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NON-CHROMATOGRAPHIC ARSENIC SPECIATION IN ALGAE, RICE, AND SHRIMP SAMPLES USING IRON-MODIFIED MAGNETIC NANOPARTICLES

FORTALEZA
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Tese apresentada ao Curso de Doutorado em Química do Programa de Pós-graduação em Química da Universidade Federal do Ceará, como parte dos requisitos para a obtenção do título de doutor em Química. Área de concentração: Química Analítica.

Orientadora: Profa. Dra. Wladiana Oliveira Matos
Co-orientadora: Profa. Dra. Gisele Simone Lopes

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EU
E aqui estou eu,
Cercado pelo mar de insegurança
Que me faz ilha inabitável
Natureza indomável.
Aqui estou Cercado de muros que ergui
Para me manter seguro,
Para me manter a salvo.
Luan Fonsêca

ABSTRACT

A new iron oxide magnetic nanomaterial functionalized with organophosphorus compound was used as solid-phase for arsenic speciation in algae, rice and shrimp samples using ICP OES and ICP-MS. The nanoparticles-based arsenic extraction/preconcentration procedure was optimized using chemometric tools. The optimum response was obtained at pH = 4.0, 15 min of extraction time, and 20 mg of nanoparticle mass The rice samples presented total As content between 0.090 and 0.295 µg kg^{-1} and inorganic As mass fraction between 0.055 and 0.109 µg kg^{-1}. The accuracy for tAs and iAs quantification was verified using CRM NIST 1568b presenting 97% and 101% of recovery, respectively. The method proposed shown satisfactory precision (RSD lower than 15% for all samples), LOD and LOQ (1.08 and 3.70 µg kg^{-1}, respectively, using ICP OES). For algae samples, the arsenic concentration quantified using ICP OES was between 9 and 11 mg kg^{-1} for tAs and between 0.332 and 0.382 mg kg^{-1} for iAs,. The accuracy of the proposed method for this matrix was evaluated using ERM CD200 obtaining 105% of recovery. Arsenic speciation analysis using liquid chromatographic coupled with ICP-MS shown that the major arsenic species found in algae are arsenosugars and the values of iAs content were similar to the proposed method using magnetic solid-phase extraction. For shrimp samples, iAs concentrations between 20 and 100 µg kg^{-1}. The proposed method was validated for accuracy using CRMs DOLT-5 and DORM-4 and LC-MS. It was obtained a detection limit of 16.4 ng kg^{-1} iAs using ICP-MS. The stability of Fe_3O_4@DTPMP nanoparticles was studied using different batches of Fe_3O_4@DTPMP nanoparticles production. The recovery of arsenic was statistically similar between the analysis, besides that, it was possible to reuse the same magnetic nanomaterial for 6 successive cycles with recoveries higher than 85% of arsenic. A proposed mechanism of adsorption was studied using FT-IR and XPS analysis with possible interaction between N from modified nanomaterial and O from the arsenic anion. The proposed method is suitable for inorganic speciation of arsenic in several matrices, presenting good accuracy, precision, relatively low cost, and acquittance to green chemistry principles.

Keywords: inorganic arsenic; magnetic solid-phase extraction; ICP-MS; ICP OES; shrimp; rice; algae.

