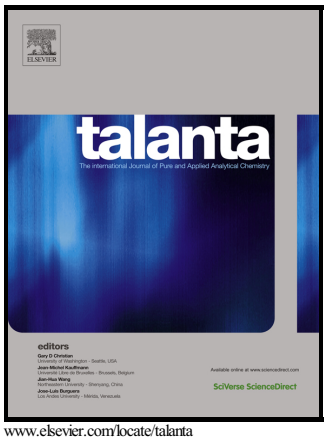
Author's Accepted Manuscript

Solvent bar micro-extraction: Improving hollow fiber liquid phase micro-extraction applicability in the determination of Ni in seawater samples

Juan J. Pinto, Mabel Martín, Belén Herce-Sesa, José A. López-López, Carlos Moreno



www.elsevier.com/locate/talanta

PII: S0039-9140(15)00267-2
DOI: http://dx.doi.org/10.1016/j.talanta.2015.04.029
Reference: TAL15529

To appear in: *Talanta*

Received date: 7 January 2015
Revised date: 6 April 2015
Accepted date: 9 April 2015

Cite this article as: Juan J. Pinto, Mabel Martín, Belén Herce-Sesa, José A. López-López and Carlos Moreno, Solvent bar micro-extraction: Improving hollow fiber liquid phase micro-extraction applicability in the determination of Ni in seawater samples, *Talanta,* http://dx.doi.org/10.1016/j.talanta.2015.04.029

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Solvent bar micro-extraction: Improving hollow fiber liquid phase micro-extraction applicability in the determination of Ni in seawater samples

Department of Analytical Chemistry. Faculty of Marine and Environmental Sciences, University of Cádiz, Puerto Real, 11510 Cádiz, Spain

Juan J. Pinto, Mabel Martín, Belén Herce-Sesa, José A. López-López, Carlos Moreno

Corresponding author: José A. López-López

e-mail: joseantonio.lopezlopez@uca.es

Telephone number: +34956016165

Abstract

During the last decade, hollow fiber liquid phase micro-extraction (HF-LPME) has become an attractive alternative in sample treatment for the analysis of trace metals in seawater. If compared with other similar methodologies, its main advantages are associated to a higher stability of the organic solution contained into the pores of the fiber, which acts as a lipophilic membrane during the extraction process. However there are some remaining problems that difficult its use, mostly related to the need of increasing the rate of analysis and improving portability.

In this paper a novel three phase solvent bar micro-extraction (3PSBME) for the fiber device has been proposed. Its main advantage is that the 3PSBME device can be left free in the sample. This way the system is portable, and no special support is needed leading the possibility of simultaneous extraction of several samples.

In this work, multivariate central composite design of experiment has been carried out to optimize Ni pre-concentration using di-2-ethylhexyl phosphoric acid (DEHPA) as extractant and HNO_3 as acceptor agent. Factors influencing exraction have been the pH in the sample and the fiber length. For seawater samples, Ni can be pre-concentrated eleven times in 140 minutes. The method presents RSD 9.42% and limit of detection 44 ng L^{-1}, using GFAAS for instrumental determination. It has been applied to determination of Ni in seawater, including a reference material CRM-403 proving its applicability.

Accepted manuscript

ACCEPTED MANUSCRIPT

Keywords: Ni, three-phase solvent bar micro-extraction (3-PSBME), trace analysis seawater, GFAAS, central composite experimental design

1. Introduction

Interest of metals analysis in surface seawater is based on their toxicity even at very low concentration [1-3]. Some metals as Ni are essential nutrients at low concentration; however they are toxic at higher level [4]. Ni can be normally found at trace level; notwithstanding it accumulates in organisms causing bioaccumulation through the trophic chain [5,6]. For this reason monitoring its concentration in natural waters and in particular in seawater is of main concern.

Despite its interest, Ni determination in seawater samples is limited by the low concentrations it normally presents and even more by the effect of saline sample matrix. For these reasons, a separation and/or pre-concentration step is normally required prior Ni quantification [7]. In this regard, several liquid and solid phase extraction based methods have been proposed for sample preparation in analysis of metals in seawater samples [8-11]; however, pre-concentration using these methods is limited and highly sensitive instrumental techniques such as inductively coupled plasma mass spectrometry are required [11]. For this reason, during the last decade popularity of micro-extraction techniques, which offer higher pre-concentration factors with lower waste generation, has importantly been increased [12,13]. Among micro-extraction techniques, liquid phase micro-extraction (LPME) has been proposed in different configurations, being hollow fiber liquid phase micro-extraction (HFLPME) the most used due to its stability against other configurations [14,15].

HFLPME can be used in a two phase or a three phase configuration. In the case of metals the most widely used is the three-phase configuration (3-HFLPME) because the metal is pre-concentrated into an aqueous solution inside the fiber, which is compatible with most of available spectroscopic techniques [15]. Metal transport takes place by means of an extractant dissolved in an organic solvent located in the pores of the fiber's walls that separate the sample from the acceptor solution [16].

