washed with tap water, immersed in Extran (48 h), rinsed with tap and deionized water, and immersed in 20% (v/v) HNO₃ for at least 24 h. Before use, these materials were thoroughly rinsed with ultrapure water.

Five types of vegetable oil samples with different origins were analyzed: soybean crude, castor crude and refined, cotton crude, and sunflower. Six biodiesel samples from different oil sources were also analyzed: soybean, canola, cotton, peanut, a mixture of soybean (90%) and castor oil (10%), and barbados nut (Jatropha curcas L.).

2.3. Procedure. For the determination of As, the samples were prepared as an oil-in-water (O/W) three-component solution (microemulsion) by mixing approximately 1 g (low dilution factor) or 0.1 g (high dilution factor) of vegetable oil or biodiesel with 1 mL of propan-1-ol plus 0.2 mL of 65% (v/v) HNO3. The final volume of 10 mL was then completed with propan-1-ol, resulting in a visually homogeneous system. In the GFAAS analysis, an injection volume of 15 µL was used. The samples were prepared in propylene flasks, and the stability of As in the samples and calibration solutions was evaluated every 30 min for 300 min. The signal was stable during the monitoring time, indicating stability of the analyte. The calibration was performed with aqueous solutions of As in 0.2% (v/v) HNO3 or using the same medium of sample solutions, i.e., propan-1-ol plus HNO3 with inorganic or organometallic As standard solutions.

3. Results and Discussion

3.1. Optimization of the Temperature Program. The pyrolysis and atomization temperatures were optimized using sample microemulsions of vegetable oil and biodiesel prepared as described in section 2.3 but spiked with 50 ng mL⁻¹ of inorganic and organic As. Palladium was used as a chemical modifier, according to previous work by Brandão et al.²⁶ A pyrolysis temperature of 1200 °C and an atomization temperature of 2400 °C were adopted, in accordance with the results shown in Figure 1. For all samples, the drying temperatures and rates were visually optimized, to permit a complete evaporation of the solvent, without the loss of the sample, at a minimum time. The pyrolysis ramp rates were

Table 1. Temperature Program for the Determination of As in Vegetable Oil and Biodiesel Samples

step	temperature (°C)	rate (°C/s)	hold (s)
drying	100	1	5
drying	200	1	5
pyrolysis	1200	100	1
autozero	1200	0	6
atomization	2400	FP^{α}	4
cleanout	2650	1000	2

"FP = maximum power.



Figure 2. Stability of arsenic (100 ng mL⁻¹) in blank and microemulsions (100× diluted) of samples of vegetable oil or biodiesel: $(\Box) n$ -propanol + HNO₃(blank) spiked with inorganic As and (\bigcirc) organometallic As, soybean biodiesel microemulsion spiked with (\triangle) inorganic As or (\bigtriangledown) organometallic As, and soybean vegetable oil microemulsion spiked with (right pointing triangle) inorganic As or (left pointing triangle) organometallic As.

Table 2. Slopes of Analyte Addition Curves and Viscosity Obtained for Vegetable Oil or Biodiesel Samples Prepared as Microemulsions at a 10× Dilution

curves	slope (L μ g ⁻¹ ± s)	$\eta \ (mm^2/s)$
inorganic As in HNO3	0.0021 ± 0.0001	
inorganic As in propan-1-ol + HNO3	0.0022 ± 0.0002	
sunflower oil	0.0019 ± 0.0002	1.1392
soybean crude oil	0.0021 ± 0.0001	1.1027
castor crude oil	0.0008 ± 0.0002	1.1875
cotton crude oil	0.0017 ± 0.0002	1.1188
soybean biodiesel	0.0021 ± 0.0001	0.8395
canola biodiesel	0.0023 ± 0.0001	0.7766
cotton biodiesel	0.0019 ± 0.0003	0.8553

chosen to avoid the small explosions that may occur with these types of samples if rapid heating is used. The modifier was injected and dried, together with the sample. The final optimized furnace program for determinations of As in vegetable oil or biodiesel samples is shown in Table 1.

3.2. Stability. The stability of As in the microemulsion medium was evaluated for a period of 300 min in the vegetable oil or biodiesel microemulsion solutions spiked with 100 ng mL⁻¹ of inorganic or organometallic As. A calibration solution containing the same As concentration was also investigated. As shown in Figure 2, the signal was stable along the monitoring time, indicating stability of the analyte in these media.

3.3. Sample Analysis and Analytical Figures of Merit. Initially, the sample microemulsions of vegetable oil and

Energy Fuels 2009, 23, 5942-5946 · DOI:10.1021/ef900709q

Table 3. Slopes and Figures of Merit Relative to the Analyte Addition Calibration Curves of Vegetable and Biodiesel Microemulsions at a 100× Sample Dilution

	ino		organometallic		
As form	slope (L μg^{-1})	R	: :	slope (L μg^{-1})	R
		Vegetable Oil			
soybean crude	0.0029 ± 0.0001	0.99	98 0	$.0029 \pm 0.0001$	0.9997
cast or crude	0.0028 ± 0.0002	0.99	99 0	$.0029 \pm 0.0001$	0.9999
cast or refined	0.0030 ± 0.0002	0.99	96 0	$.0031 \pm 0.0001$	0.9992
cott on crude	0.0029 ± 0.0001	0.99	99 0	$.0030 \pm 0.0001$	0.9986
sunflower	0.0032 ± 0.0002	0.99	99 0	$.0033 \pm 0.0002$	0.9997
		Biodiesel			
soybean 90% + castor 10%	0.0032 ± 0.0001	0.99	98 0	$.0029 \pm 0.0002$	0.9997
soybean	0.0029 ± 0.0002	0.99	99 0	$.0030 \pm 0.0002$	0.9993
peanut	0.0033 ± 0.0001	0.99	99 0	$.0030 \pm 0.0002$	0.9986
J. curcas L.	0.0032 ± 0.0002	0.99	99 0	$.0029 \pm 0.0001$	0.9999
canola	0.0032 ± 0.0002	0.99	96 0	$.0031 \pm 0.0001$	0.9992
cotton	0.0033 ± 0.0002	0.99	99 0	$.0030 \pm 0.0002$	0.9996
	c	alibration media			
	slope (L μg^{-1})	R	linear range (L μg^-) in:	strumental LOD (L μg^{-1})
0.2% (v/v) HNO ^a	0.0029 ± 0.0001	0.9998			0.5
propan-1-ol + 0.2% (v/v) HNO3b	0.0028 ± 0.0002	0.9998	25-200		0.3
propan-1-ol + 0.2% (v/v) HNO3 ^c	0.0030 ± 0.0001	0.9997	2.7 200		0.3

" Inorganic As. b Inorganic As. COrganometallic As.

