

Determination of Calcium and Magnesium in Biodiesel by Flame Atomic Absorption Spectrometry Using Microemulsions as Sample Preparation

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An alternative method for the determination of calcium and magnesium in biodiesel samples by flame atomic absorption spectrometry (FAAS) is proposed. The samples were prepared as water-in-oil microemulsions, and aqueous standards microemulsified in the same way were used for calibration. A study of long-term stability shows that the analytes remained stable in the microemulsions for at least 5 days. To assess the accuracy of the results, other methods were applied, such as recovery tests, as well as a comparison to the Brazilian Standard Method ABNT NBR 15556, which consists of the sample dilution with an organic solvent and a determination by FAAS. Limits of detection (LOD) of 0.04 and 0.1 $\mu\text{g g}^{-1}$ and characteristic concentrations of 30 and 6 $\mu\text{g L}^{-1}$ were obtained for calcium and magnesium, respectively. The method showed better LODs and precision in relation to dilution with organic solvent. The results obtained were statistically in agreement with those obtained with the Brazilian Standard Method at a 95% confidence level, and the recovery tests presented values between 92 and 117% for biodiesel samples. The main advantages observed in relation to the dilution method were the use of inorganic standards in the microemulsions for calibration, no use of carcinogenic organic solvents, and the higher stability of the analytes, besides the higher sensitivity. These advantages make the proposed method more appropriate for biodiesel routine analysis. The method was also tested for vegetable oil analysis.

1. Introduction

Because of the higher consumption of petroleum byproducts and the concerted efforts to reduce global warming caused by gas emissions, alternative fuels have attracted great attention from the worldwide market.¹ In the search for new sources of energy, biodiesel appears to be a promising alternative. Biodiesel is not only eco-friendly, because it reduces the current levels of environmental pollution both qualitatively and quantitatively, but is also a strategic source of renewable energy as a substitute for diesel oil and other crude oil byproducts.²

Fuel can be only used, without damaging engine performance, following established quality parameters.³ Calcium and magnesium concentrations are important parameters to ensure biodiesel quality. These metals normally come from the washing water, and their presence can form insoluble soap, giving rise to incrustations that can impair the functioning of a biodiesel-fueled engine.⁴

Methods for trace-metal determination in biodiesel are based on standard methods, which are certificated, although

their use reveals some difficulties. The European standard methods make use of flame atomic absorption spectrometry (FAAS) (EN 14108 and EN 14109)^{5,6} or inductively coupled plasma–optical emission spectrometry (ICP–OES) (EN 14538),⁷ with samples diluted with an organic solvent, such as xylene, cyclohexane, or petroleum ether. Nevertheless, the determination of calcium, magnesium, and phosphorus is only carried out by ICP–OES. This technique, even being a multi-element one, is very expensive because of the high cost of equipment and the high argon consumption. Its use is only justified when many elements need to be determined. Furthermore, the introduction of organic solvents in ICP–OES can extinguish the plasma,^{8–11} although some manufacturers have overcome this problem recently. Some authors have

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investigated the use of ICP techniques for biodiesel analysis.^{2,9–11} All of them reported the use of special conditions to solve the problems of the introduction of organic solvents in the plasma. Another problem related to the dilution method is the well-known low stability of the trace metals in organic solvents.^{12–15} A dry decomposition method of biodiesel samples was suggested to eliminate these problems,¹⁶ although this process is time-consuming and samples are prone to contamination.

Alternatives to the use of ICP–OES were proposed. When few elements need to be determined, the relation between the sample throughput and costs favors the application of FAAS, despite being a mono-element technique. Nowka has proposed the use of flame atomic emission spectroscopy (FAES) to determine alkali elements in biodiesel. Some advantages, such as low susceptibility to interferences and low equipment costs, were reported.¹⁷ Lyra et al.¹⁸ proposed the direct determination of phosphorus in biodiesel samples by graphite furnace atomic absorption spectrometry (GF AAS) using a solid-sampling accessory. Recently, in Brazil, following this trend toward suitable methods, a new standard method to determine Na, K, Ca, and Mg in biodiesel using dilution with organic solvents and determination by FAAS (ABNT NBR 15556)¹⁹ was published.

