Doehlert design for optimization of tin analysis in mussels: study case from Bizerte Lagoon

M. El Ati-Hellal[a*], F. Hellal[b], & A. Hedhili[a]

[a]Laboratoire de Toxicologie, Unité de Toxicologie Recherche et Environnement, 10, rue Abou El Kacem Chabbi,
Montfleury, 1008 Tunis cedex, Tunisie
[b] Département de Chimie, Institut National des Sciences Appliquées et de Technologie, B.P. 676-1080 Tunis cedex, Tunisie
[E-mail adress: mfh22002@yahoo.fr]

Experimental Doehlert design was used for the determination of the optimal conditions for tin analysis in mussel samples by Atomic Absorption Spectrometry equipped with a Transversely Heated Furnace. Five factors were chosen as being able to influence analytical signal of tin. Application of Doehlert design allowed, with minimum number of experiences, the optimization of pyrolysis and atomization temperatures (1300 and 2500 °C, respectively) and also the determination of the exact quantities of permanent modifier Ir (100 µg) and chemical modifiers Pd and $Mg(NO_3)_2$ (17 µg for both modifiers) to achieve highest sensitivity. Precision of the optimized method calculated as relative standard deviation was 5.8 %. Accuracy was checked using the reference material (NIST-SRM 2977). Analytical procedure was applied to the analysis of mussel samples collected from Bizerte lagoon. Mean concentrations varied between 0.51 and 2.72 µg.g^{-1} (dry weight).

[Keywords: Mussels, tin, experimental design, Atomic Absorption Spectrometry, chemical modifiers, Bizerte lagoon.]

Introduction

Due to their high capacity to concentrate heavy metals, mussels have been widely used as bioindicators of metal pollution in aquatic environments[1-4]. Humans that consume mussel samples could be exposed to lethal levels of toxic contaminants. Therefore, the development of rapid, sensitive and accurate methods for the analysis of trace metals is become necessary for the monitoring and control of aquatic ecosystems pollution.

Tin is among the investigated metals in environmental analysis as its organic derivatives are highly toxic, especially tributyltin (TBT) and triphenyltin (TPT) compounds[5-10]. Several analytical methods have been described in the literature to measure tin in environmental samples[11, 12]. Electrothermal Atomic absorption Spectrometry (ETAAS) has proved to be a reliable technique for this purpose[13,14]. However, the volatility of tin compounds, the complexity of its chemistry in solution and the problems of sample contamination lead to a poor sensitivity in the metal analysis with ETAAS. To overcome chemical and spectral interferences, many researchers used permanent modification with carbide forming elements (Zr, W, Nb, Ta) or noble metals (Pd, Rh, Ru, Pt, Ir) or mixed carbide forming elements with noble metals ((Zr-Ir), (Zr-Pd), (W-Ir), (W-Pd))[15-17]. In comparison with classical chemical modification, permanent modifiers provide attractive advantages such as diminution of the time of heating programs, reduction of the blank values, and increase of the tube lifetime. However, problems of poor reproducibility, multiple peaks, and overstabilization were also reported with permanent modification[16].

In this study, tin was determined in mussel samples by ETAAS. In order to find optimal conditions for tin analysis, experimental designs with Doehlert matrix were used. This multivariate matrix has some interesting qualities; it presents a uniform distribution of the points over the entire experimental range. Furthermore, compared to other second-order designs, such as the Central Composite Design, fewer experiments are required: for k factors, the minimal number of experiments is $N = k^2 + k + 1$[18].

The effects of pyrolysis and atomization temperatures, chemical modifiers $(Pd/Mg(NO_3))$, permanent modifier Ir on the tin analytical signal in mussel samples were evaluated. Tube, equipped with a transversely heated system and an integrated L'vov platform, was pre-coated with 200 µg of Zr as permanent modifier. This refractory metal reduces the

analyte oxides (SnO) catalysed by ZrC and allows the formation of tin atoms. Compared to other carbide forming elements such as Mo or W, Zr presents the best coating efficiency and a more uniform treated surface in tin analysis with ETAAS[19].