Table 4. Measured Concentrations of Inorganic or Organometallic As (μ g L⁻¹; n = 3) in Vegetable Oil and Biodiesel Sample Solution after the Addition of 50 and 150 ng mL⁻¹ by GFAAS

	inorganic	As addition	organometal	organometallic As addition	
	50 ng mL^{-1}	150 ng mL^{-1}	$50 \mathrm{ng} \mathrm{mL}^{-1}$	100 ng mL^{-1}	
		Vegetable Oil			
sovbean crude	51.7 ± 0.5	156±1	52.6 ± 0.5	155 ± 1	
castor crude	51.2 ± 0.6	150 ± 1	52.4 ± 0.7	155 ± 1	
castor refined	49.8 ± 2.1	160 ± 1	53.1 ± 1.3	148 ± 1	
cott on crude	52.3 ± 0.8	153 ± 2	52.4 ± 2.0	151 ± 2	
sunflower	51.9 ± 0.6	162 ± 2	55.2 ± 0.7	153 ± 1	
		Biodiesel			
sovbean 90% + castor 10%	48.7 ± 2.1	150 ± 2	47.3 ± 0.6	153 ± 1	
sovbean	49.8 ± 0.8	150 ± 1	54.2 ± 0.8	146 ± 1	
peanut	54.4 ± 2.1	159 ± 2	52.1 ± 0.6	153 ± 1	
L curcas L	53.1 ± 0.8	151 ± 1	50.4 ± 0.8	155 ± 1	
canola	48.5 ± 0.3	151 ± 1	51.8 ± 1.4	152 ± 1	
cott on	48.5±0.4	160 ± 1	47.9 ± 2.3	159 ± 1	

biodiesel were investigated using a $10 \times$ dilution factor, as described in section 2.3. However, with this dilution factor, it was observed that the slopes of the analyte addition curves were significantly different and related to the sample viscosity (Table 2). These results mean that analyte addition calibration ought to be used in this dilution scale. Because this mode of calibration is not desirable and there was range for further dilution, while still achieving the desired limit of detection, a dilution factor of $100 \times$ was then investigated.

Analyte addition calibration curves were prepared, spiking vegetable oil or biodiesel sample solutions with both inorganic or organometallic As. As shown in Table 3, the obtained slopes were similar and no significant difference (Student's t test, p < 0.05) is observed. The aqueous analytical curve of inorganic As in 0.2% (v/v) HNO₃ also showed the same slope, as well as that obtained with organometallic As diluted in the microemulsion medium. This means that both solutions can be used for external calibration. Linearity was observed up to a minimum of 200 ng mL⁻¹. The instrumental LODs were calculated as 3 times the standard deviation of 10 measurements of a blank divided by the slope of the calibration curve. Their values (0.5 and 0.3 ng mL⁻¹) demonstrate the excellent sensitivity of GFAAS for As. Considering the dilution factor, the LOD for the whole procedure was 0.3 mg kg⁻¹ in the original sample, well below the discussed limitation of 5 mg kg⁻¹. Average relative standard deviations (RSDs) of 3% were found for six consecutive measurements of sample solutions spiked with 150 ng mL⁻¹ of both inorganic and organometallic As.

Because no adequate certified reference material was available, accuracy was evaluated through recovery tests carried out at two levels (50 and 150 ng mL⁻¹). Spikes were performed in the original samples, and external calibration with aqueous calibration solutions was used, the results of which are shown in Table 4. The recoveries ranged from 95 to 110%, supporting the accuracy of the method. Because no available sample showed concentrations above the method LOD, the comparison with an alternative independent procedure (after acid mineralization, for instance) was not possible.

Energy Fuels 2009, 23, 5942-5946 · DOI:10.1021/ef900709q

4. Conclusions

The proposed method for the determination of As in vegetable oil and biodiesel samples by GFAAS is simple, easy, rapid, precise, and adequate for the limits expected from Brazilian legislation. The samples prepared as a three-component solution with propan-1-ol and nitric acid solution showed good stability, favoring accuracy and facilitating automation. Low-volatile and low-toxicity solvents were used. The determinations were carried out using external calibration with inorganic or organometallic As standard solutions. Recovery tests showed that the method can be used with good accuracy and precision within appropriate LODs. A study of the determination of Na, K, Ca and Mg in biodiesel samples by LS F AAS and CS HR AAS supporting the ABNT NBR 15556 norm

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Keywords: biodiesel, metals , LS-F AAS, HR-CS F AAS, ABNT NBR 15556

Introduction

Biodiesel represents today a real alternative to diesel for internal combustion engines. The use of biodiesel has the potential to lower the environmental impact of automobile transport, due to the reduction of the amount of emitted gaseous CO₂ to the atmosphere, contributing to the decrease of greenhouse gases emissions. Biodiesel is obtained by transesterification of vegetable oils (soybean, sunflower, palm, etc.) and animal fats using an excess of a primary alcohol (most commonly methanol) in the presence of a homogeneous or heterogeneous catalyst. The main reaction products are biodiesel and glycerol. Used vegetable oils are also considered a low cost source for obtaining biodiesel, permitting waste recycling. Feedstock composition will influence the properties of produced biodiesel.¹⁻⁴ and besides the traditional feedstock materials (vegetable oils or animal fat) the production of biodiesel using microalgae has been recently studied and appears to possess high production potential⁵.

For its adequate use, biodiesel must obey strict regulations and the determinations of Na, K, Mg and Ca are part of biodiesel characterization of pure biodiesel (B100) or blends with petroleum diesel (B2, B5, B10 etc). In the production process, sodium and potassium arises from the alkali catalysts (NaOH, KOH) used in the transterification reaction, while Mg and Ca come from the washing process. These elements should be removed during the biodiesel production process, but they can appear as contaminants in the final product. If present in the final product, Na, K, Ca and Mg can cause the formation of insoluble soaps, and consequently the formation of deposits in the filters of vehicles contributing to the corrosion of engine parts⁶. Thus, the determination of these elements is necessary, since their presence above certain levels can change the characteristics of use of the product. The current specification for biodiesel imposed by the Brazilian Fuel Agency (ANP)¹⁷, in consonance to the European and American regulations (EN 14108, EN 14109, EN 14538 e ASTM D6751

- 10), defines a limit of 5 mg kg⁻¹ as the maximum allowed concentration for Na + K or Ca + Mg.

