Determination of Trace Manganese and Ni in Neem Oil by ETA–AAS with Emulsion Sample Introduction

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ABSTRACT: An emulsion-based method was developed as an alternative for the introduction of oil-based samples into a graphite furnace to produce a more rapid, precise, and accurate method of analysis. The combination of emulsion sampling and electrothermal atomization for atomic absorption spectroscopy was developed and then applied to the determination of Ni and manganese in neem oil from Venezuela. The optimal concentration of neem oil in the emulsion was 30 and 4% for Ni and Mn, respectively, based on the metal concentration of the neem oil. The stability of the emulsion as a function of time was evaluated. The use of aqueous calibration solutions and the accuracy attained using this method make it attractive for the analysis of oil samples. The metal recovery was between 97 and 101%. Based on the emulsion method, the neem oil samples had 1.39 and 0.21 mg of Mn and Ni per kg of oil, respectively. The wet digestion method indicated a Mn and Ni concentration in the neem oil sample of 1.42 and 0.24 mg/kg, respectively.

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KEY WORDS: Emulsion, ETA–AAS, manganese, neem oil, Ni.

Numerous studies have been carried out on manganese uptake by plants and on Mn distribution among plant tissues (1). All of the findings indicate that Mn uptake is metabolically controlled, apparently in a way similar to that of other divalent cationic species such as Mg^{2+} and Ca^{2+}. However, passive absorption of Mn is also likely to occur, especially at high and toxic concentrations in solution. The Mn content of a plant is a characteristic not only of the plant itself but also of the pool of available Mn, which is highly controlled by soil properties. The functions of Mn in plants were reviewed by Shkolnik (2) and Boardman (3). Mn content varies with plant species and stage of growth as well as from organ to organ and for different ecosystems.

Ni is an essential trace element (micronutrient) (4). Early reports indicated that low substrate concentrations of Ni stimulate germination and growth of various crop species. It is now known that plants cannot complete their life cycles without adequate Ni supply. In most plants the Ni content in the vegetative organs is in the range of 1–10 $\mu g \cdot g^{-1}$ dry weight, and this range mainly reflects the differences between plant species in uptake and root-to-shoot transport of Ni.

The direct analysis of organic solutions for trace metals such as Mn and Ni by atomic absorption methods presents several difficulties. In general, the analytical method is less precise in organic media than in aqueous solutions. In addition, calibration is more difficult in organic media. The use of oil–water emulsions greatly reduces the time for analysis even in multilement determinations (5,6). In the electrothermal atomization atomic absorption spectroscopy (ETA–AAS) determination of metallic elements in organic matrices, the use of emulsions has the advantage that aqueous solutions can be used for the preparation of the calibration standards. Several papers have reported the use of emulsions with ETA–AAS (7–10), inductively coupled plasma (ICP)–optical emission spectroscopy (OES) (11–13), and with ICP–MS (14).

The main objective of this work is the optimization of a method using emulsions as a method of sample introduction into the graphite furnace atomic absorption spectrophotometer for the determination of Mn and Ni in neem oil. Analysis of emulsified neem oil by ETA–AAS is a good alternative to the existing methods. Furthermore, the use of aqueous standards for calibration purposes is a good addition to the methods available for the analysis of this type of sample, especially when certified standards of this type of sample are not commercially available. To our knowledge, no data have been published on the metals content in Venezuelan neem oil. Ni was chosen for analysis because this metal has a negative influence on the oxidative stability of oils and fats. Since the most important function of Mn is related to oxidation-reduction process, it is worthwhile to know its content for oil processing. This study is also part of the elemental characterization of neem oil.

EXPERIMENTAL PROCEDURES

Instrumentation. A PerkinElmer 5100ZL atomic absorption spectrometer with Zeeman background correction system and transversely heated graphite atomizer equipped with an AS-71 autosampler was used throughout this work. The temperature programs for each analyte are presented in Table 1. A multielement hollow cathode lamp for Mn and Ni was operated at 30 mA and at wavelengths of 279.5 and 232.0 nm for Mn and Ni, respectively.