In spite of its potential for metals pre-concentration, the application of 3-HFLPME for trace metals analysis in seawater has been scarcely explored. 3-HFLPME has showed its capability for selective pre-concentration of Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} using

Accepted manuscript

ACCEPTED MANUSCRIPT

the crown ether kriptofix-22DD as extractant for seawater analysis [17]. Additionally, a green HFLPME has been applied for total Ni pre-concentration in seawater, and it has been proposed as an environmentally friendly method for metals analysis [18].

Limitations of 3-HFLPME are normally associated to the need of a specific support for the fiber during extraction, leaking of acceptor solution in the connection to the support, and inconvenient for portability [12,14]. For these reasons 3-HFLPME has not been still implemented as a routine technique for sample treatment in metals analysis. In order to improve these facts, the configuration of 3-HFLPME as a three phase solvent bar recently appeared as a solution that offers better stability, portability, and the possibility to increase the number of samples that can be carried out at the same time [19]. Three phase solvent bars (3PSBME) can be obtained by thermal sealing of the ends of the fiber tube [20].

In this work, di-2-ethylhexylphosphoric acid (DEHPA) has been used as an extractant for facilitated transport of Ni from the sample to the acceptor solution. Transport takes place by coupled counter-transport in which the metal is transported from the sample to the acceptor; simultaneously, H^+ is transported from an acidic acceptor solution to the sample [21,22].

The aim of this work is the development and optimization of an extraction and pre-concentration 3-PSBME method, for analysis of Ni traces in seawater samples~~,~~ that improves operational and technical features of existing methods in terms of efficiency and portability. Multivariate optimization has been used in order to establish the relationship within the factors affecting Ni pre-concentration and to maximize the enrichment factor of the metal. Finally, the method has been applied for Ni ultra-trace analysis in seawater samples.

2. Materials and methods

2.1.Reagents and solutions

Unless otherwise stated, all chemicals were reagent grade and they were used as received without further purification.

Nitric acid for trace analysis (60%) was purchased from Scharlau (Barcelona, Spain). The extractant di-2-ethylhexyl phosphoric acid (97%) was obtained from Aldrich (Steinheim, Germany), while kerosene (99.7%), acetic acid (100%), sodium chloride (99%), potassium chloride (99%), boric acid (99%) and sodium hydroxide

Accepted manuscript

ACCEPTED MANUSCRIPT

were provided by Panreac (Barcelona, Spain). Finally, calcium chloride (99%), magnesium chloride (99.5%), sodium dihydrocarbonate (99%), potassium bromide (99.5%), sodium sulphate (99%), 1-pyrrolidine-dithiocarbamate (APDC) and diethylammonium-diethyldithiocarbamate (DDDC) were obtained from Merck (Darmstadt, Germany). Certified reference material for analysis of metals in seawater BCR-403 was obtained from the Institute of Reference Materials and Measurements in the Joint Research Centre (Geel, Belgium).

Aqueous solutions of Ni were prepared from a 1000 mg L^{-1} standard solution obtained from Fluka (Buchs, Switzerland). Deionized water of resistivity lower than 18.2 MΩ cm was obtained by a Millipore Quantum Ultrapure water supplier (Millipore, USA).

Synthetic seawater samples were prepared dissolving NaCl, $MgCl_2$, KCl, $CaCl_2$ $NaHCO_3$, HBO_3, KBr, and Na_2SO_4 in deionized water [23].

2.2.Apparatus

Polypropylene Accurel PP S6/2 hollow fibers of 0.2 μm pore size, 75% porosity, and wall thickness 450 μm, and 1800 μm internal diameter were used in this study (Membrana, Germany). Samples were stirred using an IKA-Big Squid magnetic stirrer (Ika-Werke, Germany). The pH of water samples was measured using a Jenway 4330 conducitvity & pH meter (Jenway, UK).

Ni concentration in the aqueous solutions was quantified by atomic absorption spectrometry (AAS) using an AAnalyst 200 Atomic Absorption Specctrometer (Perkin Elmer, USA), with flame atomizer (FAAS) in the case of optimization studies. For FAAS measurement a hollow cathode lamp was used as a source of radiation, as well as a deuterium lamp was used for background correction. In the case of quantification of Ni concentration in real samples a continuum source atomic absorption spectrometer model ContrAA (Analytikjena, Germany) was used, applying graphite furnace atomization (GFAAS) of the metal. Transversal heating graphite furnaces used were made in pyrolitic graphite (Analytik Jena, Germany).

Seawater samples were filtered online during collection through a 0.45 μm pore sized nylon filter and they were stored at pH<2 after acidification with HNO_3. Since Ni complexation by Cl^- is negligible it was considered to be as Ni^{2+}.

Accepted manuscript

ACCEPTED MANUSCRIPT

2.3. Set up of the three phases solvent bar micro-extraction (3PSBME)

The 3PSBME devices were prepared as follows (Figure 1) [24]:

- Fibers were cut at the desired length using a blade.
- One of the fiber's ends was thermally sealed using flat ended tweezers and a hot tip.
- The fiber was filled with the aqueous acceptor solution containing HNO_3 and the other end of the fiber was sealed to make up the solvent bar.
- Fiber pores were impregnated with the organic solution, by immersion of DEHPA solution dissolved in kerosene.
- Excess of organic solution on the solvent bar walls was rinsed with water.
- The 3PSBME bar was immersed free in the sample, which was stirred during extraction at 500 r.p.m.