In the literature, several articles have been published reporting methods for the determination of Na, K, Mg and Ca in biodiesel. Santos et al.¹⁸ reported a method for the simultaneous determination of Na, K, Mg, Ca and P in biodiesel samples by inductively plasma optical emission spectrometry (ICP OES), after a simple sample dilution with ethanol. The obtained limits of detection (LOD), in µg g⁻¹, were 0.1; 0.3; 0.005 and 0.03 for Na, K, Mg and Ca, respectively, in the original sample. Chaves et al.¹⁹ developed a method for the determination of Na and K in biodiesel by flame optical emission spectrometry (F OES). The samples were prepared as a microemulsion with propan-1-ol and aqueous acid solution. For calibration inorganic aqueous calibration solutions were used. The use of microemulsion was also proposed by Jesus et al.²⁰ for the determination of Na and K in biodiesel by AAS. The microemulsion was constituted of *n*-pentanol, Triton X-100 and water, and calibration was performed with aqueous calibration solutions. The LODs were 0.1 and 0.06 μ g g⁻¹ for Na and K, respectively. Castro e Silva et al.²¹ developed a method for the determination of Na by F OES using dry decomposition for the sample preparation. The LOD and LOQ (limit of quantification) in the original samples were 1.3 and 4.3 mg kg⁻¹, respectively.

Since Na, K, Mg and Ca determinations in biodiesel are of regulatory importance European and American normalization entities advocate the use of recommended analytical procedures for these determinations. For the determination of Na and K, European norms (EN 14108¹⁰ and EN 14109¹¹, respectively) propose sample dilution with xylene followed by flame atomic absorption spectrometry (F AAS) determination. For the determination of Mg and Ca, similar sample pre-treatment and ICP OES instrumental determination are recommended (EN 14538¹²); ASTM suggests similar procedures²⁴; in all these cases calibration is performed with organic-metallic standard solutions, diluted in xylene, and care must be taken in the viscosity matching between the calibration and sample solutions. Recently, ABNT (Associação Brasileira de Normas Técnicas – Brazilian Society for Technical Norms) has launched a series of norms, related to the determination of Na, K, Mg and Ca in biodiesel. ABNT advocates sample dilution with xylene and instrumental determination by F AAS for Na (NBR 15554)¹⁴ and K (NBR 15555)¹⁵, and ICP OES for Mg and Ca (NBR 15553)¹³, following the same trend of the European and American norms. However, ABNT norm NBR 15556 has, in addition introduced the determinations of Na and K, as well as Mg and Ca by F AAS, in contrast to European and American norms, that recommend the use of F AAS technique only for Na and K.

Even though, the recent introduction of continuous source high resolution spectrometers for atomic absorption measurements indicates the possibility of improvements in the analysis performance. High-resolution continuum source atomic absorption spectrometry (HR-CS AAS) has a number of advantages over the line source approach (LS AAS) in the determination of trace elements in complex matrices due to its improved background correction capabilities, the visibility of the spectral environment around the analytical line, the increased primary source intensity, the neighboring pixels baseline compensation and the possibility of sequential multielementar determination. Thus, limits of detection, analysis speed and accuracy are improved, in relation to the conventional LS AAS.

The present work presents the studies that have supported the proposal of the ABNT NBR 15556 Norm, and also compares the performance of LS F AAS and HR CS AAS in the application of this norm.

Experimental

Instrumentation

The measurements were carried out in two AAS spectrometers: a conventional line source atomic absorption spectrometer model 1100B (Perkin Elmer, Norwalk, CT, USA) with a flame atomizer and a continuous (deuterium lamp) background correction system. Na, K, Ca and Mg hollow cathode lamps (Varian, Monash, Austrália) were used as line sources. The continuous source atomic absorption spectrometer was a model ContrAA 300 (Analytik Jena, Jena, Germany) with a flame atomizer, a xenon short-arc lamp with in hot spot mode (as the primary continuum radiation source), an Echelle double monochromator and a CCD line detector. In the HR CS AAS a simultaneous evaluation of 200 pixels, which corresponds to a spectral environment of about \pm 0.2 nm around the central pixel is used. The instrumental parameters of both equipments are displayed in Table 1, and their optimization is discussed in the next section. The central \pm 3 pixels were used for the measurements. The samples were weighed using a microbalance Ohaus Adventurer AR 2140 (NJ, USA) with a precision of \pm 0.0001 g.

Instrumental parameters	LS F AAS			CS HR F AAS					
	Na	К	Mg	Ca	Na	К	Mg	Ca	
Lamp current (mA)	10	10	4	4		Hot spot mode			
λ (nm)	589	766.5	285.5	422.5	588.99	766.49	285.21	422.67	
Slit (nm)/number of pixels	0.8	0.8	1.2	1.2	7	7	7	9	
Integration time (s)	3.5	3.5	3.5	3.5	3.0	3.0	3.0	3.0	
Aspiration rate (mL min ⁻¹)	1.5	1.5	1.5	1.5	1.6	1.6	1.6	1.6	
Acetylene Flow (L min ⁻¹)	2.2	2.2	2.2	6.5	0.7	0.7	0.7	4.3	
Observation height (cm)	0.5	0.4	0.6	0.6	0.5	0.4	0.6	0.4	
Nitrous oxide Flow (L min ⁻¹)				7.0				10.2	
Air Flow (L min ⁻¹)	11.5	11.5	11.5		12.8	12.8	12.8		

Table 1	-	Instrumental	parameters.

Materials, reagents, solutions and samples

All reagents were of analytical reagent grade. Xylene P.A. and mineral oil (viscosity ranging from 10.8 mm²/s to 13,6 mm²/s and specific mass = 0856 g/mL, both Vetec, Rio de Janeiro, Brazil) were used. Individual Na, K, Mg and Ca 1000 µg g⁻¹ standard (Spex, Metuchen, USA), as well as an multielemental organometallic 885 µg g⁻¹ standard solution (S21+k, Conostan, Champlain, USA) were used for preparing the calibration solutions. The diluent solution (mineral oil in xylene, 120 g L⁻¹) was prepared by diluting 120 g of mineral oil with xylene to 1000 mL in a volumetric flask. This solution was used as a diluent for the calibration solutions and blanks. Biodiesel samples from different feedstock were investigated, as shown in continuation.

All plastic and glassware were washed with tap water, immersed in Extran (48 h), rinsed with tap and deionized water, and immersed in 20% (v/v) HNO_3 for at least 24 h. Before use, these materials were thoroughly rinsed with ultrapure water.