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TABLE 1
Furnace Heating Program Used (temperature,ºC/ramp time, s/hold time, s)

	Analyte			
	Ni		Mn	
Step	Aqueous	Emulsion	Aqueous	Emulsion
Dry	110/1/20	110/1/20	110/1/20	110/1/20
	130/5/30	130/5/30	130/5/30	130/5/30
Pyrolysis	500/20/10	500/20/10	500/20/10	500/20/10
	1100/20/10	1100/20/10	1200/20/10	1200/20/10
Atomization	2300	2300	1900	1900
Cleaning	2400	2400	2400	2400

Reagents. A stock Mn solution of 1000 mg/mL was prepared from metallic manganese in HNO_3 (Baker Analyzer). A 1000 µg/mL Ni solution was prepared from a Titrisol solution supplied by Merck (Darmstadt, Germany). Nitric acid was Merck Suprapure grade. Suprapure Sulfuric acid (J.T. Baker), hydrogen peroxide (Merck), and high-purity (99.95 %) argon were used. The surfactants used were Triton X-100 (J.T. Baker) and Tween-80 (Merck), and the cosurfactant was *p*-xylene (J.T. Baker). All glassware was cleaned in nitric acid before use. Ultrapure water was obtained from a Milli-Q water system (Millipore).

Sample. A neem oil sample from Venezuela was used in this work. The oil was returned to the laboratory after it was extracted and processed on the plantation.

Sample emulsification procedure. Neem oil (0.4000 g) was weighed in a glass container. Then, *p*-xylene (0.4000 g) was introduced to reduce the viscosity of the oil and facilitate the interaction between oil and the nonionic surfactant. The optimized concentration of Triton X-100 (0.3000 g) was added, and then the mixtures were shaken manually to mix the components. Milli-Q water was added with continuous agitation to make a mixture weighing10.000 g in total. The mixture was placed in an ultrasonic bath for 20 min. Phase separation was not observed at least for 24 h. These conditions were suitable for Mn analysis. Emulsion preparation for Ni analysis was dictated by the analyte concentration present in the oil sample.

Sample digestion. Neem samples were digested using a wet acid method. An oil sample (5.0000 g) was accurately weighed and placed in a beaker. A total of 5 mL of suprapure nitric acid was added and the mixture was heated for 5 h. Then, 6 mL of suprapure sulfuric acid was added and heating continued for about another 6 h. Finally, hydrogen peroxide (5 mL) was added to the solution, producing a clear solution. The digested contents were quantitatively transferred to a 100-mL volumetric flask and diluted with deionized water.

RESULTS AND DISCUSSION

Sample emulsification. Oil-in-water (O/W) emulsions were prepared using a specific sequence to guarantee their stability. Two main factors were taken into account during the preparation: the mixing sequence of the components and the type of agitation. These factors affect the drop size of the emulsion. (5). The optimized mixing sequence included mixing the oil sample with the required amount of the cosurfactant, adding surfactant, and ultrasonicating at room temperature for 20 min. The cosurfactants tested were methanol, 2-butanol, and *p*-xylene. The cosurfactant *p*-xylene was best for reducing oil viscosity and facilitating the interaction between the oil and the nonionic surfactant. A solution containing 4 % (vol/vol) *p*-xylene was selected for further experiments.

Three parameters were evaluated during emulsion preparation: surfactant, cosurfactant, and emulsion stability. The stability of the emulsion was evaluated as a function of the time the neem oil remained uniformly dispersed in the solution. Other factors studied were the relative concentrations of oil, water, surfactant, and cosurfactant and the technique used to mix and homogenize the components. Emulsions prepared with Triton X-100 were found to be more stable than those prepared with Tween-80.

A central composite design 2^2 (15) was used to study the influence of oil content and surfactant concentration on the Mn and Ni atomic absorption signals. Each factor was examined at two levels. For oil these levels were 1 and 30%, and for surfactant the levels were 1 and 10%.

Probability plots were used to identify significant effects. Negligible effects, which are due primarily to random error, tend to fall along a straight line on this plot. The probability graph (Fig. 1) shows that only one effect on the Ni atomic absorption signal, the oil content in the emulsion, is far from the line. The surfactant concentration and the interactions among the factors are negligible. Similar results were obtained for Mn.

The optimal absorption signal for both analytes was determined by using the one-factor-at-a-time method. Figure 2 shows the effects of surfactant and oil concentration on the Ni atomic absorption signal. Maximum absorption signal was obtained at 30% (w/w) oil content and 10% (w/w) Triton X-100. Results for Mn were obtained using the same procedure as for Ni. The optimal Mn signal was obtained with 4% (w/w) oil and 3% (w/w) Triton X-100. The difference in O/W emulsion concentration for Mn and Ni is due to the concentration of analytes present in the original oil sample.