RESUMO

Um novo nanomaterial ferromagnético funcionalizado com composto organofosforado foi utilizado como fase sólida para especiação de arsênio em amostras de algas, arroz e camarão por ICP OES e ICP-MS. O procedimento de extração/pré-concentração de As usando nanopartículas foi otimizado por meio de ferramentas quimiométricas. A melhor resposta foi obtida em pH = 4,0, tempo de extração de 15 min e 20 mg de massa de nanomaterial. As amostras de arroz apresentaram teor de As total entre 0,090 e 0,295 μg kg^{-1} e fração em massa de As inorgânico entre 0,055 e 0,109 μg kg^{-1}. A exatidão para quantificação de tAs e iAs foi verificada utilizando CRM NIST 1568b apresentando 97% e 101% de recuperação, respectivamente. O método proposto apresentou precisão (RSD inferior a 15% para todas as amostras), LOD e LOQ (1,08 e 3,70 μg kg^{-1}, respectivamente, utilizando ICP OES) satisfatórios. Para as amostras de algas, a fração em massa de arsênio foi entre 9 e 11 mg kg^{-1} para tAs e 0,332 e 0,382 mg kg^{-1} para iAs utilizando ICP OES. A exatidão do método proposto foi avaliada com ERM CD200 com 105% de recuperação. Análise de especiação de arsênio usando cromatografia líquida acoplada ao ICP-MS mostrou que a principal espécie nas amostras de algas são os arsenoaçúcares e fornece valores de concentração de iAs semelhantes ao método proposto usando extração em fase sólida magnética. Para as amostras de camarão, os iAs extraídos com nanopartículas apresentaram concentrações entre 20 e 100 μg kg^{-1}. O método proposto foi validado quanto à exatidão usando os CRMs DOLT-5 e DORM-4 and LC-MS. Foi obtido um limite de detecção de 16,4 ng kg^{-1} iAs usando ICP-MS. A estabilidade das nanopartículas de Fe_3O_4@DTPMP foi estudada em diferentes lotes e a recuperação de arsênio foi estatisticamente semelhante entre as análises, além disso, foi possível reutilizar o mesmo nanomaterial magnético por 6 ciclos sucessivos com recuperações superiores a 85%. Um mecanismo de adsorção proposto foi estudado usando análise FT-IR e XPS com possível interação entre N e O do ânion arsênico. O método proposto é adequado para o uso da especiação inorgânica de arsênio em diversos tipos de matrizes, apresentando boa acurácia, precisão, custo relativamente baixo e conformidade com os princípios da química verde.

Palavras-chave: arsênio inorgânico; extração magnética em fase-sólida; ICP-MS; ICP OES, camarão; arroz; algas.

SUMÁRIO

1. INTRODUCTION

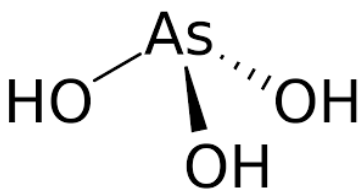
1.1 Arsenic

Arsenic (As) is a metalloid occurring in the oxidation states +V (arsenate), +III (arsenite), 0 (arsenic) and -III (arsine). However, the most common inorganic As species in the environment are arsenate and arsenite. While organic species are the predominant species in biological systems (BISSEN AND FRIMMEL, 2003). The As toxicity depends on its chemical form. Data obtained from experiments with rats indicate that the lethal dose of the most toxic As species are: As (III),4.5 mg kg^{-1}; As (V),14-18 mg kg^{-1}; monomethylarsenous acid (MMA),1220 mg kg^{-1}; dimethylarsenic acid (DMA),1800 mg kg^{-1} (CAVA-MONTESINOS et al., 2005; NRIAGU, 1994, IARC, 2012). As can be seen, inorganic forms of As have higher toxicity compared to organic species. Other As species such as arsenobetaine and arsenocholine are not considered toxic for living beings.

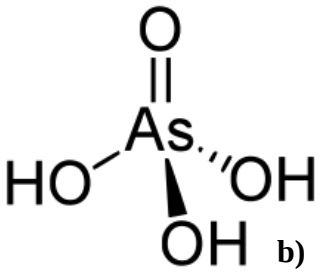
Arsenic is in the environment through natural and anthropogenic sources. It is found in Earth's crust in concentrations between 1.5 and 5.0 mg kg^{-1} mainly in sulfide forms on 200 minerals, sedimentary and volcanic rocks, besides that, geothermal and volcanic activity release As in air, water, and soils (RAHAMAN et al., 2021). The mainly anthropogenic via for As release is related to mining, coal, and petroleum extraction as well the use of arsenic-containing pesticides, herbicides, insecticides, and phosphate fertilizer (OZTURK et al., 2022; RAHAMAN et al., 2021).