Procedure

The samples were prepared in accordance to the procedure described by ABNT NBR 15556: Approximately 1.0 g of biodiesel was accurately weighed in a 15 mL polypropylene flask and dilute to 10 mL with xylene, and homogenized. Calibration solutions were prepared from appropriate dilution of the standard solutions with the mineral oil in xylene solution (120 g L⁻¹) for matching their viscosity with those of the sample solutions. The samples solutions were then analyzed by F AAS according to the instrumental parameters shown in table 1.

Results and discussion

Viscosity adjustment of the calibration solutions using mineral oil

Special attention must be given to the question of the viscosity matching between the sample and calibration solutions, also considering that biodiesel of different origins present different viscosities. Since the dilution factor of the samples were previously defined (1+9), in order to minimize the viscosity differences between biodiesel samples and calibration solutions, mineral oil was added to the calibration solutions to promote this adjustment: The viscosity of mineral oil in xylene solutions with different concentrations were measured and the results obtained are shown in figure 1. The viscosities of sample solutions of biodiesel of different origins (1+9) diluted in xylene were also measured and showed viscosity values ranging from 0.82 to 0.94 cP. According to figure 1, for this range of viscosity, the mineral oil in xylene solution with the closest viscosity matching corresponds to a concentration of 120g/L, which was then used as diluent and final medium for the calibration solutions.



Figure 1 – Variation of the viscosity of mineral oil in xylene solutions as function of the mineral oil concentration. Remember that the viscosity of pure xylene is 0.64 Cp.

Parameters optimization

Observation height (measured by the burner height), flame composition (measured by the acetylene flow) and aspiration rate were optimized using a complete (3³) factorial planning. At first, the line source equipment was used. The sensitivity, measured by the slope of the calibration curves taken in each of the studied conditions was used as response. The levels are shown in table 2.

		Levels		Levels			
Factors		Na, K e Mg		Са			
	-1	0	1	-1	0	1	
1 - Burner height (cm)	0	0.5	1	0	0.5	1	
2 - Acetylene flow (L min ⁻¹)	2	2.5	3	4	5.5	7	
3 - Aspiration rate (mL min ⁻¹)	1	1.5	2	1	1.5	2	

Table 2 – Factors and their respective levels in the 3³ factorial planning.

Pareto charts for the 4 elements are displayed in Fig 2. For Na, the significant effects were the aspiration rate (linear), observation height (linear), and acetylene flow (linear). For K besides these three effects the interaction between aspiration rate (linear) and acetylene flow (linear) was also significant. For Mg, the significant effects were the aspiration rate (linear), the observation height (linear) and the interaction between the observation height (linear) and the acetylene flow (linear) and the acetylene flow (linear). For Ca, only the aspiration rate (linear) and the acetylene flow (linear) were significant.



Figure 2 – Pareto charts relatively to the factorial planning of the determination of Na, K, Mg and Ca in biodiesel by F AAS; 1 = burner height (cm), 2 = acetylene flow (I min⁻¹) and 3= aspiration rate (mL min⁻¹).

Fig 3 shows some of the response surfaces for the studied elements. In the case of K, Mg and Ca, it is possible to observe that the critical point was out of the studied range. However, due to practical limitations, it was not logical to perform further experiments in the direction of the indicated maximum sensitivity. For instance, the surface response for K indicates that the maximum response must be expected at larger aspiration rates. However, aspirations rates above 1.5 would lead to a too lean flame (an organic solvent is being aspirated), and a correspondent increased noise. Similar considerations should be made to Mg and K as well. Thus, table 1 displays the optimum values, taking into account these practical aspects. Similar optimization was performed with the continuous source equipment, and the optimized parameters are also displayed in table 1.

The calibration curves were prepared according to the ABNT NBR 15556 norm, with calibration solutions of 0.10, 0.20, 0.30, and 0.40 mg/L.



Figure 3 – Response surfaces arosen from the optimization experiment in the determination of Na, K, Mg and Ca in biodiesel by F AAS.

Calibration

Since biodiesel of different origins present different compositions (and consequently, different properties, such as viscosity and density), the matrix influence was investigated by observing the slopes of analyte addition curves with biodiesel samples of different feedstocks. Calibration curves in xylene were also investigated. The analyte addition calibration curves were prepared by spiking biodiesel samples with appropriate microvolumes of the organometallic standard solutions and diluting (1+9) with xylene and the external calibration curve were prepared as described in section procedure. The results (Figures 4 and 5) show that no multiplicative matrix effects were observed (F AAS and HR-CS F AAS) in relation to the external calibration curve prepared in 120 g.L⁻¹ mineral oil in xylene solution. Thus, external calibration using this calibration curve could be performed. Note that the added concentrations were well below the limits permitted to biodiesel by the legislation. This was due to the fact that most of the samples received in the laboratory are in this concentration range. A further experiment was made using a 3.0 mL min⁻¹ aspiration rate. In this case, the slopes values for the analyte addition curves were different when compared with the slopes values of the external calibration curve in xylene, confirming that the lower values displayed in Table 2 must be used.

Sample and sample solution stability

Literature²² indicates that metal solutions in organic media may prove unstable, and analytical signal tends to decrease with time. Calibration and sample solutions prepared as described in item procedure proved unstable, dropping down to up to 18% of their original concentration value after 2.5 h standing in glass or plastic flasks. Similar results were found by Jesus et al.²⁰, who proposed the stabilization of these solutions by microemulsion formation. Thus, due to this lack of stability, it is important to analyze the calibration and sample solutions as soon as possible after their preparation, making it difficult the use of the whole autosampler carrousel. In relation to stability of the original sample, no information was found in the literature, considering the determination of these metals in biodiesel. Thus, the following experiment was made: a biodiesel sample was prepared in the laboratory by the transesterification of a soy vegetable oil with methanol, using NaOH as catalyst. According to the usual procedure²³, the obtained product should be washed 3 times. However, only one washing procedure was performed, in order to obtain a product with a high Na content. Just after its preparation, the biodiesel sample obtained was analyzed each 24 h, using for calibration freshly prepared calibration solutions. As can be seem in Fig 6, the Na content dropped along the measurements, while K concentration kept itself reasonably constant. This behavior must be taken into consideration for an accurate analysis.



Figure 6 – Na and K response stability in biodiesel samples prepared with NaOH and KOH as catalysts, respectively.

Accuracy studies for the determination of Mg and Ca

Since the determination of Mg and Ca in biodiesel by AAS, proposed in the present study, is in contrast to well established norms, that recommend the use of ICP OES for this determination, a comparative study was performed. Biodiesel samples were analyzed by the proposed AAS procedure and the usual EN 14538 procedure (ICP OES determination). No significant difference was found between the results obtained by both procedures (Fig 7 e 8) confirming their equivalence.