New methods for trace-metal determination in biodiesel samples using emulsions/microemulsions as sample preparation were recently proposed. This technique has been successfully applied for the preparation of fuel samples, because of the homogeneous dispersion and stabilization of the oil microdroplets in the aqueous phase, which brings the viscosity close to that of an aqueous solution and reduces the organic load of the system.⁸ An oil-in-water (O/W) emulsion was proposed for the determination of Co, Cu, Fe, Mn, Ni, and V in diesel and biodiesel by electrothermal vaporization–inductively coupled plasma–mass spectrometry (ETV–ICP–MS),¹¹ As by GF AAS,²⁰ and Cu, Pb, Ni, and Cd by GF AAS,²¹ while water-in-oil (W/O) microemulsions have been proposed for determination of Na and K in biodiesel by FAES²² and FAAS.¹⁴ The main advantages reported by the aforementioned authors are higher stability of analytes in the emulsified medium, feasibility of using inorganic standards for calibration instead of expensive and instable organometallic standards, and sample preparation speed and non-use of carcinogenic solvents.

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The goal of this work was to investigate a simple, fast, and low-cost method for calcium and magnesium determination in biodiesel samples, using microemulsions as sample preparation and FAAS as a quantification technique instead of ICP–OES. Investigations about the stability of the analytes in the microemulsions, optimization of instrumental parameters, and recovery tests were carried out. The performance and accuracy of the proposed method were assessed comparing the results to the adopted standard method for metal determination in biodiesel.

2. Experimental Section

2.1. Instrumentation. Measurements were carried out with an AAS 6 Vario atomic absorption spectrometer (Analytik Jena AG, Jena, Germany). Hollow cathode lamps of calcium (422.7 nm) and magnesium (285.2 nm) were employed as radiation sources, both operating at 4.0 mA and 1.2 nm of bandwidth. The fuel used was acetylene (White Martins, Brazil), and nitrous oxide (White Martins, Brazil) and compressed air were used as the oxidant for calcium and magnesium, respectively. Instrumental parameters, aspiration rate, and acetylene flow rate were optimized to obtain the maximum and stable absorbance signals. An analytical balance (Gilbertini, Italy) was used for the weighing of samples and reagents.

2.2. Reagents and Samples. All reagents used were of analytical grade. The distilled water was purified by a Milli-Q water purification system (Millipore, Bedford, MA), resulting in a specific resistivity of 18.2 M Ω cm. The containers and glassware were previously cleaned with 3 mol L⁻¹ nitric acid and rinsed with distilled water. The nitric acid (Merck, Germany) was purified in a quartz sub-boiling still (Kürner Analysentechnik, Roseheim, Germany). The surfactant Triton X-100 (Union Carbide, Danbury, CT) and the co-surfactants *n*-butanol and *n*-pentanol (Merck, Darmstadt, Germany) were used for microemulsion preparation. Base mineral oil (High Purity Standards, Charleston, SC) was used in the standards for calibration to simulate the samples in the microemulsions and in the dilution with xylene. Calcium and magnesium aqueous stock solutions (1000 mg L⁻¹) were prepared from calcium hydroxide and magnesium chloride (Merck, Darmstadt, Germany), respectively. Xylene (Nuclear, Brazil) was used as a solvent to dilute the samples and standards. Calcium and magnesium organic standards diluted in base mineral oil from calcium cyclohexanebutyrate (1000 \pm 0.008 mg g⁻¹) and magnesium 2-ethylhexanebutyrate (0.997 \pm 0.003 mg g⁻¹) (Specsol, São Paulo, Brazil) were used for the calibration in the dilution with xylene method.

The commercial biodiesel samples tested were obtained from different sources: soybean (B-92, B-116, and B-200), castor bean (B-13, B-14, and B-15), animal fat (P-05 and F-01), sunflower (B-48 and B-147), and mixtures of soybean/castor bean (B-128, B-178, and B-179). Three vegetable oil samples (soybean, sunflower, and rapeseed) were also analyzed.