The arsenite and arsenate forms are the most common species and are found in solutions usually in the form of their acids, the structures of these As species are shown in Figure 1.

Figura 1: Structure of a) Arsenious acid (As(III)) e b) Arsenic acid (As(V))



a)



b)

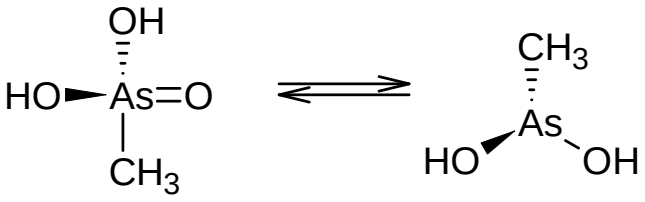
Source: Author himself

In aqueous solution, arsenious acid and arsenic acid are weak triprotic acids with pKa_1, pKa_2 and pKa_3 values of 9.2; 12.1; 13.4, and 2.2; 7.0; 11.5, respectively (BISSEN AND FRIMMEL, 2003). The double bond between As and O in arsenic acid molecule makes deprotonation easier due to the resonance effect that stabilizes the ion formed. Therefore, in aqueous environments, arsenic acid is more predominant than arsenious acid.

Organic forms of As are found in terrestrial and marine organisms (figure 2) (AMARAL, NÓBREGA AND NOGUEIRA, 2013; BARNET et al., 2021; DÌAZ et al., 2019; ZHANG et al., 2020) as monomethyl arsenic acid (MMA), dimethyl arsenic acid (DMA), very common in plants; arsenobetaine (AsB) and arsenocholine (AsC), ordinary in marine organisms; as well trimethylarsine oxide (TMAO), thiol-arsenical compounds (-SAs), arsenosugars. Recently, it was revealed a new class of As species present in lipid fractions of biological systems called arsenolipids (CHEN & ZHANG, 2019; COELHO, 2019; DÌAZ et al., 2019; HUSSAIN et al., 2019). There are a lot of unknown As species in several samples.

Figure 2: Structure of a) Monomethyl arsenic acid (MMA) b) Dimethyl arsenic acid (DMA) c) Trimethyl arsine oxide (TMAO) d) Arsenocholine e) Arsenobetaine.

a)



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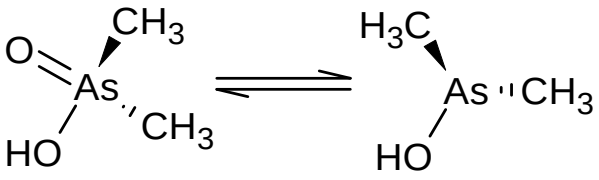

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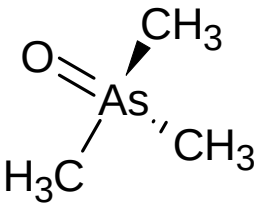

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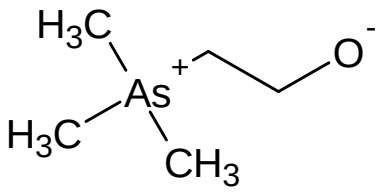
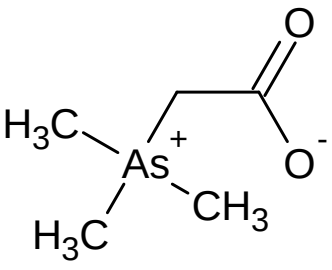


e)



Source: Author himself

Arsenic can cause acute or chronic toxicity and its metabolism is not completely understood yet (BALALI-MOOD et al., 2021). Inorganic arsenic species have acute toxicity well known, moreover, methylated species metabolized in the human body may be so cytotoxic as inorganic ones (OZTURK et al., 2022; RAHAMAN et al., 2021; VERGARA-GERÓNIMO et al., 2021).