Figure 7 – Comparison of the Ca content in biodiesel samples prepared from different feedstocks by the proposed NBR 15556 AAS procedure (\blacksquare) and by ICP OES according to the EN 14538 norm (\blacksquare).



Figure 8 – Comparison of the Mg content in biodiesel samples prepared from different feedstocks by the proposed NBR 15556 AAS procedure (■) and by ICP OES according to the EN 14538 norm (■).

Analytical figures of merit and accuracy

These experiments were performed as described in the Experimental section both by LS F AAS or HR-CS F AAS at the optimized conditions for each instrument. In both instruments, the linearity was observed up to at least 0.4 mg L⁻¹. The instrumental limits of detection (LOD) were calculated as three times the standard deviation of 10 measurements of a blank (in the present case a solution of mineral oil in xylene) divided by the slope of the respective calibration curve for each analyte. The LOD was calculated for the original sample, considering the dilution factor. According Table 3, the LOD obtained using the HR-CS F AAS showed improvements for all elements evaluated, in comparison of the limits obtained using LS F AAS, although they are all well below the limits determined by the legislation. Larger sensitivities for Na, K and Mg were also observed for the continuous source system, and this is probably due to the better fitting of the primary source spectral shape to the absorption line profile promoted by this equipment for these elements. Regarding the analysis time, using the HR-CS F AAS spectrometer, the time was 5 times reduced due to the capacity of sequential determination of instrument. However, there is no impediment for performing all these determinations by LS F AAS.

	HR CS	FAAS	LS F AAS		
	Slope (mg kg ⁻¹)	LOD* (mg L ⁻¹)	Slope (mg kg ⁻¹)	LOD* (mg L ⁻¹)	
Na	1,639	0.009	0.853	0.02	
К	0.508	0.002	0.305	0.05	
Mg	2,479	0.005	1,850	0.01	
Ca	0.197	0.03	0.242	0.16	

Table 3 - Figures of merit.

* LOD calculated for the sample (dilution 1:9)

The repeatability and intermediate precision were established by the analysis of calibration solutions with concentrations within the working range. They were analyzed along 7 successive days, with 5 replicates for each concentration level, which were 0.15, 0.25 and 0.35 mg L^{-1} . The results obtained for both equipments are displayed in table 4.

			F	IR CS FAA	S			
Repeatability (C.V. %)					Intermediate precision (C.V. %)			
mg L ⁻¹	Na	ĸ	Mg	Ca	Na	к	Mg	Са
0.15	0.77	1.97	0.65	1.12	1.33	3.44	0.80	2.04
0.25	0.67	2.08	0.58	0.77	2.94	3.57	2.04	5.10
0.35	0.71	1.60	0.78	1.40	1.69	3.13	3.52	6.36
				LS FAAS				
	Repea	atability (C	.V. %)		Inter	mediate pr	ecision (C.	V. %)
mg L ⁻¹	Na	ĸ	Mg	Ca	Na	к	Mg	Са
0.15	0.52	1.20	0.35	3.04	1.87	1.56	0.96	3.22
0.25	0.23	0.62	0.29	1.11	2.93	1.27	0.50	7.95
0.35	0.37	0.83	0.20	0.82	1.60	1.63	0.53	7.92

Table 4 – Repeatability and intermediate precision (CV, %) obtained for HR CS F AAS and LS F AAS at three concentration levels (n=5, 7 days of measurements).

Biodiesel samples of different origins were analyzed as recommended by the NBR ABNT 15556 norm. The determinations were conducted in parallel in both spectrometers, by two operators in distinct order to avoid any influence of the stability of the samples or environmental factors. Concordant results were found (paired t test, p<0.05) for all elements in the analysis of samples by the two instruments. It is important take into consideration that the samples and calibration solutions must prepared just before the analysis, in order to avoid stability problems of the diluted solutions, already described in the literature²⁰. The results obtained are presented in table 5.

Table 5 – Concentrations (mg kg⁻¹) for Na, K, Mg and Ca obtained by HR-CS F AAS and LS F AAS in biodiesel samples (n=3).

	HR-CS	FAAS	LS F AAS		
	Soybean	Castor	Soybean	Castor	
Na	0.54 ± 0.01	0.93 ± 0.04	0.59 ± 0.01	0.98 ± 0.06	
к	-	9.20 ± 0.60	-	10.00 ± 0.30	
Mg	0.59 ± 0.08	-	0.47 ± 0.03	-	
Ca	-	2.11 ± 0.02		2.09 ± 0.01	

Since no certified reference material is available, accuracy was first assessed by recovery tests, performed with 5 biodiesel samples of different origins. The samples were spiked with 2 and 3 mg L^{-1} of Na, K, Mg and Ca, respectively. Recoveries ranged from 91 to 103%, as displayed in Table 6.

Table 6 – Recoveries (%) of Na, K, Mg and Ca at two concentration levels in the analysis of biodiesel samples from different origins by the NBR 1556 norm (n=5).

				HR CS FAAS	5			
		2 mg L ⁻¹				3 m	g L ⁻¹	
	Na	к	Mg	Ca	Na	к	Mg	Ca
Palm	99.76%	95.62%	101.15%	94.03%	99.21%	101.65%	99.78%	100.12%
Castor	96.95%	114.30%	101.65%	90.25%	99.95%	113.67%	104.09%	95.47%
Sunflower	100.84%	103.49%	100.50%	96.15%	96.18%	89.15%	86.82%	101.83%
Soybean	99.21%	97.54%	102.23%	91.95%	99.07%	114.97%	106.99%	104.87%
Peanuts	98.26%	98.77%	103.41%	95.75%	99.60%	101.60%	103.87%	104.84%
				LS FAAS				
		2 mg L ⁻¹				3 m	g L ⁻¹	
	Na	к	Mg	Ca	Na	к	Mg	Ca
Palm	91.51%	92.22%	92.48%	94.19%	91.59%	89.71%	90.66%	92.59%
Castor	93.99%	116.22%	92.87%	105.59%	92.93%	104.14%	89.38%	103.45%
Sunflower	92.81%	106.34%	91.57%	82.22%	93.24%	104.14%	91.65%	89.68%
Soybean	95.63%	104.45%	93.32%	93.41%	95.36%	100.38%	91.91%	93.56%
Peanuts	97.98%	95.98%	93.83%	92.84%	95.59%	92.53%	89.19%	94.00%

Accuracy was also assessed by the participation in an interlaboratorial exercise promoted by ASTM.