Materials and Methods
All experiments were carried out with a novAA 400 Atomic Absorption Spectrometer (Analytik Jena, Jena, Germany), with deuterium background correction, equipped with a Transversely Heated Graphite Furnace and an MPE60 Autosampler (Analytik Jena). Stabilized temperature platform furnace (STPF) conditions were applied throughout this study. Platform of the graphite furnace was pre-treated with Zr or Zr-Ir. The Zr coating on the integrated platform was performed by injecting 40 µL aliquots of a 200 µg.mL^{-1} Zr solution and submitting the tube to a specific temperature program[20]. This procedure was repeated 25 times to obtain a deposit of 200 µg of Zr, as permanent modifier. The Zr-Ir coated integrated platforms were first treated with Zr and then further treated with a standard solution of 100 µg.mL^{-1} Ir as indicated above. All the measurements were carried out at the analytical wavelength 224.6 nm (slit 0.8 nm, lamp current 6 mA) by using a hollow cathode lamp (Analytik Jena). 20 µL of the digested sample and 10 µL of the chemical modifier were pipetted into the graphite tube. Accuracy of the applied analytical procedure for the determination of tin was tested using the reference material NIST-SRM 2977 mussel tissue, obtained from National Institute of Standards and Technology. The optimized heating program for the graphite tube is given in Table 1. Integrated absorbance was applied for the evaluation of signals. Purge gas of the Atomic Absorption Spectrometer was argon (99.9 %) (Air liquide, Tunis, Tunisia).

Table 1: Temperature program for the determination of tin in mussels.

Step[*]	Temperature (°C)	Ramp time (s)	Hold time (s)	Ar flow rate (mL.min^{-1})
Drying 1	100	5	5	250
Drying 2	140	15	5	250
Pyrolysis	1300	5	10	250
Atomization	2500	0	4	0
Cleaning	2600	1	4	250

[*] Zr-Ir permanent modifier and Pd+ Mg(NO$_3$)$_2$ were employed throughout.

A closed vessel microwave digester, MARS 5 CEM (CEM Corporation, Mattews, NC, USA), with a temperature control device, was used. Deionised water (Millipore, Bedford, MA, USA) of 18 MΩ.cm resistivity was used for the preparation of solutions. Nitric acid 65% and hydrogen peroxide 32% (Suprapur, Merck, Darmstadt, Germany) were used for total tin determination. The following 1000 mg.L^{-1} stock solutions were applied: Tin (IV) (Scharlau, Barcelona, Spain) in 5 mol.L^{-1} hydrochloric acid; zirconium (Spectracer, Paris, France) in 5 % v/v HCl and 0.5 % v/v HF; and ruthenium (Carlo Erba, Milan, Italy) in 5 % v/v hydrochloric acid. A standard solution of Mg(NO$_3$)$_2$ (10 g.L^{-1}) was prepared from Mg(NO$_3$)$_2$ (99 %) (Acros organics, New Jersey, USA). A 10.00 g.L^{-1} palladium solution was made by dissolving Pd powder (99.99 %) (Acros organics) in the minimum volume of aqua regia, followed by evaporation to near dryness and dilution with 5 % v/v nitric acid[21]. To avoid metal contamination, all glassware and material for analysis were rinsed with deionized water, kept for 2 days in 10 % (v/v) nitric acid solution, and rinsed several times before use.
Mussels (*Mytilus galloprovincialis*) were collected from three mussel farming stations of the Bizerte Lagoon situated in the Tunisian country. This lagoon is subject to intensive industrial activities. It is connected to a large harbor that may pollute marine organisms by organotin compounds[22]. Immediately after their arrival to the laboratory, the mollusks were washed with sea and deionized water and the whole soft tissues were excised from the shells, rinsed again in deionized water and dried at 70 °C. Dried material was ground to a fine powder in a stainless steel mill and stored at -20 °C until analysis. Sample aliquots (0.5 g) were digested with 7 ml of concentrated nitric acid and 2 ml of hydrogen peroxide in a microwave oven for 30 min and diluted to 25 ml with deionized water. Blank digests were treated in the same way.

Results and Discussion
The use of chemical modification is one of the approaches adopted to resolve difficulties encountered during tin analysis, such as the volatility of tin compounds before atomization[19]. Considering the literature, the amounts of permanent modifier Ir and chemical modifiers Pd, Mg(NO$_3$)$_2$ were first selected as factors being able to influence the analytical signal of tin. Performances of Ir as permanent modifier

were evaluated successfully in the analysis of trace elements by ETAAS, especially in complex matrices such as sediments, plants or petroleum refinery streams[23-25]. On the other hand, the efficiency of Pd/Mg(NO$_3$)$_2$ as conventional modifier has been proved in many research articles, where comparisons between the usually called "universal modifier" with other chemical or permanent modifiers were performed[15, 17, 21].