Optimization of furnace program. To find appropriate conditions for the determination of the analytes in an emulsified oil matrix, pyrolysis and atomization curves were established using O/W emulsions with the platform atomization technique and compared with those obtained for an aqueous analyte standard. Previously, a semiquantitative analysis of the oil sample

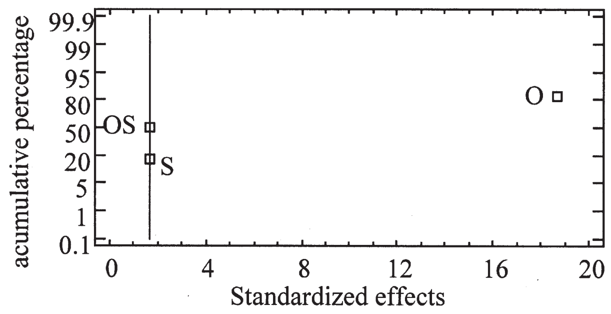

FIG. 1. Normal probability curve obtained for Ni in neem emulsion using electrothermal atomization–atomic absorption spectroscopy. O, oil concentration; S, surfactant concentration; OS, O × S interaction.

by ICP–OES was performed to determine the approximate concentrations of Ni and Mn in the sample solution.

The minimum temperature used for pyrolysis was 950°C and it was varied to 1300°C; the atomization temperature was held fixed to that recommended by the instrument manufacturer, 2300°C. The pyrolysis curves for Ni in the emulsified oil samples and in an aqueous standard are shown in Figure 3. A significant decrease in signal occurred above 1150°C for both matrices. The optimal pyrolysis temperature was chosen as 1100°C. Optimization of the atomization temperature for the oil emulsion and for the aqueous solution is also represented in Figure 3. This was varied between 2000 and 2400°C, at the previously chosen pyrolysis temperature. The optimal atomization temperature was found to be 2300°C.

The optimal furnace parameters for Mn were 1200°C for pyrolysis and 1900°C for atomization (Fig. 4). Similar pyrolysis and atomization curves were obtained with the emulsion and aqueous solutions (Figs. 3, 4); thus, calibration against aqueous standards is feasible. Also, less sample preparation is required, simplifying the analytical procedure.

Analytical figures of merit. Calibration curves were established using the optimized experimental parameters developed here and the instrumental parameters previously set. Aqueous standard calibration and standard addition curves were compared to check the possibility of using the former for the quantification of the oil emulsions. Table 2 shows these results. Linearity was observed up to an analyte concentration of 30 $\mu g \cdot L^{-1}$ for Mn and up to 100 $\mu g \cdot L^{-1}$ for Ni.

The difference between the slopes was not significant ($P < 0.05$) when the *t*-test was applied (16). The accuracy of the proposed method was checked by spike-and-recovery experiments at 2 $\mu g \cdot L^{-1}$ for Mn and 10 $\mu g \cdot L^{-1}$ for Ni. Average recoveries obtained were 96.97% for Mn with a relative SD (RSD) of 0.4% and 101.14% for Ni with a RSD of 1.2%.

Analysis of neem oil sample using the proposed method. The analysis of Mn and Ni in neem oil, extracted from seeds of plants grown in Venezuela, was carried out by the pro-

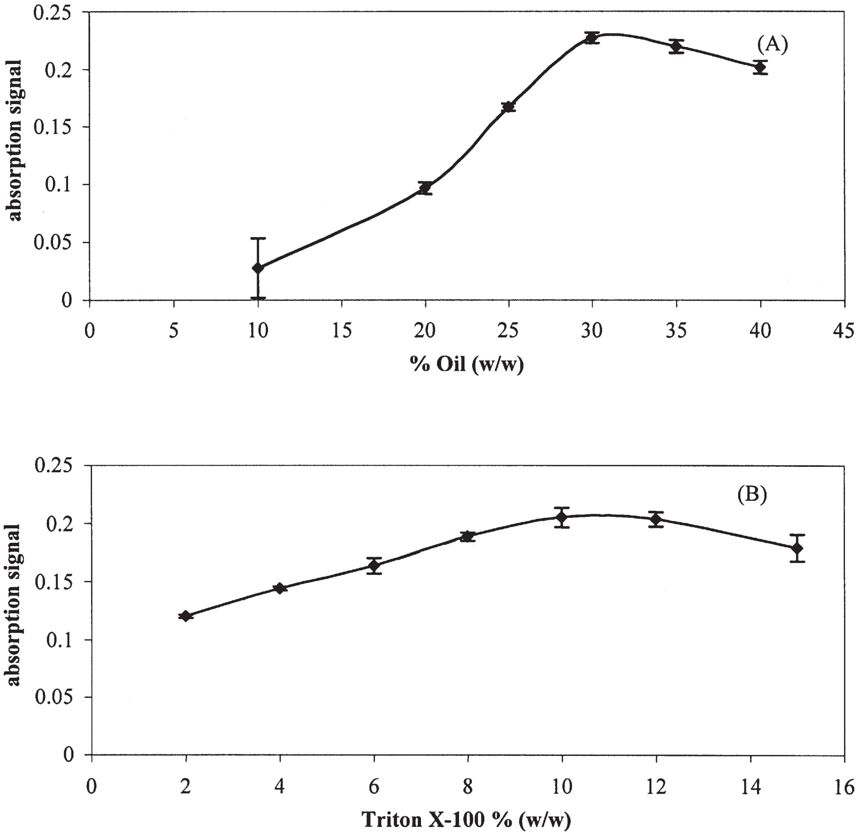

FIG. 2. Optimization of (A) oil-in-water emulsion and (B) Triton X-100 concentrations for Ni.