 Table 7 – Resultados obtidos por programas interlaboratorial em biodiesel.

NBR 15556							
Concentration (mg Kg ⁻¹)							
	Biodiesel 1 Biodiesel 2 Biodiesel 3						
Na	4.41 ± 0.03	2.42 ± 0.03	0.30 ± 0.00				
K	0.11 ± 0.03	< 0.05	< 0.05				
EN 14108 (Na) e EN 14109 (K)							
Concentration (mg Kg ⁻¹)							
	Biodiesel 1	Biodiesel 2	Biodiesel 3				
Na	5.03 ± 0.08	2.93 ± 0.04	0.39 ± 0.03				
К	0.15 ± 0.02	< 0.05	0.060 ± 0.04				

Conclusions

Na, K, Mg and Ca could be determined in biodiesel samples obtained from different feedstock by F AAS, with limits of detection adequate for biodiesel characterization according to the current legislation. A 120g/L mineral oil in xylene solution provided the adequate medium for the preparation of the calibration solutions, due to the viscosity matching between calibration and sample solutions. In this way response in biodiesel samples of different origins was equivalent and external calibration could be performed, by the dilution of standard organic solutions in the 120g/L mineral oil in xylene medium. In comparison to the determination of these metals in aqueous medium, much lower aspiration rates should be used, otherwise the flame became too lean and multiplicative matrix effects present. Magnesium and Ca concentrations found in biodiesel samples of different origins by the studied F AAS procedure, were equivalent to those found by ICP OES following the EN 14538 norm.

The use of HR-CS F AAS provided higher sensitivity and better limits of detection. However, the main advantage was in relation to the analysis time, which was reduced in 5 times due the possibility of sequential determination offered by the instrument. Recovery tests showed that both instruments can be used with good accuracy and precision within appropriate limits of detection for these determinations.

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Figure 4 – Analyte addition and external calibration curves in xylene obtained using LS F AAS.
(+) xylene; (×) castor biodiesel; (■) soybean biodiesel; (*) canola biodiesel; (●) 90% soybean
+ 10% castor biodiesel; (●) cotton biodiesel and (▲) babassu biodiesel.



Figure 5 – Analyte addition and external calibration curves in xylene obtained using HR- CS F AAS. (+) xylene; (×) castor biodiesel; (■) soybean biodiesel; (*) canola biodiesel; (●) 90% soybean + 10% castor biodiesel; (●) cotton biodiesel and (▲) babaçu biodiesel.

Determination of silicon in vegetable oil and biodiesel by high-resolution continuum source flame atomic absorption spectrometry (HR-CS F AAS)

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In this study, a simple procedure for the determination of Si in vegetable oils and biodiesel of different origins by high-resolution continuum source flame atomic absorption spectrometry is proposed. The samples were diluted with xylene (1+9) and, external calibration was performed with calibration solutions prepared with xylene and base oil, used for viscosity matching. Since no adequate certified reference material is available, accuracy was assessed by recovery tests. Recoveries at 3 and 5 mg L⁻¹ levels using organic Si standard spiked solutions ranged from 93 to 113%. The limit of detection at the optimized conditions in the original sample was approximately 1.0 mg kg⁻¹, which is adequate for the analysis of vegetable oils and biodiesel according to the expected in the Brazilian legislation in discussion.

Keywords: silicon; vegetable oil; biodiesel; HR-CS F AAS.

Most of all energy consumed worldwide comes from oil, coal and natural gas. These sources are limited and estimates lead to scenarios in which they will come near to exhaustion in a near future. Their use is also the main cause of CO_2 release into the atmosphere, contributing to the increase of the greenhouse effect and global warming. Therefore, the search for alternative energy sources turns very important¹. In this context, biodiesel appears as an alternative in the replacement of regular diesel in compression ignition engines. Its use was tested in the late 19th century, producing satisfactory results in diesel engines²⁻⁴. The use of agricultural fuels in diesel engines is very attractive in environmental and security contexts, since they are a renewable energy source, lower the CO_2 release and reduce the world oil dependence⁴⁻⁶.

Biodiesel is a mixture of fatty acid esters, derived from the transesterification of triglycerides of oils and fats with short chain alcohols, resulting in glycerol as a coproduct. The transesterification is performed in the presence of catalysts, and homogeneous alkaline catalysts are the most utilized, since they are more efficient, promoting high yields. Among these, alkoxides are more active, leading to yields of 98%. However, they are also the most sensitive to the presence of water. Sodium and potassium hydroxides, although less active, present lower costs and promote satisfactory yields, and, therefore, are the most widely used.^{7,8}

Brazilian Law n° 11.097/2005 imposes the mandatory addition of 2% biodiesel to diesel (B2) from January 2008 onwards, along with a progressive biodiesel increment, reaching B5, to be implemented until 2013. In parallel, the National Energy Policy Council (CNPE), by its resolution n° 2/2008, imposed the addition of 3% biodiesel to diesel (mixture B3) from July 1, 2009 onwards. This measure, while also reducing the contribution of mineral diesel in the Brazilian energy matrix, ensures biodiesel market

and production. These deliberations are part of the National Biodiesel Program, created alongside the laws. The raw materials available for biodiesel in Brazil is extremely diverse: The warmer areas of the country are favorable for the cultivation of castor beans, palm (palm), babaçu, peanuts, *Jatropha curcas L*, sunflower, cotton and soybean. All these vegetables can produce their respective vegetable oil that can be processed into biodiesel. Also, a well established cattle meat productive chain assures the availability of large amounts of animal fat, a raw material for biodiesel production too.

This diversity underlines to the need of ensuring biodiesel quality. The specifications for B100 to be blended with conventional diesel fuel are established by the Brazilian National Agency of Petroleum, Natural Gas and Biofuels (ANP) by Resolution N° 07/2008, which has replaced Resolution N° 42/2004, turning the quality parameters of Brazilian biodiesel stricter. Inorganic contaminants in biodiesel, if present above certain levels, will degrade the performance and integrity of the engine, the quality of emissions or cause changes in the biodiesel characteristics during transport and storage. Thus, among the tests required for biodiesel specification, the determination of metals such as Na, K, Mg and Ca is important, since these elements have the ability to react with esters or free fatty acids and form insoluble soaps. These soaps can cause filter clogging and damage to the injection system (deposits)¹⁰. Current Brazilian specifications for biodiesel imposed by ANP define a limit of 5 mg kg⁻¹ as the maximum permissible concentration for Na+K or Ca+Mg, and 10 mg kg⁻¹ for P⁷. European¹¹⁻¹³, as well as Brazilian, norms¹⁴⁻¹⁵ recommend the determination of Na, K, Mg and Ca in biodiesel by spectrometric techniques such as F AAS and ICP-OES. Today many approaches for the determination of these elements in biodiesel samples exist, and several articles describing methods have been published, some of them slight variations of the norms, always taking into consideration the sensitivity and accuracy required for this kind of analysis¹⁶⁻²¹.

