Total and inorganic mercury determination in biodiesel by emulsion sample introduction and FI-CV-AFS after multivariate optimization

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An automated procedure for total and inorganic mercury determination in biodiesel by CV-AFS was studied. The samples were introduced directly as oil-in-water emulsions in a flow injection manifold followed by cold vapor generation coupled to atomic fluorescence spectrometry (FI-CV-AFS). After irradiation with an UV source, organic mercury (*e.g.* $MeHg^+$ and $PhHg^+$ among others) was decomposed. Mercury vapors were generated using an acidic $SnCl_2$ solution in a continuous flow system and were then determined. This strategy reduced sample handling, avoiding sample contamination and analyte losses. The limit of detection was calculated as 0.2 μg Kg^{-1} (0.03 μg L^{-1} for the emulsions) and the relative standard deviation was better than 8% at levels of 3.0 μg L^{-1} in the emulsion, calculated from the peaks obtained. The accuracy was verified by comparing the results with a total microwave-assisted digestion.

Introduction

Biodiesel is a renewable, biodegradable and non-toxic fuel derived from biological sources such as vegetable oil or animal fat through transesterification. It can be used in diesel engines and can supplement fossil fuels as the primary transport energy source and significantly reduce the emissions of carbon monoxide, carbon dioxide, sulfur dioxide and hydrocarbons.[1–4]

Among others, As, Cd, Hg, Se and Tl are released into the environment due to combustion of fuel in automobiles and are an important source of atmospheric pollution.[5–7] Mercury is a high toxic element and methylmercury (MeHg) is particularly important due to its toxicity and its high proportion among organomercury species in the environment.[8,9]

As a result, increasingly sensitive, accurate and rapid analytical techniques are required to monitor Hg species in different environmental samples.[10]

The use of emulsions as sample preparations has been applied for trace metal determination in petroleum derivates such as automobile fuels.[11,12] This sample pre-treatment has been used with the main atomic spectrometric techniques and, interestingly, applications were carried out when it was associated to cold vapor atomic fluorescence or absorbance spectrometry (AFS or AAS).[13–18] The use of aqueous standards for calibration instead of expensive and instable organometallic standards was also possible.[4]

Procedures involving optimization by multivariate techniques have been increasingly used as they are fast, more economical and effective, and allow more than one variable to be optimized simultaneously.[19–22]

Analysis of biodiesel is becoming of great importance. So far, there are several reports that describe methods for elemental determination in this new fuel,[3,23] however, this is the first report that depicts the determination of Hg(II) and MeHg in biodiesel and, even more, this is the first time that AFS has been employed to analyze such type of samples.

This study was focused in developing a method with low sample handling and low risks of contamination and analyte losses for Hg(II) and MeHg determination in biodiesel samples by emulsion formation followed by flow injection cold vapor generation atomic fluorescence spectrometry (FI-CV-AFS). A fractional factorial design was used as a multivariate strategy for the evaluation of the effects of several variables at once, and a further optimization was done with a central composite design (CCD). A microwave-assisted digestion was also applied for sample pre-treatment for comparative purposes.

2. Experimental

2.1. Instrumentation

The measurements were carried out with an atomic fluorescence spectrometer, AI 3300, Aurora Instruments (Vancouver, British Columbia, Canada). A hollow cathode lamp for Hg from Aurora Instruments (Vancouver, British Columbia, Canada) was employed as excitation source. The experimental conditions for the determination of Hg are described elsewhere.[9]

The microwave digestions were performed with a domestic microwave oven Philco (Ushuaia, Argentina) equipped with a magnetron of 2450 MHz and Milestone hermetically sealed 1 cm wall thickness polytetrafluoroethylene reactors (100 mL internal volume).

An ultrasonic bath (Astrason Ultrasonic Clear, Farmingdale, NY, USA) was employed for emulsion preparation.

The UV digester was made as follows: a 400 W Hg vapor lamp (15W G15T8 UV-C LONG LIFE high pressure Hg, PHILIPS) that ignited with a suitable starter and chock surrounded by a 3 m PTFE tubing.