Tin analysis depends not only on the nature and the quantity of modifiers, but also on the electrothermal program used in the graphite tube. In this program, pyrolysis and atomization temperatures represent the most critical factors. In fact, the optimal pyrolysis temperature leads to the best atomization efficiency. The selection of this temperature must realize a compromise between the stabilization of the analyte and the elimination of the interactions due to the decomposition of the matrix. Furthermore, the optimal atomization temperature must be sufficient to involve the complete vaporization and atomization of the metal, by avoiding the memory effects being able to appear later on. However, the application of a too high atomization temperature (> 2600 °C) can reduce the selectivity of atomization and the tube lifetime.

According to the data mentioned above, the pyrolysis and atomization temperatures in the Zr-coated graphite furnace, the quantities of regular modifiers Pd and Mg (as nitrates) and the amount of permanent modifier Ir were selected as factors that could influence the analytical signal of tin.

The optimization of the ETAAS determination of tin was carried out using Doehlert design[18]. Applications of Doehlert designs in analytical chemistry were described in the determination of: tin nitrogen in bean samples[26]; copper in natural food samples by Flame Atomic Absorption Spectrometry[27]; vanadium and copper in seawater by Inductively Coupled Plasma Optical Emission Spectrometry[28] or arsenic in petroleum refinery streams by ETAAS [23]. Doehlert designs were also used for the optimization of the arsenic extraction from mussel samples with low power focused microwaves[29].

Y : measured response representing the tin absorbance;
U_1: first factor representing the quantity of chemical modifier Pd;

U_2: second factor representing the quantity of chemical modifier Mg;
U_3: third factor representing the pyrolysis temperature;
U_4: fourth factor representing the atomization temperature;
U_5: fifth factor representing the quantity of permanent modifier Ir.

Coded variables were used to compare the effects of the different factors in the experimental field concerned. Factors U_i we transformed into coded variables X_i according to the equation model #1:

$$X_i = \frac{(U_i - \overline{U_i})}{\Delta U_i}$$

(1)

Where,

X_i : value of the coded variable i;
U_i : value of the factor i;
$\overline{U_i}$: value of the factor i in the center of the experimental field;
ΔU_i : range of variation of the factor i.

All the experiments were carried out with a real mussel sample. Factors and experimental domain are listed in Table 2.

Table 2: Factors and Experimental domain for the determination of tin in mussels.

Factor	Unit	Associated variable	Low level	Upper level
Quantity of chemical modifier Pd	µg	X1	10	20
Quantity of chemical modifier Mg	µg	X2	10	20
Pyrolysis temperature	°C	X3	400	1800
Atomization temperature	°C	X4	1800	2500
Quantity of permanent modifier Ir	µg	X5	0	200

As shown in Table 2, the quantities of regular modifiers Pd and Mg varied respectively from 10 to 20 µg. Thus, the mean amount used for both modifiers was 15 µg, which corresponds to the mass generally injected into the graphite furnace for chemical modification. As the tube was previously coated with 200 µg Zr, we considered that a maximum level of 200 µg Ir was sufficient for the permanent modification of tin (Table 2). The selected domains for pyrolysis and atomization temperatures were large enough to let an easier determination of the optimal conditions of the temperature program.

In order to have the highest possible quality forecast, an experimental plan proposed by Doehlert was chosen (Table 3). In our case,

thirty-three experiments, within three experiments in the center, were carried out to estimate the coefficients of the model. Each run was carried out three times and the average values of the absorbance are also shown in Table 3.

The analytical responses obtained in each experiment were used to estimate the coefficients of the second-order polynomial model (Table 4). If a mathematical approach is adopted, the experimental response Y can be represented by a

Table 3: Experimental Plan for the analysis of tin in mussels by ETAAS.