The vegetable oils used in the production of biodiesel can assimilate several metals from soil and water along the vegetal growing cycle, and by contact with the metallic tubing and walls of the transport or storage system. Arsenic, for example, may be present because it is absorbed from the soil by the plant, or it may be incorporated during the industrial production and storage processes. Fertilizers or insecticides are also a potential As source²².

Among the strategies for the determination of metals in vegetable oils and biodiesel²² are the prior mineralization of the sample, performing the instrumental analysis in aqueous medium, the formation of emulsions or microemulsions²⁴⁻²⁶, which also allow calibration with aqueous solutions, the direct analysis by GF AAS,²⁷ and dilutions with a suitable solvent, in this case using organometallic standards in the calibration solutions¹⁶⁻¹⁹. These methods usually involve atomic spectrometry instrumental determinations, due to their adequate sensitivity, accessibility and widespread use.

Regarding silicon, specifications for this element are under discussion, and the expected limit will probably be near the 5 mg kg⁻¹ threshold²³. Thus, analytical procedures that can detect Si in these samples at this level will be necessary, and, so far, no recommended methodology is described for biodiesel, and only one analytical study was found in the literature³.

High-resolution continuum source flame atomic absorption spectrometry (HR-CS AAS) is a recent breakthrough in relation to the conventional line source AAS, due to its enhanced background correction capabilities, the visibility of the spectral environment around the analytical line, enhanced dynamic range and improved limits of
detection. Also, sequential analysis is made possible, what is especially advantageous if multielemental analysis is necessary, as it happens in biodiesel characterization. Thus, the objective of the present study is to take advantage of HR-CS AAS for the determination of silicon in vegetable oils and biodiesel samples.

2. Experimental Section

2.1. Instrumentation

Silicon measurements were conducted with a continuum source atomic absorption spectrometer model ContrAA 300 (Analytik Jena, Jena, Germany) with a flame atomization system. This equipment is provided with a xenon short-arc lamp, operating in hot spot mode as the continuum radiation source (190 – 850 nm), an Echelle double monochromator and a CCD line detector. The analytical wavelength of 251.611 nm was adopted, with a simultaneous evaluation of 200 pixels, corresponding to a spectral environment of approximately \pm 0.2 nm around the central pixel. Atomization was performed using an acetylene-nitrous oxide flame, formed at gas flows of 280 and 613 L/h, respectively. The aspiration rate was 1.1 L min⁻¹, adjusted for all samples and calibration solutions, and the read time was 5 s. All measurements were made in absorbance. Samples were weighed in a Ohaus Adventurer AR 2140 (Pine Brook, NJ) microbalance with a precision of 0.0001 g.

2.2. Materials, reagents, solutions and samples

Acetylene and nitrous oxide (both 99.99%) were provided by Linde (Rio de Janeiro, Brazil). All reagents were of analytical reagent grade. Analytical reagent grade xylene and mineral oil (viscosity = $75 \text{ mm}^2/\text{s}$) were from Vetec, Rio de Janeiro, Brazil. An organometallic standard solution (Conostan, Houston, USA) with Si concentration

of 1000 mg kg⁻¹ was diluted to 100 mg L⁻¹ Si using xylene as diluent. The calibration solutions were obtained from adequate microvolume dilutions of this solution in a 120 g L⁻¹ mineral oil solution in xylene for viscosity adjustment. The 120 g L⁻¹ solution of oil in xylene was prepared diluting 120 g of mineral oil to 1000 mL in a volumetric flask with xylene. Six types of vegetable oil samples from different origins were analyzed: three types of soybean oil (crude, milled and degummed), and castor, corn and sunflower oils. Six biodiesel samples from different oil sources were also analyzed: soybean crude, canola, cotton, peanut, barbados nut (*Jatropha curcas L.*) and a mixture of soybean (90%) and castor (10%).

2.3. Analytical Procedure

Approximately 1.0 g of vegetable oil or biodiesel was accurately weighed in a 15 mL polypropylene flask and diluted to 10 mL with xylene. Calibration solutions were prepared from the intermediary (100 mg L⁻¹) Si organometallic solution and diluted with the stock solution of oil in xylene (120 g L⁻¹), in order to match the viscosity of these solutions with those of the diluted samples. These solutions were then analyzed by HR-CS AAS, as shown in Table 1.

3. Results and Discussion

3.1. Optimization of the instrumental parameters

Among the most important non-spectral variables to be evaluated in the flame atomic absorption technique are flame stoichometry, defined by the flow of the flame gases, the rate of sample aspiration and the observation height in the flame. In the case of sample introduction of aqueous solutions these variables are well defined, but in the case of organic solutions, their values can differ greatly. In previous studies, the need to work with the most possible oxidizing flame was verified, in order to compensate for the fuel supply originating from the calibration solutions and samples (diluted in xylene), as well as a minimum aspiration rate, for the same reason. The choice of the observation height was done manually, by verifying which height generated the maximum absorbance. Given that procedures present in Brazilian norms for the determination of Na, K, Ca and Mg indicate a 1 + 9 dilution of the sample with xylene, and, since we sought to use the same sample solution in the determination of Si, this same dilution was chosen in the present study.

For external calibration, as occurs for other procedures in which sample pretreatment consists only of a dilution with an organic solvent, it is necessary to adjust the viscosity of the calibration solutions, as shown in Fig. 1. From this study, a 120 g L^{-1} mineral oil solution in xylene was chosen as the diluting medium, since in this way the calibration solutions show a final viscosity similar to those of the diluted samples. Table 1 summarizes the optimized conditions

3.2. Calibration studies

In order to define the calibration mode, the slope of an external calibration curve was compared to those obtained from analyte addition curves relative to samples of vegetable oil or biodiesel from different origins were compared. The results are presented in figs. 2 and 3. No significant differences (t test, p<0.05) were observed between these slopes. Table 2 shows the slope values. Possible spectral interferences were also analyzed, since the equipment allows the visibility of the spectral environment around the analytical line, and were found absent.