2.3. Sample and Standard Preparation and Analysis. The microemulsions were obtained mixing 0.86 g of biodiesel, 0.62 g of Triton X-100, and 150 μ L of water, and the final volume of 5 mL was filled with the co-surfactant (about 2.6 g of *n*-butanol or *n*-pentanol). Nitric acid (1.4 mol L⁻¹) was added within the water component to increase the stability of the analytes. In the standards for calibration, inorganic standards were also added within the water component to obtain standards between 0.0 and 2.0 mg L⁻¹ of calcium or magnesium and base mineral oil was added to simulate the samples. For the determinations according to the dilution method (reference method ABNT NBR 15556),¹⁹ 1.0 g of biodiesel was diluted with xylene to the final volume of 10.0 mL. In the standards for calibration, base mineral oil substituted the samples and organic standards were added to obtain concentrations in the range of 0.0–2.0 mg L⁻¹.

Table 1. Analytical Figures of Merit for the Determination of Calcium and Magnesium in Biodiesel by FAAS Using Microemulsions and Dilution with Xylene Methods^a

analyte	method	linear regression equation (<i>R</i>)	<i>C</i> ₀ (mg L ⁻¹)	LOD (μg g ⁻¹)	LOQ (μg g ⁻¹)
calcium	dilution with xylene	$A = 0.1249c - 0.0059$ (0.9981)	35	0.1	0.3
	microemulsion	$A = 0.1456c - 0.0021$ (0.9998)	30	0.04	0.1
magnesium	dilution with xylene	$A = 0.5267c + 0.0146$ (0.9939)	8	0.04	0.1
	microemulsion	$A = 0.6816c + 0.0589$ (0.9990)	6	0.005	0.02

^a*R*, correlation factor; *C*₀, characteristic concentration.

Table 2. Determination of Calcium and Magnesium in Biodiesel Samples by FAAS^a

Ca concentration (mg kg ⁻¹) (mean ± SD, <i>n</i> = 3)			Mg concentration (mg kg ⁻¹) (mean ± SD, <i>n</i> = 3)		
sample	DX	ME	sample	DX	ME
B-13	5.1 ^b	5.2 ± 0.1	B-13	2.9 ^b	2.9 ± 0.04
B-15	5.4 ^b	5.6 ± 0.2	B-92	7.6 ± 0.6	8.0 ± 0.2
B-128	1.7 ^b	2.1 ± 0.04	B-178	1.9 ± 0.04	1.9 ± 0.04
B-147	4.3 ^b	5.0 ± 0.04	B-116	1.5 ^b	1.6 ± 0.03
B-179	2.8 ^b	2.3 ± 0.1	B-179	0.9 ^b	0.7 ± 0.01
B-200	3.1 ± 0.1	3.1 ± 0.04	B-200	0.5 ^b	0.4 ± 0.01
P-05	<0.3	<0.1	F-01	<0.1	<0.02

^aDX, dilution with xylene (reference method = ABNT NBR 15556)¹⁹; ME, microemulsion (proposed method). ^bResults provided by the Atomic Absorption Laboratory at Pontifical Catholic University of Rio de Janeiro, Brazil.

Recovery experiments with aqueous standards have been carried out, using three biodiesel samples and three vegetable oil samples, to investigate potential matrix effects. In the microemulsion method, the microemulsified samples were spiked with inorganic standards added to the water component. In the dilution method, the samples were weighed in the flask, spiked with organic standards, and then diluted with xylene. Different amount of standards were added, giving the final concentration of 0.5, 1.0, or 1.5 mg L⁻¹. The stability of the analytes was tested for both methods, through the daily monitoring of the analytical signal of standards and samples.

3. Results and Discussion

In previous work,¹⁴ a phase diagram of biodiesel/water/Triton X-100 and *n*-pentanol mixtures was constructed to obtain the region of formation of microemulsion with the components. The microemulsion formation was evidenced by its transparency and homogeneity in a single phase after mixing the components. It was observed that the hydrophobic phase (biodiesel) not exceeding 20% in mass was not stabilized when more than 8% (w/w) of water was used and the emulsion formation was achieved. The microemulsion formation was achieved for surfactant/co-surfactant ratios in the range of 1:1–1:8, considering the percentage of water not exceeding 8% in mass. Concerning the amount of biodiesel, it was possible to stabilize up to 90% (w/w) sample using 8% (w/w) water. The use of a maximum amount of sample in the microemulsion is desirable to increase the analysis sensitivity, but it also increases the viscosity, reducing the nebulization efficiency. Therefore, the amount of 20% (w/w) was adopted. The mass proportion adopted for the microemulsion preparation for biodiesel analysis was 57.6% (w/w) *n*-pentanol or *n*-butanol, 20% (w/w) biodiesel, 14.4% (w/w) Triton X-100, and 8% (w/w) water. This composition was also efficient for the microemulsion formation with vegetable oil or mineral oil (replacing the biodiesel). The use of *n*-butanol or *n*-pentanol as a co-surfactant did not show significant differences in terms of sensitivity, indicating that both co-surfactants could be employed.