Symptoms of As acute poisoning are muscle pain, weakness, nausea, vomiting, abdominal pain, and diarrhea (COSTA, 2019). Arsenite interferes with cellular energy metabolism by inhibiting of the bihydrogenated pyruvirate enzyme interacting with lipid acid (COSTA, 2019; PAUL et al., 2017). Arsenate can cause oxidation by phosphorylation and inhibiting NAD+ (Nicotinamide and adenine dinucleotide) decrease the energy during mitochondrial respiration and inhibiting the synthesis of ADP (Adenosine Diphosphate)(COSTA, 2019; VERGARA-GERÓNIMO et al., 2021).

Some studies clarified the mechanisms for chronic disease related to As toxicity. This element is metabolized in the liver prior to mobilization and excretion. At the cellular level, As species have distinct interactions, As(III) use aquaglyceroporin 7, aquaglyceroporin 9 channels, and the glucose transporters type 1 (GLUT1) for enters in the cell (OZTURK et al., 2022; RAHAMAN et al., 2021). As(V) use the phosphate transporters due to its similarity with phosphate (COSTA, 2019; RAHAMAN et al., 2021).

It is important to mention that an increase in organic character in As represents better solubility in lipidic fractions and the capability of causing chronic disease, as diabetes type 2 and hypertension due to the oxidative stress caused in cells and organs(OZTURK et al., 2022; RAHAMAN et al., 2021).

The main source of As intake are natural water and food (31% of the daily ingestion) being rice and seafood the principal products of As contamination (OZTURK et al., 2022). The presence of this element in water could be explained for rock erosions, and in

food, some animal and plant species have the capability of bioaccumulated and/or biotransformed As (PETURSDOTTIR, SLOTH E FELDMANN, 2015).

This element is not considered essential, and acute toxicity (poisoning) effects could be noticed with ingestions higher of 10 mg kg^{-1} day^{-1}, however, adverse effects could occur with ingestions from 1 mg kg^{-1} day^{-1} (INSTITUTE OF MEDICINE (U.S.). Panel on Micronutrients., 2001). Ingestions greater than 10 µg kg^{-1} day^{-1} is set as uptake limit (UL) due to the chronic issue caused for this element. (INSTITUTE OF MEDICINE (U.S.). Panel on Micronutrients., 2001).

1.2 Chemical Speciation

Metals and metalloids are commonly found in biological matrices, however the role that they play in these systems depends on the chemical form in which they are found and/or their concentration ranges (WANG et al., 2016). Thus, the analysis of the total analyte content may not be sufficient to understand its functioning in biological and environmental systems, as well as its toxicity. In this aspect, chemical speciation becomes a new frontier to be reached in Analytical Chemistry.

The term speciation has its origin in biological sciences and analytical chemistry brings as a concept that specific forms of a chemical element must be analyzed/considered individually. This individualization may be necessary for several reasons: only one chemical species of an element ispotentially toxic, or only certain forms are used in the biochemical processes of living beings, etc. (CORNELIS et al., 2003).

The International Union of Pure and Applied Chemistry (IUPAC) defines chemical speciation analysis as the qualitative and/or quantitative analysis of different chemical species of an analyte in a sample (FELDMANN, RAAB E KRUPP, 2018; TEMPLETON et al., 2000). In that same document, the definition of chemical species is

given as the specific form of a chemical element defined by its isotopic composition, oxidation state or electronic distribution state as well as metal complexes molecules, and molecular structures (TEMPLETON et al., 2000; TEMPLETON E FUJISHIRO, 2017).

Due to the extensive possibilities of chemical speciation analysis and the fact of the same element can be found in different forms, this area of Analytical Chemistry remains a challenge. Chemical speciation analysis means to quantify only a fraction of the total concentration of elements, usually in traces quantity. New analytical procedures and strategies are required, especially when considering that, often, the total concentration of a chemical element cannot be quantified by the individual sum of the chemical species determined in the matrix (TEMPLETON et al., 2000).