After extraction experiment was completed, the fiber was extracted from the sample and the acceptor solution was transferred into a vial for further determination of Ni by AAS. Acceptor solution was recovered cutting the ends of the fiber and flushing the acceptor solution into a vial. Hollow fibers were discarded after use to avoid memory effects. In all cases, two aliquots of the same sample were extracted. Moreover, for each aliquot of sample, three solvent bars were immersed as internal replicates.

2.4. Instrumental analysis of Ni

Instrumental determination of Ni was carried out by atomic absorption spectrometry.

In the case of optimization studies, FAAS using acetylene as fuel and air as oxidizer was employed for quantification. Measurements in this case were conducted using a wavelength of 232.0 nm and a D_2 lamp was used for background correction Determination of Ni by FAAS was possible because initial concentration of Ni in the sample was 0.1 mg L^{-1}. Samples were diluted to a volume of 1.5 mL using deionized water, to have enough volume of sample as to be measured by FAAS. Standard solutions of Ni were prepared according to HNO_3 concentration of analyzed samples to reproduce sample matrix.

Accepted manuscript

ACCEPTED MANUSCRIPT

Analysis of Ni in real samples was conducted by GFAAS using argon as inert gas in a Continuum Source Atomic Absorption Spectrometer. In this case the acceptor solution was directly recovered into a vial for measurement. A volume of 20 µL of sample was injected for analysis, and three replicates of each aliquot of acceptor solution were analyzed. Measurements were carried out applying 800ºC for ash and 2200ºC for Ni atomization. Registration of Ni absorbance at 232.0 nm was completed using a digital detector between pixels 98-102. Under these conditions a limit of detection of 0.13 µg L^{-1}, calculated as three times the standard deviation of 10 independent blanks divided by the slope of the calibration curve, and a linear range 0.2-10 µg L^{-1} were obtained [25].

2.5.Quantification of Ni transport efficiency

Ni transport through 3PSBME using DEHPA takes place by coupled counter-transport. It consists in the simultaneous transport of Ni^{2+} from the sample to the acceptor solution while H^+ is transported from the acceptor solution to the sample [21]. Summarizing, a gradient of [H^+] between the acceptor solution and the sample is the driving force of metals transport and pre-concentration. This means that an acidic acceptor is required, and that pH in the sample cannot be equal or lower than in the acceptor solution. Taking these factors into account, multivariate optimization has been chosen in order to clarify possible interactions between the variables affecting Ni transport [26].

Efficiency of the proposed 3PSBME system for Ni pre-concentration was measured in terms of enrichment factor (*EF*), which can be defined as the ratio between Ni concentration in the acceptor solution after a certain time ($[Ni]_t$) and the initial concentration of Ni in the sample ($[Ni]_0$) (Equation 1).

$$EF = \frac{[Ni]_t}{[Ni]_0} \qquad \text{Equation 1}$$

2.6.Multivariate optimization of Ni pre-concentration

Multivariate optimization using a response surface method was applied for enhancement of Ni pre-concentration using the *EF* as a response factor. All calculations were conducted using software Statgraphics 5.1 (Statpoint Technologies Inc., USA). In particular, a central composite experimental design (CCD) was chosen [26]. It consist in a two level (2^n) factorial design in which *n* factors are tested in 2 levels, augmented with N_c centered points equally separated from the minimum and maximum level to be

Accepted manuscript

ACCEPTED MANUSCRIPT

tested, and $2 \cdot n$ star points, one at either extreme of each factor and at the center of all the other factors. In conclusion the number of experiments required to complete a composite central design is represented by equation 2. [27]. The factors considered for optimization in this work were: pH in the sample, concentration of DEHPA in the organic solution, HNO_3 concentration in the strip solution, time of experiment (t), and length of the solvent bar (L). In this case 5 factors have been optimized using 3 center points, resulting in a total of 45 experiments. Two replicates of the experimental design have been conducted and the order of the experiments was randomized.

$$2^n + 2n + N_c \qquad \text{Equation 2}$$

As a result of the CC-D design, a mathematical model was obtained. In this model response factor (*EF*) is represented as a function of the experimental factors ($EF = f(pH, [DEHPA], [HNO_3], t, L)$). A mathematical model that relates the response of the system as a function for the selected variables can be obtained. This model was used to calculate the optimized value for each variable. Finally, the fit of the model to the experimental results was evaluated by analysis of the variance (ANOVA). Results from ANOVA were used to interpret which factors presented a significant effect on *EF* [25].

3. Results and discussion

3.1.Capability of 3PSBME for Ni transport

The first step was the evaluation of hollow fibers suitability, in the configuration of 3PSBME, for Ni extraction. All experiments in this section were conducted during 90 minutes and an initial concentration of 0.1 mg L^{-1} Ni in the sample was used. Therefore, Ni in aqueous solutions was measured by FAAS. First, the volume of acceptor solution inside a 4-cm-lengt solvent bar was measured after an extraction experiment to evaluate stability of the system and to detect possible leaks in the sealing of the fiber ends. For 10 different experiments a RSD of 10% was obtained and as a result, an average volume of 55±5 µL was recovered, showing the physical-chemical stability of the system during extraction, in particular referred to thermal sealing of the fiber ends.