The optimization of instrumental parameters was carried out with calcium and magnesium microemulsion standards, with a concentration of 0.5 mg L⁻¹. The aspiration rate used

to give best sensitivity values for both analytes was 2.0 mL min⁻¹. The fuel/oxidant ratios for calcium and magnesium were 0.613 and 0.206, respectively. The instrumental parameters were also optimized for the reference method based on the dilution with xylene,¹⁹ and the best sensitivity and stable signals were obtained with the minimal aspiration rate possible to use in the equipment (2.8 mL min⁻¹) and minimal fuel/oxidant ratio of 0.380 for calcium and 0.059 for magnesium.

The stability investigations showed that the analytes from standards and samples remained stable for at least 5 days in the microemulsions prepared with *n*-butanol or *n*-pentanol. In the dilution with xylene, there was only a slight decrease of the analytical signal in a period of 6 h, but in the period of 5 days, there was a decrease of about 40% in the analytical signal for both standards and samples. The higher stability of the analytes in the microemulsions is due to the addition of nitric acid that avoids the adsorption of the metals in the walls of flasks.

The figures of merit obtained for both methods are presented in the Table 1. When the sensitivity of the calibration curves is compared, it can be seen that the microemulsion method showed a slightly better sensitivity, as reflected in the characteristic concentration (*C*₀). Moreover, the limits of detection (LOD) and limits of quantification (LOQ) obtained for calcium and magnesium with the use of microemulsions were 3 and 5 times better, respectively, than the results obtained by the dilution with xylene. This feature is due to the higher amount of sample used for microemulsion preparation but also the lower fluctuation in the signal measurements, which reflected also in a better relative standard deviation (RSD). According to the Brazilian regulations, the maximum level allowed for calcium plus magnesium in biodiesel samples is 5 mg kg⁻¹, which is comfortably higher than the limits obtained in this work.

Because no adequate certified reference material was available yet, accuracy was evaluated through the comparison to the Brazilian Standard Method ABNT NBR 15556. The results obtained by both methods for calcium and magnesium in biodiesel samples from different sources are summarized in Table 2. For both methods, the samples were prepared in triplicates (*n* = 3) and all determinations were also carried out in triplicates (*n* = 3). As can be seen, at least three samples are

out of specification. The paired *t* test was applied to the data of Table 2 and showed that the results of both methods are not significantly different at the 95% confidence level. The method was also applied for analysis of vegetable oil samples following the same procedure used for biodiesel samples. The three vegetable oil samples (soybean, sunflower, and rapeseed) analyzed presented no measurable calcium and magnesium concentrations, considering the LOD of both methods.

Recovery experiments have also been carried out with vegetable oil and biodiesel samples from different sources, such as soybean, castor bean, and animal fat, as described in section 2.3. The experiment for biodiesel samples resulted in recoveries ranging from 92 (± 4.1) to 117% (± 1.7) for microemulsions and from 92 (± 0.7) to 118% (± 1.5) for dilution with xylene methods. For vegetable oil samples, the recoveries ranged from 86 (± 1.3) to 95% (± 0.1) for microemulsions and from 82 (± 1.2) to 105% (± 1.2) for dilution with xylene. This study was important particularly for vegetable oil analysis by the proposed method and also by the dilution with xylene method, once it was not possible to compare the results because of the low concentration of calcium and magnesium in these samples. These results show that the matrix of the samples investigated does not have significant influence on

the sensitivity of the added standards, indicating that both methods could be used for vegetable oil analysis.

4. Conclusion

The proposed method based on microemulsions as sample preparation for the determination of calcium and magnesium in biodiesel samples was very satisfactory. The main advantages compared to the dilution method are the use of inorganic standards in the microemulsions for calibration, non-use of carcinogenic organic solvents, and the higher stability of the analytes allied with the higher sensitivity. As a consequence, the method presented in this work is an excellent alternative for biodiesel routine analysis. Besides, the feasibility of using FAAS instead of the ICP–OES technique is economically appealing.

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