Prior to any chemical speciation analysis, sample preparation methods are required for most of the samples. It is well discussed in the literature that sample preparation step is a dare in Analytical chemistry because it represents the stage more expensive, time-consuming, and more insertion of errors in an analytical sequence.

In speciation analysis, sample preparation gains other challenges still to be solved. The methods should be aggressive to extract the species of the analyte from the matrix but cannot convert the original species. Besides that, the step of storage and period of time between sample preparation and analysis as well the stability of species are important variables to be studied in speciation research.

1.3 Chemical Speciation Analysis

The development of techniques more and more accurate, sensitive, such as ICP-MS and new calibration methods increases the capability to perform chemical speciation analysis (BUTLER et al., 2016). In addition, the development of separation techniques has become possible to separate different chemical species being an essential step to this type of

analysis. However, the largest issue in chemical speciation is the validation step, mainly because there are no available certified reference materials for speciation analysis or, there are for limited analytes (MARSCHNER et al., 2019; PETURSDOTTIR, SLOTH E FELDMANN, 2015; SPERLING E KARST, 2018; WAHLEN et al., 2004).

A chemical speciation analysis could be performed in two different ways according to the objectives that want to be reached (FELDMANN, RAAB E KRUPP, 2018). The bespoken methods are focused on analysis and quantification of well-known species, generally, they are supporting in literature data or previous experiments. In the other hand, the nontarget methods have as objective the search and identification of new species and their quantification.

In the bespoken methods the analyte measured generally has chemical characteristic different for each species, as distinct toxicity, and the most part of this species are regulated by local and international legislation. As example, inorganic As in rice has limits in almost all regular agencies around the world (INSTRUÇÃO NORMATIVA-IN N° 88, DE 26 DE MARÇO DE 2021, 2021; CHINA, 2013; European Parlamento, 2015.; (FAO), 2018), however this same analyte continues without a limit legislation in algae and crustacean samples in a major part of countries.

Speciation analysis for As species is focused on the new methods for quantification of known species and the discovery of new compounds (FELDMANN, RAAB E KRUPP, 2018). Arsenic compounds have different chemical properties and could be analyzed for several procedures based in specific characteristic (BISSEN E FRIMMEL, 2003; HU et al., 2019). The most common As characteristic considered is toxicity. it is classified as the toxic species, such as inorganic forms; potentially toxic, as arsenosugars and arsenolipidics; and the non-toxic as arsenobetaine (FELDMANN E KRUPP, 2011).

On the other hand, the separation of As species can also be done according to their

solubility: water soluble and liposoluble species. The last one has an increase in studies with the discovery of a new As class, the arsenolipids, with compounds as toxic as inorganic species (PÉTURSDÓTTIR et al., 2018; WITT et al., 2017).

The complete speciation of As requires powerful separations methods and high sensitivity techniques of quantification. With the improvement of the analytical instrumentation, atomic spectrometry techniques have increased their sensitivity, especially with the use of mass spectrometry associated with an inductively coupled plasma (ICP-MS) (BUTLEr et al., 2016). However, the selectivity for various chemical forms is still a challenge, and it is generally necessary to use separation methods coupled with atomic spectrometry techniques to perform the chemical speciation analysis (GONZALVEZ et al., 2010).

The techniques of gas chromatography (GC) or liquid chromatography (LC) coupled to a detector are widely used in the separation of chemical species (RANI, SHARMA E MALIK, 2017). But it should be noted that GC is limited to volatile species and the long analytical path taken by the species with the use of chromatography reduces the sensitivity of the method, requiring extremely sensitive detectors (GONZALVEZ et al., 2009). In addition, chromatographic techniques coupled to sensitive detectors are an awfully expensive instrumentations, which require analysts with in-depth knowledge of the use of these techniques. Thus, this type of analysis is restricted to specialized research laboratories.