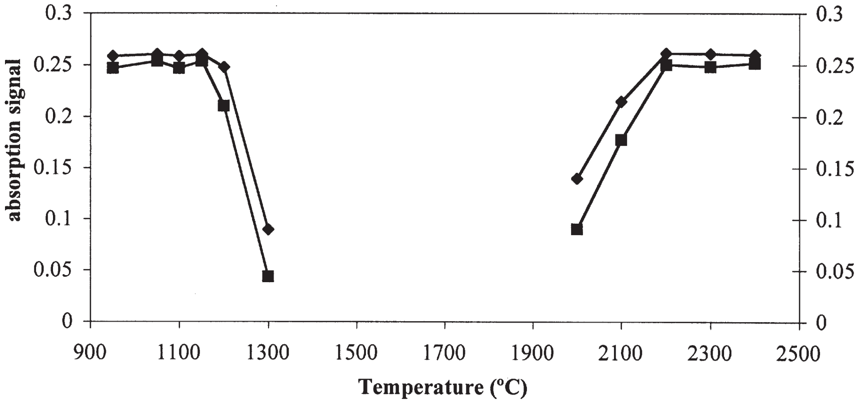

FIG. 3. Pyrolysis and atomization curves for Ni. (◆) Aqueous solution (70 µg·L^{-1}) and (■) neem oil emulsion.

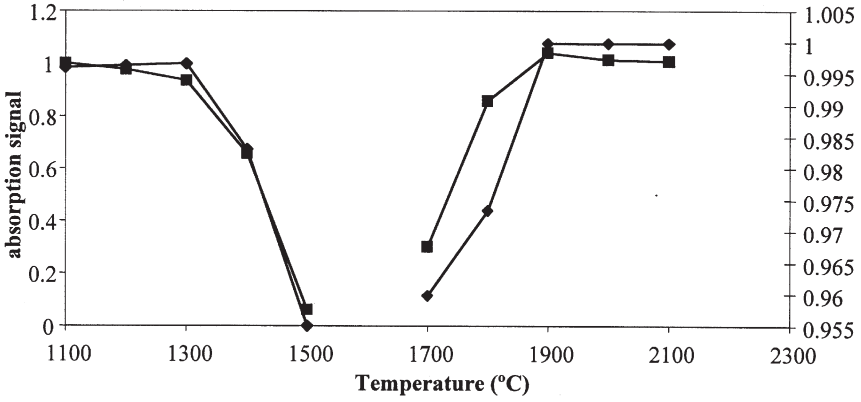

FIG. 4. Pyrolysis and atomization curves for Mn. (◆) Aqueous solution (40 µg·L^{-1}) and (■) neem oil emulsion.

TABLE 2
Comparison[a] of the Aqueous Standard Calibration and Standard Addition Curves and Their Significance

		Regression line $y = aC + b$			
Element	Calibration curve	$a \pm sa$	t exp slope	$b \pm sb$	r^2
Ni	Aqueous	0.0034 ± 0.0009	0.06	0.0027 ± 0.0001	0.9981
	Standard additions	0.0033 ± 0.0001		0.027 ± 0.002	0.9970
Mn	Aqueous	0.0099 ± 0.0003	1.58	0.0052 ± 0.0001	0.9984
	Standard additions	0.0109 ± 0.0005		0.554 ± 0.002	0.9925

[a] Definitions: *a*, slobe; *b*, intercept; *C*, concentration; *r*, correlation coefficient; *sb* and *sa*, SD for the slope (*b*) and the intercept (*a*), respectively *t* exp slope, experimental value (*t*) calculated when the significance *t*-test was applied.

posed method and by the traditional method of wet acid digestion. The concentration of Mn was 1.39 ± 0.06 and 1.42 ± 0.04 mg kg^{-1} for the emulsion and wet acid digestion, respectively. The concentration of Ni was 0.21 ± 0.01 and 0.24 ± 0.06 mg kg^{-1} for the emulsion and wet acid digestion, respectively. The difference between the values obtained by the two methods was not significant at the 95% confidence level (16).

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