Moreover, the enrichment factor was evaluated using pH = 4.7 in the sample, 0.9 M DEHPA in the organic solution and 1M HNO_3 in the acceptor solution [18]. As a result, an enrichment factor of 8.62±1.41 was obtained for 10 extraction experiments, proving the repeatability of the system.

Accepted manuscript

ACCEPTED MANUSCRIPT

3.2.Multivariate optimization of 3PSBME for Ni pre-concentration

Central composite design was used for optimization of the different factors in order to maximize the *EF* of Ni. Application of CCD considers the use of star points that are beyond the upper and lower limits of the experimental range. In this case the distance from the center point to the star points was 1.57. This fact conditioned the selection of the experimental range to ensure that experimental conditions for star points can be achieved.

In particular, for pH, slightly acidic conditions are normally required for metals transport using DEHPA; additionally, 1 M acetic acid/acetate buffer was used to keep pH in the sample during extraction experiments [21,22]. In the case of DEHPA, concentration range is limited by an increase of viscosity of the organic solution at concentrations higher than 0.9M [18]. Selection of time of extraction was conducted considering that for longer times of experiment the analytical applicability of the system would be limited. The upper and lower levels for each factor, center points and star points are showed in table 1. During optimization, all the experiments were carried out with samples containing 0.1 mg L^{-1} Ni, and therefore, Ni concentration in the acceptor solution was analyzed by FAAS.

Once the experimental results were obtained, they were analyzed and the effect of each factor on the *EF* was evaluated. First, the fit of the model to the experimental results was evaluated analyzing the residual values for each experiment, showing a 77% fit. Thus, ANOVA was used to evaluate the influence of factors and their interactions, as well as curvature of the response surface for *EF* in the experimental range [25]. This information can be summarized in a Pareto chart representing the standardized effect of each factor (Figure 2). The threshold line marks the value for an effect to present significant influence on the response for a 95% level of confidence. As a result, pH in the sample, and curvature for time and fiber length presented significant effects on *EF*.

In the case of pH in the sample, this result supports the fact that the transport of Ni through the organic solution takes place by coupled counter transport, being the pH a key factor to set the optimum gradient of H^+ concentration between the sample and the acceptor solution. Regarding fiber length and time of extraction, a significant effect of quadratic factor has been observed. Therefore, these must be rigorously controlled during application of the method.

Accepted manuscript

ACCEPTED MANUSCRIPT

Equation of the surface of response was estimated and *EF* for certain value of the factors could be calculated (equation 3). Moreover, the optimum value for every variable was calculated. Optimized values were sample pH= 6.14, DEHPA concentration 0.87 M, HNO_3 concentration 1.86 M, time of experiment 136.91 min and fiber length 3.18 cm. Predicted enrichment factor under optimized conditions was 81.04.

$$EF=-190.56+27.09 \cdot pH-4.08 \cdot [DEHPA]-70.41 \cdot [HNO_3]+0.51 \cdot t+80.16 \cdot L+$$

$$7.60 \cdot pH \cdot [DEHPA]+4.93 \cdot pH \cdot [HNO_3]+0.06 \cdot pH \cdot t+1.04 \cdot pH \cdot L$$
$$+29.84 \cdot [DEHPA] \cdot [HNO_3]+0.25 \cdot [DEHPA] \cdot t-6.84 \cdot [DEHPA] \cdot L+0.17 \cdot [HNO_3] \cdot t$$
$$-5.24 \cdot [HNO_3] \cdot L-0.06 \cdot t \cdot L-4.21 \cdot pH^2-47.70 \cdot [DEHPA]^2+22.30 \cdot [HNO_3] \ -0.01 \cdot t-8.98 \cdot L^2$$

Equation 3

Optimized values are summarized in table 1. As it can be observed, the highest *EF* could be obtained near to the highest value of pH in the sample and for the highest concentration of H^+ in the acceptor. It is agree with the fact that coupled counter-transport of Ni is favored by a gradient of H^+ from the acceptor solution to the sample, as a driving force. However, at pH higher than 6.14 *EF* decreased because DEHPA presented the highest yield at relatively acidic pH values [22] (Figure 3). In the case of DEHPA concentration, the results of multivariate optimization presented an increase of extraction efficiency with increasing concentration of DEHPA; notwithstanding 0.87 M was considered as optimum because at higher concentration viscosity of the organic solution increases, leading to difficulties in fibers set up. Regarding fiber length, it is important to note that higher enrichment factors have been obtained for shorter fibers, as it was expected due to a smaller internal volume of acceptor solution. However, due to difficulties of setting up and handling fibers shorter than 3.18 cm, this fiber length showed to be optimum. Finally, for time of extraction an increase of extracted Ni was observed with time until 136.91 min. For longer times a decrease of Ni pre-concentration was observed that could be associated to a loss of organic solution from the fiber pores [16]. In spite of it could appear as a long time of pre-concentration, the use of 3PSBME allowed setting up several experiments simultaneously, because the fiber is free in the sample, without the need of any support. In particular, up to 10 samples were processed at the same time, so time of extraction per sample was 14 minutes.