N°Exp	Pd (µg)	Mg (µg)	T_{pyr} (°C)	T_{atom} (°C)	Ir (µg)	Absorbance* Y
1	20	15	1100	2150	100	0.2020
2	10	15	1100	2150	100	0.0000
3	18	20	1100	2150	100	0.2120
4	13	10	1100	2150	100	0.0000
5	18	10	1100	2150	100	0.0850
6	13	20	1100	2150	100	0.0930
7	18	17	1800	2150	100	0.2310
8	13	13	400	2150	100	0.0000
9	18	13	400	2150	100	0.0000
10	15	18	400	2150	100	0.0000
11	13	17	1800	2150	100	0.0000
12	15	12	1800	2150	100	0.0000
13	18	17	1275	2500	100	0.5180
14	13	13	925	1800	100	0.0310
15	18	13	925	1800	100	0.0000
16	15	18	925	1800	100	0.0000
17	15	15	1625	1800	100	0.0000
18	13	17	1275	2500	100	0.2890
19	15	12	1275	2500	100	0.2410
20	15	15	575	2500	100	0.2180
21	18	17	1275	2220	200	0.2040
22	13	13	925	2080	0	0.0000
23	18	13	925	2080	0	0.0380
24	15	18	925	2080	0	0.0000
25	15	15	1625	2080	0	0.0420
26	15	15	1100	2430	0	0.2390
27	13	17	1275	2220	200	0.0770
28	15	12	1275	2220	200	0.0300
29	15	15	575	2220	200	0.1050
30	15	15	1100	1870	200	0.0000
31	15	15	1100	2150	100	0.0783
32	15	15	1100	2150	100	0.0789
33	15	15	1100	2150	100	0.0776

* Negative values of absorbance obtained were converted to zero in the experimental plan.

quadratic equation of the response surface:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} \sum_{j=1}^{n} b_{ij} X_i X_j$$

(2)

Y : is the integrated absorbance of tin;
b_0 : is a constant corresponding to the value of Y when the X_i are equal to zero;
b_i : is the estimation of the main effects of the factor i ;
b_{ii} : is the estimation of the second order effect of the factor i;
b_{ij} : is the estimation of the interactions between factor i and factor j.

It was possible to verify that the generated model provided a good theoretical description of the experimental data (r^2 = 0,994).
Analysis of results (Table 4) showed that the atomization temperature would be the most influential factor on the tin absorbance in mussel samples.
Quadratic term of this factor was also significant. The considerable effect of atomization temperature on the magnitude of absorbance signals was also noticed in previous researches related to the determination of trace metals by ETAAS[13,23,30]. Design allowed contour plot graphs to be obtained. This is an efficient way of locating the optimum, and also for interpreting the interactions between the

studied variables. Fig. 1 shows the response surface developed by the model for pyrolysis and atomization temperatures at fixed quantities of Pd, Mg and Ir modifiers (17, 17 and 100 µg respectively). From the analysis of the isoresponse curves at the chosen experimental

field delimited by circle, we concluded that highest absorbance signals are obtained at elevated values of atomization and pyrolysis temperatures.

Table 4: Coefficients values of the second order polynomial model

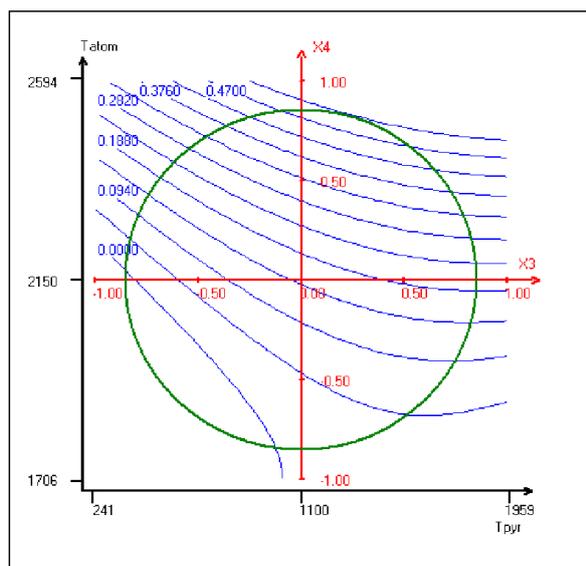| b0	b1	b2	b3	b4	b5	b11	b22	b33	b44	b55
0.078	0.100	0.066	0.047	0.197	0.013	0.023	0.018	−0.070	0.140	−0.026
b12	b13	b23	b14	b24	b34	b15	b25	b35	b45	
0.020	0.135	0.080	0.123	0.108	0.063	−0.010	0.052	−0.085	−0.002	

1

Fig. 1 Contour plot graph obtained with the variation of the absorbance signal versus *Tpyr* and *Tatom* for quantities of *Pd*, *Mg* and *Ir* fixed at 17, 17 and 100 µg respectively.