In the absence of reference materials for these kinds of samples, and lacking samples with silicon content high enough for comparing the proposed procedure with an independent one, the accuracy of the method was evaluated by recovery tests. Additions of Si as an organometallic salt in concentrations of 3 and 5 mg L^{-1} were performed on the original samples of vegetable oil or biodiesel. The results, presented in Table 3 show that recoveries ranged between 93 and 113%, acceptable for these concentration levels.

3.4 Figures of merit

The working range of the calibration curve was selected based on the range of analyte concentrations (maximum permissible concentration of 5 mg L^{-1}) that should be analyzed. However, this curve was found to be linear up to, at least, 50 mg L^{-1} . From the calibration curve, the characteristic concentration, limit of detection (LOD) and linear determination coefficient were obtained.

The LOD value in the present work was estimated from the following equation $(1)^{28}$:

$$LOD = (3x S_B)/S$$
 Eq. (1)

where S_B is the standard deviation of 10 measurements of the blank and *S* is the slope of the calibration curve, in the best working conditions. In the case of the LOD the dilution factor (10 times) for the sample was taken into account, what certainly underestimates the uncertainty associated with this step. However, since this is usually much lower than the uncertainty of instrumental measurement, in this level of concentration, it may be considered negligible. Table 4 presents the figures of merit obtained.

The precision of the method was evaluated by estimating the repeatability and reproducibility. For repeatability, four concentration levels were investigated (1.5; 2.5;

3.5 and 4.5 mg L^{-1}). For reproducibility, the procedure was repeated for six consecutive days, in which two analysts were alternated. The method proved to be repetitive for all the studied levels, since the coefficients of variation were all below 3%. The same occurred with the reproducibility, with all coefficients of variation below 5.5%. According to Wood²⁹, for the concentration levels taken for the present study, the coefficients of variation for repeatability and reproducibility should be lower or equal to 13,6%, which occurred in the present study.

4. Conclusions

Results indicate that it is possible to perform the determination of Si in vegetable oil and biodiesel by HR-CS F AAS, after simple sample dilution with xylene, using external calibration with a calibration solutions prepared in a solution of base oil in xylene, for viscosity adjustment. The procedure is fast and simple, resulting in good precision and accuracy. The limit of detection (LOD) in the original sample, was of 1.0 mg kg⁻¹. This value is adequate to meet the Brazilian specifications (still under discussion), that indicate a possible maximum permissible limit of 5 mg kg⁻¹.

5. References

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Figure 1 - Viscosity of the calibration solutions as function of the mineral oil concentration in xylene. In the square, the viscosities of the sample solutions as presented to the instrument (10x diluted in xylene) are shown: Vegetable oil: (\diamond) castor, (\square) sunflower, (\triangle) soybean crude, (\times) soybean milled, (*) soybean degummed and (\bullet) corn; Biodiesel: (\triangle) soybean crude, (\diamond) castor, (-) peanut, (\bigcirc) *Jatropha curcas L*, (\bullet) canola and (\square) cotton.



Figure 2 – Analyte addition curves for samples of vegetable oil in comparison to the (+) external calibration curve: (\diamond) castor, (\Box) sunflower, (\triangle) soybean crude, (×) soybean milled, (*) soybean degummed, and (\diamond) corn.



Figure 3 –Analyte addition curves for biodiesel samples in comparison to the (+) external calibration curve: (\triangle) soybean crude, (\diamondsuit) castor, (-) peanut, (\bigcirc) *Jatropha curcas L*, (\bigcirc) canola, and (\Box) cotton.

Parameter	
Wavelength (nm)	251.611
Number of pixels	200
Acetylene flow (l h ⁻¹)	280
Nitrous oxide flow (l n ⁻¹)	613
Aspiration rate (mL min ⁻¹)	1.1

Addition curves	Slope (mg L ⁻¹)
Vegetable oil	
Soybean (crude)	$0.0077 (\pm 0.0002) \text{ x} + 0.0015 (\pm 0.0003)$
Soybean (degummed)	$0.0078 (\pm 0.0001) \text{ x} + 0.0021 (\pm 0.0004)$
Soybean (milled)	$0.0076 (\pm 0.0002) \text{ x} + 0.0022 (\pm 0.0003)$
Sunflower	$0.0077 (\pm 0.0005) \text{ x} + 0.0031 (\pm 0.0005)$
Corn	$0.0075 (\pm 0.0003) \text{ x} + 0.0023 (\pm 0.0003)$
Castor	$0.0074 \ (\pm \ 0.0003) \ x + 0.0018 \ (\pm \ 0.0003)$
Biodiesel	
Soybean (crude)	$0.0071 (\pm 0.0007) \text{ x} + 0.0008 (\pm 0.0001)$
Castor	$0.0073 (\pm 0.0003) \text{ x} + 0.0007 (\pm 0.0001)$
peanut	$0.0076 (\pm 0.0005) \text{ x} + 0.0007 (\pm 0.0001)$
Jatropha curcas L	$0.0073 (\pm 0.0005) \text{ x} + 0.0016 (\pm 0.0003)$
canola	$0.0074 (\pm 0.0002) \text{ x} + 0.0011 (\pm 0.0001)$
cotton	$0.0073 (\pm 0.0002) \text{ x} + 0.0018 (\pm 0.0002)$
Xylene + mineral oil	0.0074 (± 0.0004) x

Table 2 – Slopes and intercepts of the external and analyte addition Si calibrationcurves in vegetable oil and biodiesel samples.

	3 mg L^{-1}	5 mg L^{-1}
Vegetable Oil		
Soybean (crude)	3.3 ± 0.1	5.7 ± 0.2
Soybean (degummed)	3.4 ± 0.1	5.6 ± 0.1
Soybean (milled)	3.2 ± 0.3	5.2 ± 0.2
Sunflower	3.3 ± 0.2	5.8 ± 0.4
Corn	3.1 ± 0.5	4.9 ± 0.3
Castor	3.4 ± 0.4	5.5 ± 0.2
Biodiesel		
soybean 90% + castor 10%	3.3 ± 0.2	5.1 ± 0.2
Soybean (crude)	3.4 ± 0.3	5.2 ± 0.3
Peanut	2.9 ± 0.4	5.5 ± 0.4
Jatropha curcas L	3.2 ± 0.6	5.6 ± 0.4
Canola	3.2 ± 0.4	5.3 ± 0.3
Cotton	2.8 ± 0.1	5.1 ± 0.3

Table 3 - Measured concentrations of organic Si (mg L⁻¹, n=3) in vegetable oil and biodiesel after the addition of 3 and 5 mg L⁻¹ 5 to the original sample by HR-CS F AAS.

Table 4 – Figures of merit.

50
0.63
< 0.9990
0.10
1.0