Accepted manuscript

ACCEPTED MANUSCRIPT

3.3. Application to Ni analysis in seawater samples

Regarding application to Ni pre-concentration in seawater samples, it is important to note that Ni transport using DEHPA as extractant has been reported to be affected not by ionic strength of seawater but by the presence of Ca^{2+} and Mg^{2+} ions [18,21]. Taking this into account, synthetic seawater samples were prepared following the Grashoff protocol and spiked with a known concentration of Ni of 10 µg L^{-1} [23]. This way the extent of interferences found in seawater samples, including Ca^{2+} and Mg^{2+}, could be evaluated. For this type of samples, *EF* was calculated using a series of samples of known concentration of Ni from 0.1 mg L^{-1} to 1 µg L^{-1} offering a value of 10.96±1.05 for six independent samples of each concentration. This EF was used for Ni analysis in seawater samples. Additionally, the method was applied for recovery analysis of Ni in a real seawater sample collected from the North Morocco Mediterranean Sea (N35º24'396'', W005º01'726''). This sample was spiked with 2 µg L^{-1} Ni and analyzed by the proposed method obtaining a recovery of 91% Ni. Additionally, spiked sample was analyzed by the reference method of liquid-liquid extraction with APDC/DDDC [28], proving that there are no significant differences between the expected and analyzed concentrations of Ni [25]. This fact supports the reliability of the estimated *EF* for seawater samples.

Once the applicability of 3PSBME using DEHPA was tested, methodological blanks were analyzed to calculate the limit of detection of the method. For instrumental determination of Ni, GFAAS was used due to the low concentration of Ni in the acceptor solution after extraction that did not allow measurement by FAAS. In this case synthetic seawater samples were used, without addition of Ni. Concentration of Ni in blank samples was 0.043±0.001 µg L^{-1} for ten blank samples. Limit of detection was calculated as the average concentration of ten independent blank samples plus three times their standard deviation, after extraction and analysis, giving a value of 0.044 µg L^{-1} [16] (Table 2).

Finally, capability of the method for determination of Ni in seawater samples was evaluated. On the one hand, a sample collected from the so-called San Pedro River which is an arm-of-the-sea located in the Bay of Cadiz (SW, Spain) was analyzed. This sample was collected using clean sampling techniques and it was stored at pH<2 until it

Accepted manuscript

ACCEPTED MANUSCRIPT

was used for analysis [29]. For this sample the method was compared with the reference method of liquid-liquid extraction with APDC/DDDC [28]. On the other hand, a certified reference material BCR-403 from the IRMM of the Joint Research Center collected in the North Sea was analyzed. In both cases a *t* test was used for comparison of results obtained by the proposed method and the results from the reference method and the reference material respectively [25]. The null hypothesis that there no exist significant differences between the reference concentration and the analyzed concentration for a 95% confidence level was accepted if the calculated *t* value was smaller than the critical value of t (t_c).

As it can be observed in table 3, the null hypothesis of the t test is accepted in both cases proving the accuracy of the proposed method and its applicability to Ni monitoring, at trace level in seawater. Additionally, the number of samples that can be treated simultaneously by 3PSBME is higher than for traditional extraction methods and other HFLPME based methods because no support is required for the fiber during Ni extraction. Finally reproducibility of the method in the determination of Ni in seawater samples at the level of sub μg L^{-1} was 9.42%, calculated as standard deviation of the analyzed concentrations for real samples (Table 2).

Conclusions

In comparison with other hollow fiber micro-extraction techniques, 3-PSBME simplifies the extraction process and allows simultaneous extraction of higher number of samples and also samples processing cost.

Multivariate optimization has allowed the identification of the main factor that affects Ni transport by DEHPA through 3PSBME. Among chemical variables, pH has appeared to be particularly important; however for selection of a proper fiber length a balance between reduction of internal fiber volume and reproducibility has to be considered.

Finally, the proposed method appears as an accurate alternative for multi-sample treatment in ultra-trace determination of Ni in seawater samples; which minimized operational requirements improving analytical features of existing methods.

Acknowledgments

The present work was financed by the Spanish Ministry of Economy and Competitiveness (CTM-2010-15618 and CTM-2013-47549-P).