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Table 1 Variables and levels used for fractional factorial design

Factor	Name	Units	Low Actual	Central Actual	High Actual	Low Coded	Central Coded	High Coded
(*A*)	HNO_3	mL	1.00	1.50	2.00	−1	0	1
(*B*)	T-X100	mL	1.00	2.00	3.00	−1	0	1
(*C*)	Ultrasound	min	5.00	7.50	10.00	−1	0	1
(*D*)	H_2O	mL	1.00	2.00	3.00	−1	0	1

Table 2 Variables and levels used for central composite design

Factor	Name	Units	Low Actual	Central Actual	High Actual	Low Coded	Central Coded	High Coded
(*A*)	HNO_3	mL	1.00	2.50	4.00	−1	0	1
(*B*)	H_2O	mL	1.00	2.50	4.00	−1	0	1

2.2. Reagents

A mercury standard stock (1000 μg mL^{-1}) was prepared from mercury(II) chloride, Merck (Darmstadt, Germany). Methyl- and phenyl-mercury stock solutions (Merck) were prepared in ethanol and methanol, respectively. $SnCl_2 \cdot 2H_2O$ from Sigma (St. Louis, MO, USA) in 10% (v/v) HCl (Merck) was used as reducing agent. It was prepared by dissolving the salt in concentrated HCl, heating for 10 min and diluting with water. Nitric acid was provided by Fluka Sigma-Aldrich (Steinheim, Germany). Triton X-100 was obtained from Tokyo Kasei Industries (Chuo-Ku, Tokyo, Japan) and biodiesel from local stores, the same for kerosene.

Ultrapure water (18.2 MΩ cm^{-1}) was obtained from Barnstead EASY pure RF water system (Iowa, USA).

2.3. Sample treatment

2.3.1. Emulsion formation. For the preparation of each sample, 1 mL (0.88 g) of biodiesel was precisely weighed and 3 ml of concentrated HNO_3 was added. This mixture was kept for 30 min, followed by addition of 1.5 mL Triton X-100. This surfactant was used because it was readily available and it has already been proven to be a good dispersant for the preparation of oil-in-water emulsions.

Table 3 Fractional factorial design (2^{4-1} + 3)

Run	(*A*)	(*B*)	(*C*)	(*D*)	*F*
1	−1	1	−1	1	4.9
2	1	1	1	1	6.5
3	1	−1	1	1	5.4
4	0	0	0	0	18
5	0	0	0	0	20.7
6	0	0	0	0	22
7	−1	−1	−1	−1	3.5
8	−1	1	1	−1	5.8
9	1	1	−1	−1	12
10	1	−1	−1	1	4.3
11	1	−1	1	−1	11.5

This mixture was then placed in an ultrasonic bath for 10 min and, after that, a desired volume of ultra pure water was added and analyzed immediately by FI-CV-AFS. Blanks were treated in the same way. All further determinations were made according to the results of the optimization procedure.

2.3.2. Microwave-assisted digestion procedure. For the preparation of each sample, 0.5 g of biodiesel was introduced into the PTFE reactors. Then, 2.0 mL of concentrated nitric acid and 1 mL of hydrogen peroxide were added and the reactors were closed. The samples were digested applying different microwave powers, *i.e.* at 350 W (5 min), 350 W (5 min), 550 W (5 min), 750 W (5 min), 0 W (20 min). The concentration was obtained directly from calibration graphs after correction of the fluorescence signal with an appropriate reagent blank. Spiked samples were also analyzed.

2.4. Experimental setup

A flow injection scheme was employed in order to accomplish the determinations. This flow injection manifold, which included two peristaltic pumps, Tygon tubing and a two six-port rotary valve with a PTFE sample loop, was described previously.[9] The sample loop volume was optimized and fixed at 100 μL. The Hg fluorescence (transient signal) was recorded, and its height was proportional to the mercury concentration in the emulsions. Before the determinations, the sample tubing was on-line irradiated with an UV source in order to achieve $MeHg^+$ decomposition and its subsequent determination. Inorganic Hg was determined without UV irradiation.[24]

2.5. Multivariate optimization

2.5.1 Fractional factorial design (FFD). For the evaluation of four variables at two levels plus three replicates of a central point, a FFD with $2^{(4-1)} + 3 = 11$ experiments is described. These (extra) experiments were carried out in order to estimate the experimental error as well as the curvature of the experimental domain.

The variables: amount of nitric acid added (*A*), volume of surfactant added (*B*), time of ultrasound (*C*), and amount of

water added (*D*), were regarded as factors, while the intensity of Hg fluorescence (*F*) was the dependent variable (Table 1).

2.5.2. Central composite design (CCD). A spherical CCD was used, consisting of 19 experiments; *i.e.* two replicates of 4 factorial points and 4 star points, and three replicates of the central point. The experiments were combinations of the independent variables in the following ranges: nitric acid volume 1–4 ml and water volume 1–4 ml (Table 2). These ranges were selected based on prior knowledge about the system under study. On the other hand, the amount of Triton X-100 and the ultrasonic time were fixed at 1.5 mL and 10 min, respectively. All experiments were performed in random order to minimize the effects of uncontrolled factors that may introduce a bias in the measurements.