At low values of atomization temperatures, the signal was practically absent.
In fig. 2, variation of quantities of Pd and Mg modifiers versus absorbance, at fixed levels of atomization, pyrolysis temperatures and Ir modifier (1300 °C, 2500 °C and 100 µg respectively) showed that the addition of both chemical modifiers increased significantly the selected response. The stabilizing effect of palladium that prevents pre-atomisation losses of volatile elements, with considerable background reduction, was described in many research articles [30-32]. Mixed with magnesium nitrate, it constitutes a "universal modifier" used for the direct determination of the majority of volatile compounds with satisfactory analytical performances. According to the obtained results, the chosen optimum working conditions for tin analysis in mussel samples were: quantity of chemical modifier Pd, 17 µg; quantity of chemical modifier Mg, 17 µg; pyrolysis

temperature, 1300 °C; atomization temperature, 2500 °C and quantity of permanent modifier Ir, 100 µg.
The precision of the optimized method, evaluated through the analysis of ten replicates of the same mussel sample and calculated as the relative

standard deviation (R.S.D.), was 5.8 %. The sensitivity was studied by means of the detection (LOD) and quantification (LOQ) limits, expressed respectively as three and ten times the standard deviation of ten measurements of the blank divided by the slope of the standard addition graph. The LOD and LOQ were 0.069 µg.g^{-1} and 0.232 µg.g^{-1}, respectively.
Accuracy was checked using the reference material (NIST-SRM 2977). Results (1.35 ± 0.16 µg.g^{-1}) were in good agreement with reference values (1.47 ± 0.27 µg.g^{-1}), obtained as part of the NRCC interlaboratory comparison exercise.
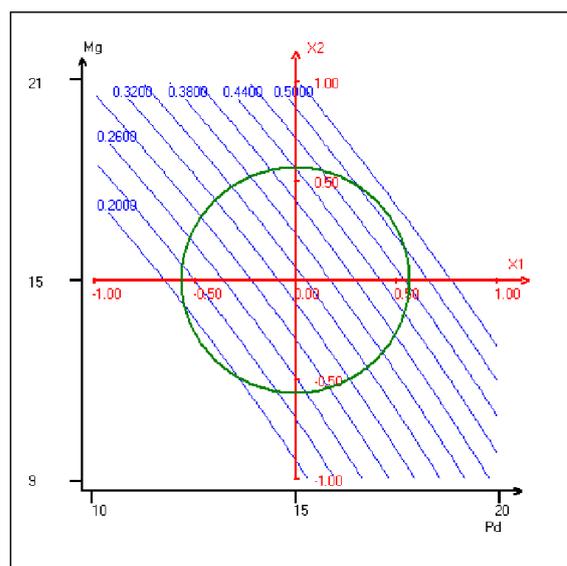

Fig. 2 Contour plot graph obtained with the variation of the absorbance signal versus *Mg* and *Pd* quantities for values

of *Tpyr* and *Tatom* fixed at 1300 and 2500 °C respectively and a quantity of *Ir* fixed at 100 μg.

The optimized methodology was applied to the analysis of mussel samples collected from Bizerte lagoon. Total tin concentrations are listed in Table 5. Levels of tin in the mussel samples ranged between 0.51 and 2.72 μg.g^{-1}

(dry weight). Highest values, found in Menzel Abderrahmen stations (table 5) might have been derived from extensive anthropogenic imputs or contamination by organotin antifouling paints. Compared to data reported in literature, total tin concentrations were relatively low[33].

Table 5: Total tin concentrations obtained in mussel samples collected from Bizerte lagoon

Station	Menzel Abderrahmen			Menzel DJemil			F.M.B.		
Sample	MA1	MA2	MA3	MJ1	MJ2	MJ3	FM1	FM2	FM3
Tin (μg.g^{-1})	1.89±0.09	2.72±0.13	2.34±0.11	1.39±0.07	1.66±0.08	1.12±0.05	0.51±0.03	0.78±0.04	1.24±0.06

Conclusion

Optimization by Doehlert design allowed the determination of optimal conditions for tin analysis in mussel samples with a minimum number of experiences. Interactions between factors were also estimated. Analysis was performed with ETAAS, where the best operating conditions required the combination of both permanent (Zr + Ir) and chemical (Pd + Mg(NO$_3$)$_2$) modifiers to avoid tin losses during the pyrolysis stage. Optimum atomization and pyrolysis temperatures were also selected (T$_{atom}$ = 2500 °C and T$_{pyro}$= 1300 °C). High sensitivity and good precision were obtained with the developed method that was applied for analysis of mussel samples collected from Bizerte lagoon.

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