Accepted manuscript

ACCEPTED MANUSCRIPT

References

1. Wurl,O., Obbard, J.P. A review of pollutants in the sea-surface microlayer (SML): a unique habitat for aquatic organisms. Mar. Pollut. Bull. 48 (2004) 1016-1030.
2. Fu, J., Wang, H., Billah S.M.R., Yu, H., Zhang, W. Heavy metals in seawater, sediments, and biota from the coastal area of Yancheng City, China. Environ. Toxicol. Chem. 33 (2014) 1697-1704.
3. De los Ríos, A., Pérez, L., Ortiz-Zarragoitia, M., Serrano, T., Barbero, M.C., Echevarría-Erasun, B., Juanes, J.A., Orbea, A., Cajaraville, M.P. Assessing the effect of treated and untreated discharges to estuarine and coastal waters applying selected biomarkers on caged mussels. Mar. Pollut. Bull. 77 (2013) 251-265.
4. Kennish, M.J. Estuarine and marine pollution. CRC Press Inc. Boca Ratón, USA (1997)
5. Malea, P., Kevrekidis, T. Trace element patterns in marine macroalgae. Sci. Total Environ. 1 (2014) 144-157.
6. Ryan, S., McLoughlin, P., O'Donovan, O. A comprehensive study of metal distribution in three main classes of seaweed. Environ. Pollut. 167 (2012) 171-177.
7. Larsen M.M, Sondergaard, J., Asmund, G., Parmentier, K., Vermaercke, P. Marine monitored parameters, matrices and related techniques. Trace metals, in, Quevauviller, P., Roose, P., Verreet (Eds), Chemical Marine Monitoring. Policy framework and analytical trends. Wiley, Sussex, pp 71-100 (2011)
8. Wang, B., Lee, C., Ho, T Trace metal determination in natural waters by automated solid phase extraction system and ICP-MS: The Influecne of low level Mg and Ca. Talanta, 128 (2014) 337-344.
9. Zereen F., Yilmaz, V., Arslan Multielement solid phase extraction using a chelating resin of styrene divinylbenzene copolymer and application for the analysis of seawater and fish otoliths by inductively coupled plasma mass spectrometry (ICP-MS). Anal. Lett, 47 (2014) 58-76.
10. Komjarova, I., Blust, R. Comparison of liquid-liquid, solid-phase extraction and co-precipitation preconcentation methods for the determination of cadmium, copper, nickel, lead and zinc in seawater. Anal. Chim. Acta 576 (2006) 221-228.
11. López-López, J.A., Mendiguchía, C., Pinto, J.J., Moreno, C. Liquid membranes for quantification and speciation of trace metals in natural waters. Trac-Trend. Anal. Chem. 29, (2010) 645-653.

Accepted manuscript

ACCEPTED MANUSCRIPT

12. Spietelun A., Marcinkowski L., de la Guardia M., Namiesnik J. Green aspects, developments and perspectives of liquid phase microextraction techniques, Talanta, 119 (2014) 34-45
13. Dadfarnia, S., Shabani, A.M.H. Recent development in liquid phase microextraction for determination of trace level concentration of metals- A review. Anal. Chim. Acta., 658 (2010) 107-119.
14. Pabby, A.K., Sastre, A.M. State-of-the-art review on hollow fibre contactor technology and membrane-based extraction processes. J. Mem. Sci., 430 (2013) 263-303.
15. Pena-Pereira, F., Lavilla, I., Bendicho, C. Miniaturized preconcentration methods based on liquid-liquid extraction and their application in inorganic ultratrace analysis and speciation: A review. Spectrochim. Acta. B 64 (2009) 1-15.
16. López-López, J.A., Jonsson, J.A., García-Vargas, M., Moreno, C. Simple hollow fiber liquid membrane base pre-concentration of silver for atomic absorption spectroscopy. Anal. Methods, 5 (2014) 1462-1467.
17. Slaveykova, V.I., Karadjova, I.B., Karadjov, M., Tsalev, D.M. Trace metal speciation and bioavailability in surface waters of the Black Sea coastal area evaluated by HF-PLM and DGT. Environ. Sci. Technol. 43 (2009) 1798-1803.
18. Vergel, C., Montoya-Mayor, R., Mendiguchía, C., Moreno, C. HF-LPME as a Green alternative for the preconcentration of nickel in natural waters. Anal. Bioanal. Chem. 3 (2012) 665-670.
19. Anthemidids A.N., Mitani C. Advances in liquid phase micro-extraction tenchniques for metal, metalloid and organometallic species determination, Curr. Anal. Chem., 9 (2013) 250-278
20. L. Guo, H.K. Lee Ionic liquid based three-phase liquid-liquid-liquid solvent bar microextraction for the determination of phenols in seawater samples, J. Chromatogr. A, 1218 (2011) 4299-4306.
21. Irigoyen, L., Moreno, C., Mendiguchía, C., García-Vargas, M Application of liquid membranes to simple preconcentration for the spectrophotometric determination of cadmium in seawater. J. Mem. Sci., 274 (2006) 169-172.
22. Mendiguchía, C., García-Vargas, M., Moreno, C. Screening of disolved heavy metals (Cu, Zn, Mn, Al, Cd, Ni, Pb) in seawater by a liquid-membrane-ICP-MS approach. Anal. Bioanal. Chem., 391 (2008) 773-778.

Accepted manuscript

ACCEPTED MANUSCRIPT

23. Grasshoff, K., Ehrhardt, M. Kremling, K Methods of seawater analysis. Second edition. Verlag Chemie, Weinheim, Germany (1983)
24. López-López J.A., Pinto J.J., Mendiguchía C., Vergel C., Manuel M.V., Moreno C. (2013) Spanish Patent Application: 201300122.
25. Miller, J.N, Miller J.C Statistics and chemometrics for analytical chemistry fourth edition. Pearson education, Madrid. (2002)
26. Morgan, E. Chemometrics: Experimental Design. Wiley, Belmont, USA (1995)
27. Montgomery, D.C. Design and analysis of experiments. Wiley, Belmont, USA (1991)
28. Bruland K.W., Franks R.P., Knauer G., Martin J., Sampling and analytical methods for the determination of copper, cadmium, zinc and nickel in seawater, Anal. Chim. Acta 105 (1979) 233-245.
29. López-López, J.A., García-Vargas, M. Moreno, C. A chemometric approach to the evaluation of atmospheric and fluvial pollutant inputs in aquatic ecosystems: The Guadalquivir River estuary as a case study. Environ. Pollut. 159 (2011) 1136-1143.