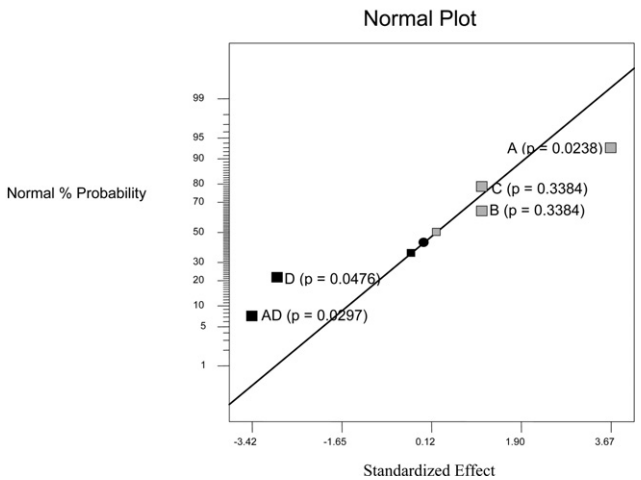

Fig. 1 Normal probability plot of the standardized effects of the estudied factors: *A*, volume of HNO_3; *B*, mass of T-X100; *C*, time of ultrasound and *D*, volume of H_2O. (Positive effect and negative effect; *p*-values obtained by ANOVA are included).

3. Results and discussion

3.1. Multivariate optimization

Four factors were selected for examination by the proposed FFD. All these factors were selected since they might have some influence on mercury release from the organic matrix under analysis. These considerations were of major importance, since the final goal of this study was Hg determination in oil-based samples by means of a simple calibration.

Analysis of variance (ANOVA) and *p*-values were used to check the significance of the effects at a 95% confidence level. The results of this study (Table 3) are visualized in a normal plot (Fig. 1). Since the amounts of HNO_3 (*A*) and H_2O (*D*) are the only two factors with some sort of effect upon the Hg fluorescence (at the studied levels), the effect of the aliased terms $AD + BC$ may be attributed only to the interaction between those two factors. These results were in concordance with the experimental observations and previous knowledge; *i.e.* the nitric acid is responsible for releasing the mercury species from the organic matrix, and the water is added in order to form a stable three-component solution that contains Hg. Consequently, the effect of HNO_3 on the fluorescence was expected to be positive. On the other hand, the negative effect of water should be interpreted as a result of a diluting effect. The negative effect of the interaction HNO_3–H_2O may not have a straightforward interpretation; however, it seems that the diluting effect is the main cause of the changes observed in fluorescence.

After screening out the variables, a central composite design was used. Those factors that showed no effect on the analytical response were fixed at convenient values as follows: ultrasound time = 5.0 min and T-X100 = 1.5 mL for all runs. The model coefficients were calculated by backward multiple regression[19] and validated by the analysis of variance (ANOVA). In this case, a modified cubic model is the one that better explain the behavior of the Hg response under the studied conditions. In this study, the lack of fit was not significant ($p > 0.05$) and the predicted *R*-squared was of 0.9673 which was in reasonable agreement with the adjusted *R*-squared of 0.9816, showing a good relationship

Table 4 Central composite design and experimental data

Run	(*A*)	(*B*)	*F* (experimental)	*F* (predicted)	Residual
1	1.00	1.00	9	8.1	0.9
2	1.00	1.00	8	8.1	−0.1
3	4.00	1.00	18.5	18.9	−0.4
4	4.00	1.00	20	18.9	1.1
5	1.00	4.00	16.4	16.3	0.1
6	1.00	4.00	17	16.3	0.7
7	4.00	4.00	14.6	13.9	0.7
8	4.00	4.00	13.9	13.9	0.2
9	0.38	2.50	8	8.9	−0.9
10	0.38	2.50	9	8.9	0.1
11	4.62	2.50	22	23.6	−1.6
12	4.62	2.50	24.5	23.6	0.9
13	2.50	0.38	15.5	16.6	−1.1
14	2.50	0.38	17	16.6	0.4
15	2.50	4.62	8	8.1	−0.1
16	2.50	4.62	7.5	8.1	−0.6
17	2.50	2.50	29	30.5	−1.5
18	2.50	2.50	31	30.5	0.5
19	2.50	2.50	31.5	30.5	1.0

Table 5 Criteria for the optimization of the individual responses

Factor	Goal	Lower limit	Upper limit	Solutions
Volume of HNO_3/mL	Is in range	1	4	3.04
Volume of H_2O/mL	Maximize	1	4	2.56
Fluorescence	Maximize	7.5	31.5	31.3