High Performance Liquid Chromatography coupled with ICP-MS technique is the most usual method for total speciation of As due to high separation efficiency coupled with an extremely sensitive technique (FELDMANN, RAAB E KRUPP, 2018). Several methodologies have been developed for biological fluids, environmental samples, food samples, specially, rice, seafood and algae (AMMANN, 2011; NINH, NAGASHIMA AND SHIOMI, 2006; PEDERSEN AND FRANCESCONI, 2000; REID et al., 2020). The use of

these methodologies is not trivial and requires special procedures for As speciation. Frequently are necessary graduation of elution in mobile phase due to the difference of polarity of the As compounds, besides that anionic and cationic column are demanded for separation of some species (AMMANN, 2011; NINH, NAGASHIMA AND SHIOMI, 2006; PEDERSEN AND FRANCESCONI, 2000; REID et al., 2020).

The amount of innumerous As compounds, with special attention to those present in marine organisms, represents an important issue for speciation. There are no standards for some As compounds and some of them are even known As species. Co-elution of known and unknown As species is a risk during the chromatographic separation of As species (PÉTURSDÓTTIR, ÁSTA HEIRÚN et al., 2012; SLOTH, LARSEN AND JULSHAMN, 2005; WAHLEN et al., 2004).

Inorganic As speciation have other problems for analysis by HPLC-ICP-MS. Arsenic(III) can co-elute with arsenobetaine and the use of hydrogen peroxide to promote the oxidation of As(III) to As(V) is a strategy to confirm As(III) presence, but this increases the time of analysis (SLOTH, LARSEN AND JULSHAMN, 2005). Furthermore, certified reference material for As species is only available for few matrices, as rice, and frequently present divergent results depending of the analytical technique applied (MARSCHNER et al., 2019).

Recently, Pétursdóttir & Gunnlaugsdóttir, 2019 used hydride generation (HG) coupled with HPLC-ICP-MS system for iAs quantification in seafood with LOD of 0.0004 mg kg^{-1} with selectivity for the inorganic species. The same procedure was used in several seafood CRM showing conflicting results when the HG was not used (MARSCHNER et al., 2019). There is evidence that some As species are coeluted with DMA (MARSCHNER et al., 2019). Even though the use of HG prior to the As species quantification being a good alternative for iAs speciation, an extra step increases the cost of analysis, the time consuming

and the waste generation.

It is unquestionable that HPLC-ICP-MS is a powerful technique for As speciation analysis, however, it requires high trained analysts, use of huge volume of solvents, and expensive equipment. Meanwhile, total speciation is not necessary. The quantification of a group of species is sufficient for decision making (GONZALVEZ et al., 2009, 2010). For arsenic, the inorganic forms content it is enough to provide information about toxicity. Thus, simpler chemical speciation methods which target on specific groups of species can be applied dispensing with the need for chromatography separation step.

1.4 Non-chromatographic methods

Since elemental speciation analyzes methods that use chromatography to isolate the analyte species requires high-cost analytical instrumentation, few laboratories of routine analyzes and even in academia have the infrastructure to carry out chemical speciation analysis (SPERLING AND KARST, 2018). The chromatography step is critical in chemical speciation and entails an increase in the final cost of the analysis, in addition to the high consumption of time and reagents. In recent years non-chromatographic methods have emerged as a trend towards the development of robust methods for analyzing target analytes (BUTLER et al., 2016).

Non-chromatographic methods for speciation are generally lower cost, faster, fit green chemistry guidelines, and can be used for speciation using less expensive detection techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP OES) (CAVA-MONTESINOS et al., 2005; ESPERANZA et al., 2017; GONZALVEZ et al., 2010; WELNA AND POHL, 2017).