Accepted manuscript

ACCEPTED MANUSCRIPT

Figure captions

Figure 1. Set up of the three-phase solvent bar micro-extraction system

Figure 2. Pareto chart of normalized factors. Effects higher than (2.08) were considered as significant. Positive effect: ■, negative effect: ■.

Figure 3. Surface of response for EF with pH and [DEHPA] at fixed time=90 min, L=4 cm, and [HNO_3]=1M

Accepted manuscript

Figure 1

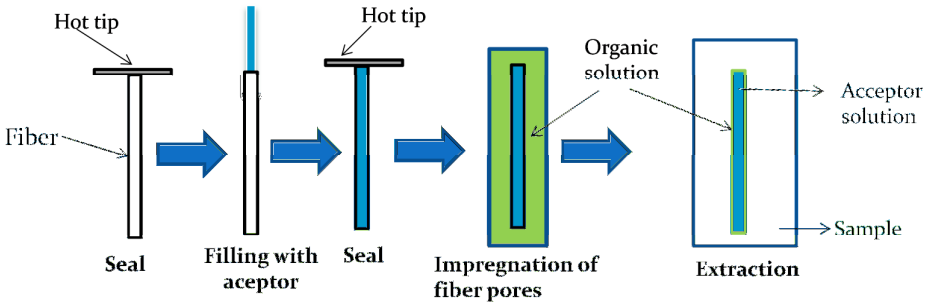
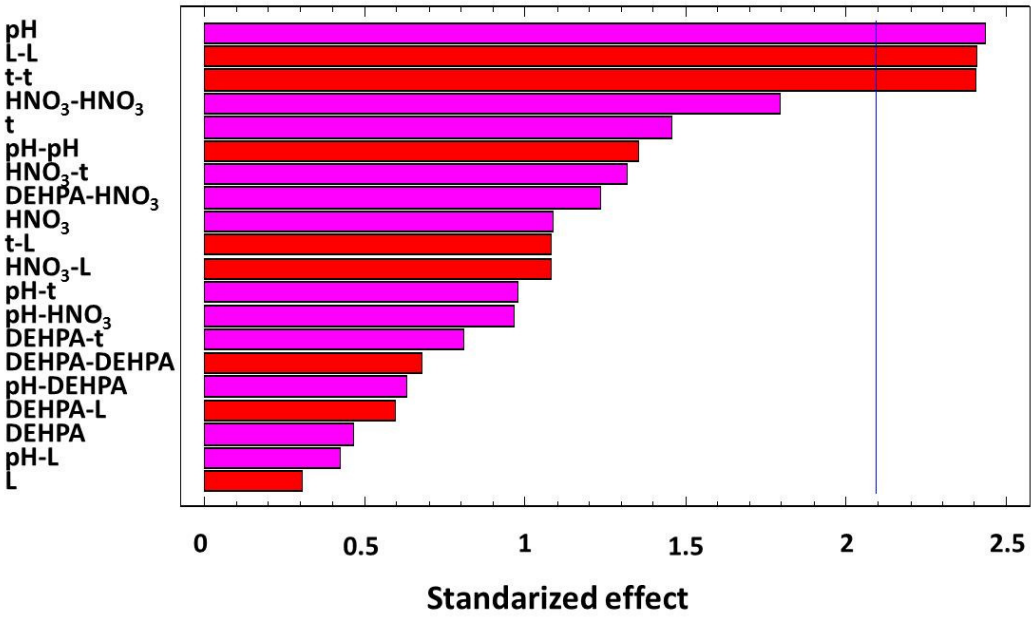
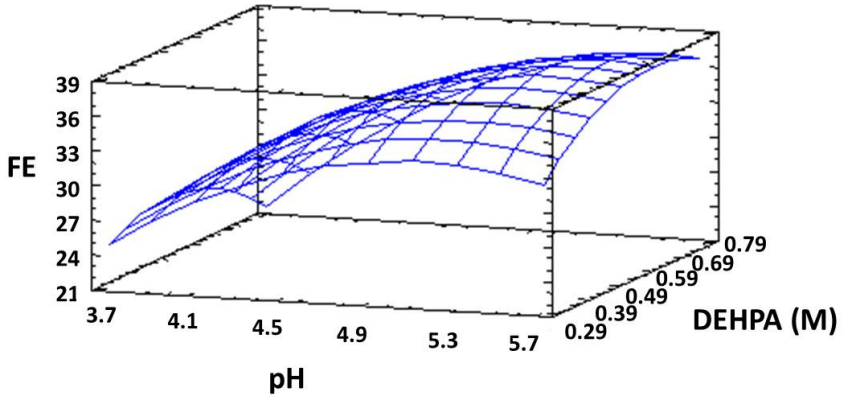


Figure 2



ACCEPTED MANUSCRIPT

Figure 3



Accepted Manuscript

ACCEPTED MANUSCRIPT

Table 1. Experimental conditions for each factor used for optimization by central composite design of experiment.