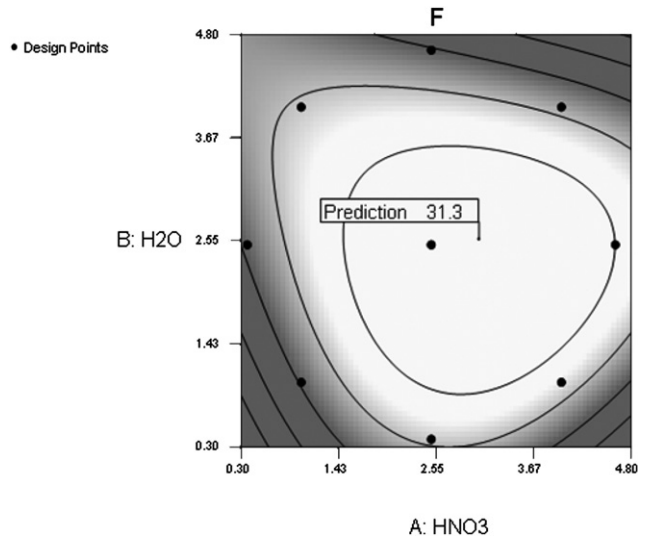

Fig. 2 Contour plot of the response surface.

between the experimental data and the fitted model. The adequate precision of 32.227 attained indicated an adequate signal. Table 4 shows the experimental data compared with the predicted data together with the residual errors.

Table 5 shows the followed criteria for the optimization which were selected with the aim of achieving the best sensitivity but with enough water content in order to avoid matrix effects when calibrating with aqueous standards. Following the conditions and restrictions previously discussed, the response surface for the intensity of fluorescence was obtained (Fig. 2).

The experimental conditions corresponding to one maximum in the function were: 3.04 mL HNO_3 and 2.56 mL of H_2O. The response value corresponding to these conditions is a fluorescence of 31.3. The suggested values during the optimization procedure were experimentally corroborated.

3.2. Analytical performance and application to real samples

Mercury was determined in all samples by the standard addition method. The calibration covered a concentration range from 1.0 to 15 μg L^{-1} Hg in the emulsions (base value + added value).

Table 6 Mercury levels in soya biodiesel and other fuel samples

	Emulsion		Microwave digestion
Sample	Inorganic Hg/μg Kg^{-1}	Total Hg/μg Kg^{-1}	Total Hg/μg Kg^{-1}
Biodiesel I	0.5 ± 0.1	2.2 ± 0.2	2.6 ± 0.4
Biodiesel II	2.9 ± 0.2	3.7 ± 0.1	3.3 ± 0.3
Kerosene	N.D.[a]	1.1 ± 0.2	0.8 ± 0.2

[a] N.D.: not detected.

The precision (3.0 μg L^{-1} of Hg in the emulsion), evaluated as the average relative standard deviation (RSD%), was better than 8%. The detection limit, determined applying the 3σ concept of calculation ($n = 10$), was of 0.2 μg Kg^{-1} (0.03 μg L^{-1} in the emulsions). The accuracy was verified by comparing with a total microwave-assisted acid digestion (Table 6). A comparison with other recent works is given in Table 7.

The proposed method was applied to two biodiesel samples from the same source and another fuel sample. Then, those samples were enriched with different levels of Hg(II) and MeHg and re-analyzed and the recoveries were satisfactory. Furthermore, the sum of the concentration obtained for Hg(II) and for MeHg matched the total Hg concentration found after a microwave digestion.

4. Conclusions

The proposed method based on the emulsion formation was shown to be an efficient alternative for total and inorganic mercury determination in biodiesel samples. The method, based on the excellent sensitivity attainable by AFS and on a soft room-temperature treatment of samples, is a safe and comfortable methodology for operators, reduces the reagent consumption and time of analysis, and avoids the risk of sample contamination and analyte losses.

This simple and fast method of sample preparation allows the use of aqueous standards for calibration and the adequate limits of detection, making this sample preparation suitable for routine application. Moreover, the procedure has the potential to be extended to similar samples, such as naphtha and alcohol among others.

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Table 7 Procedures for Hg determination in biological and environmental samples by AFS

Type of samples	Detection limit	Relative standard deviation (%)	Sample amount	Analytical strategy	References
Biodiesel	0.2 μg Kg^{-1}	<8	0.88 g	Emulsion formation	This work
Blood serum	0.025 μg L^{-1}	3.9	1 mL	Slurry sampling	9
Natural waters	16 pg L^{-1}	4–10	7 mL	On-line digestion	18
Human hair	1.2 ng L^{-1}	1.8	0.5 mL	Intermittent flow	25
Milk	11 μg Kg^{-1}	3.4	1 g	Slurry sampling	26

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