Non-chromatographic methods are limited to a small number of species or groups and provide less information than coupled chromatographic methods (GONZALVEZ et al.,

2009, 2010). However, most of the time a simple information is enough for analytical decision making. For example, iAs species are much more toxic than organic species; therefore, for decision making, information about the presence or content of iAs in the sample would be sufficient. In the same sense, organic tin species are more harmful to the environment than inorganic ones (ZHANG et al., 2021). Another example is the case of chrome, where its trivalent species is essential to organisms and the hexavalent species has carcinogenic potential (CORNELIS et al., 2003).

Hydride generation is an alternative for enhance sensibility in arsenic analysis (CAVA-MONTESINOS et al., 2003; PÉTURSDÓTTIR et al., 2014), it is based in the formation of a volatile compound with the analyte which is carried to detection method. The formation of a gas phase eliminates the nebulization step in optical methods increasing the amount of analyte in atomizer, therefore, increasing the sensitivity.

Hydride generation methods are often used as non-chromatographic methods for As speciation analysis due to the distinct kinetics rates of hydride generation of As species (CAVA-MONTESINOS et al., 2003; GONZALVEZ et al., 2009; VICENTE-MARTÍNEZ, CARAVACA AND SOTO-MECA, 2020; WELNA E POHL, 2017). In general, tetrahydroborate is used as reducing and at high pH values only As(III) species form hydride. For As(V) quantification, itis reduced to As(III) prior to hydride formation. Thus, it is kinetically possible to do iAs speciation analysis. (D'ULIVO, 2004).

Other way to increase the sensibility of the analytical method and go without chromatographic separation step is applying preconcentration methods. This sample preparation strategy can makes possible the use of less-sensitive techniques of detection, such as FAAS and ICP OES, for chemical speciation analysis. Besides, analytical and are, generally, in accordance with green chemistry guidelines.

1.5 Nanomaterials in Analytical Chemistry

Nanomaterials are becoming an important tool for non-chromatographic analysis. The knowledge base of nanomaterials appeared in the field of theoretical physics in the 60’s and today this type of material is inserted in almost all fields of human knowledge.

The theoretical physicist Robert Feynman is often called the father of nanotechnology because he created the concepts of this science when made the following statement during a speech for the American Physics Society: "There is plenty of room at the bottom". In this way, said, he has introduced the concept that there are a lot of things to do in miniaturizing systems (FEYNMAN, 1960).

Nanomaterials are specific types of structures where at least one of the dimensions is smaller than 100 nm. Tt is the small size that provides to this type of material chemical and physical characteristics different from those obtained in larger scale materials (RATNER AND RATNER, 2003). These differences are related to surface phenomena, once, nanomaterials have a ratio surface/volume much higher than observed in large-scale material. Besides that, the phenomenon of quantum confinement provides different interactions with radiation and processes of as absorption and emission of light. (TAHIR, RAFIQUE AND SAGIR, 2021)

Nanoscale materials can be classified in different ways such as origin, nature, dimension, or homogeneity. It can be from natural origin, made accidentally or by engineering materials using physical (top-down) or chemical (bottom-up) methods (FECHINE, 2020). Considering the nature of this material, it can be organic or inorganic, with hybrid materials still existing. The dimensionality of the material is of great importance and there may be nanostructures with zero, two or three dimensions, such as metallic nanoparticles, carbon nanotubes and nanostructured materials (metal-organic framework), respectively (BÜYÜKTIRYAKI, KEÇILI AND HUSSAIN, 2020). The dimensionality of material is

affected by quantum confinement, when a material is 0D means that there is this phenomenon in all dimensions of the material. For 1D material, it has quantum confinement in 2 dimensions.

The use of nanomaterials in analytical chemistry has been growing in recent years. Some authors have already described that the impact of nanostructures in the last 20 years has caused a change in the frontiers of analytical sciences (CALLE & ROMERO-RIVAS, 2018). In the beginning, nanomaterials were used as an analyte with the idea of developing methods for its quantification, and this interest in the use of nanomaterials as an analytical tool evolved.

In recent years, the use of nanomaterials as an analytical tool has generated interest from several research groups, especially in their application in chromatography, sample preparation methods and as sensors.