Factor	Lower star point	Lower level	Center point	Higher level	Higher star point	Optimum value
pH	2.97	3.70	4.70	5.70	6.42	6.14
DEHPA (mol L^{-1})	0.14	0.30	0.51	0.70	0.87	0.87
HNO_3 (mol L^{-1})	0.10	0.5	1.00	1.50	1.90	1.86
Time (min)	11.20	40.00	80.00	120.00	148.90	136.91
Fiber length (cm)	2.20	3.00	4.00	5.00	5.70	3.18

Accepted manuscript

ACCEPTED MANUSCRIPT

Table 2. Figures of merit of the proposed method

Enrichment factor	10.96
Limit of detection	44 ng L^{-1}
Reproducibility	9.42%
Time of processing per sample	14 minutes
[1]Bias	9.21%

[1] Calculated from recovery analysis of Ni in comparison with the reference method of liquid-liquid extraction for San Pedro River sample and from the reference value of the CRM-403.

Accepted manuscript

ACCEPTED MANUSCRIPT

Table 3. Accuracy of the proposed method for Ni analysis in seawater samples

Sample	[Ni] $_{\text{this method}}$ (µg L^{-1})	[Ni] $_{\text{reference}}$ (µg L^{-1})	t	H_0
[1]Seawater	6.96±0.54			
[1] Seawater (spiked 2 µg L^{-1})	8.79±0.49	8.96	[4]-0.79	Accepted
San Pedro River	0.61±0.07	[2]0.72	[4]-3.51	Accepted
BCR-403	0.31±0.04	[3]0.26±0.02	[5]-2.06	Accepted

[1]Mediterranean Sea North Morocco (N35º24'396'', W005º01'726'')

[2]Liquid-liquid extraction with APDC/DDDC and ICP-MS quantification

[3]Certified concentration IRMM- JRC

[4] $t_{c\ (\alpha=0.05,\ n=2)} = 4.30$

[5] $t_{c\ (\alpha=0.05,\ n=14)} = 2.14$

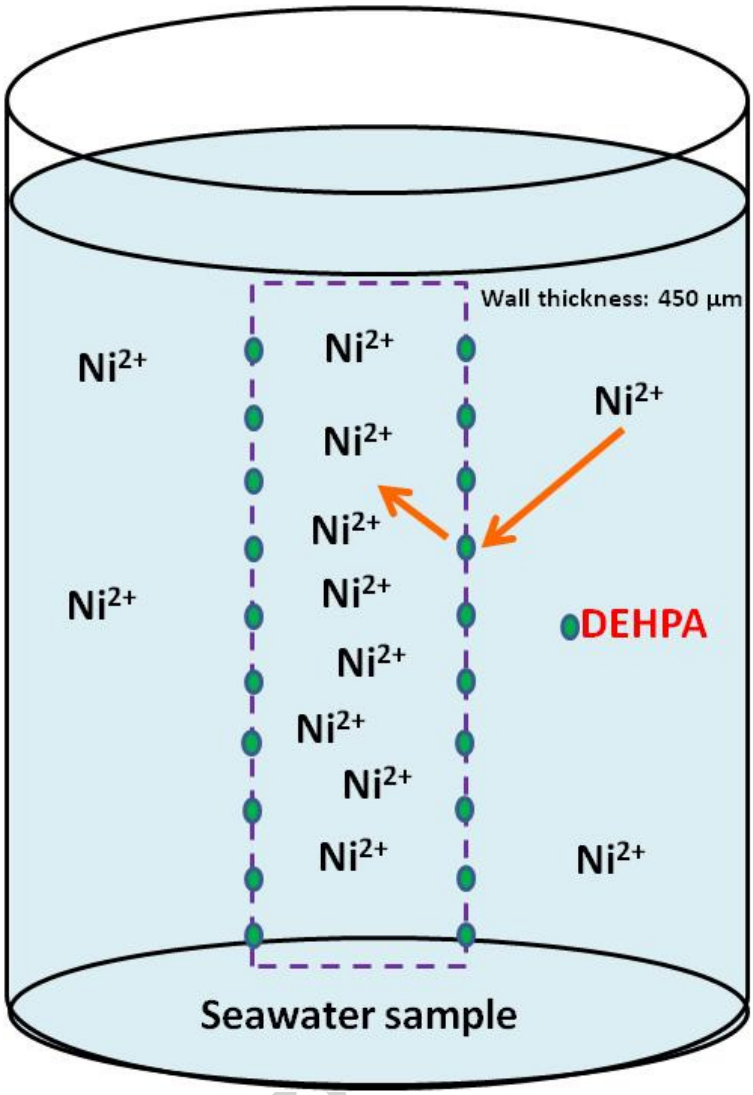
Accepted manuscript

ACCEPTED MANUSCRIPT

Highlights

- 3-phase solvent bar micro-extraction is suitable for Ni determination in seawater
- Ni can be determined at ng L^{-1} level in seawater using 3-phase solvent bar
- Sample pH and fiber length are a key factors in the extraction of Ni by DEHPA

Accepted manuscript



Accepted Manuscript