Between 2010 and 2017, nearly 4000 scientific articles were published by various groups using nanomaterials in sample preparation steps. The most common application is in solid phase extraction (SPE), cloud point extraction (CPE), dispersive liquid-liquid extraction (DLLE) among other extraction method. These methods were developed for quantification of the most diverse trace elements and organic substances (CALLE & ROMERO-RIVAS, 2018).

The use of nanomaterials as nanosorbents in solid phase extraction for pre-concentration of trace elements is a constantly growing field (BENDICHO, BENDICHO-LAVILLA E LAVILLA, 2016; KARADJOVA et al., 2016; ZARBIN, 2007). Compared to conventional sorbents, nanomaterials have several advantages, such as high surface area, which increases their sorption capacity and reaction kinetics (ZARBIN, 2007). The use of this type of material for chemical speciation analysis has been explored, but with few applications for real samples analysis. The use of nanomaterial for chemical speciation analysis is still scarce compared to its use for preconcentration.

The new trends for materials such as nanosorbents are to improve selectivity, sensitivity, extraction efficiency and reaction kinetics, in addition to the seek for faster and easier to handle analytical methods. In this way, the use of magnetic materials brings several advantages for the development of analytical methodologies due to their ease of magnetic separation and the possibility of surface functionalization, which guarantees selectivity possibilities. The possibility to improve the selectivity of these materials is of great interest for chemical speciation, especially for non-chromatographic methodologies.

A review of methods using iron-based nanomaterial for As speciation analysis are summarized in table 1.

Table 1: Review of Fe_3O_4 nanomaterial in As speciation analysis.

Functionalizing	Species	Samples	Technique	LOD ($\mu g\ L^{-1}$)	Ref.
$Ni_{0.5}Zn_{0.5}Fe_2O_4$	As(III) / As(V)	Water	Spectrophotometric UV-Vis	0.15 (As(III)) / 0.10 (As(V))	(AFKHAMI et al., 2020)
PSAH[1]	As (III) / As (V)	Sea Water, water	On-line HG-ICP-MS	2.7	(MONTORO LEAL et al., 2018)
MPA[2]-APTS[3]	As (III) / As (V) / iAs	Tap water, rice	ETAAS	0.01	(POURGHAZI, AMOLI-DIVA E BEIRAGHI, 2015)
-	As(III) / As(V) / MMA	Herbal Tea	Proportional Equations – ETAAS	0.02	(LÓPEZ-GARCÍA et al., 2018)
SiO_2 – MPA SiO_2 – APTS	As(III) / As(V)	Tap, River, Lake and Rain water	ICP-MS	0.0235 (As(III)) / 0.0105 (As(V))	(FAIZ *et al.*, 2022)

[1] [1,5-bis(2-pyridyl)3-sulfophenylmethylene]thiocarbonohydrazide)

[2] 3-mercaptopropionic acid

[3] 3-aminopropylthiethoxysilane

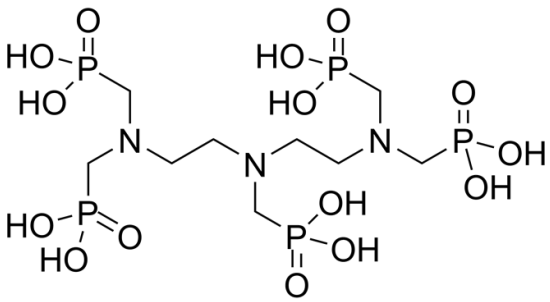
Source: Author himsel

It is necessary to explore more the application of magnetic nanoparticles for chemical speciation analysis, in particular, for biological and environmental samples.It can be an interesting alternative to iAs analysesin seafood and algae samples, since the level of arsenobetaine content in these samples is extremely higher than the inorganic As content which often causes problems for speciation analysis using conventional HPLC-ICP-MS systems.

1.6 Diethylenetriamine-penta(methylphosphonic) acid (DTPMP)

Diethylenetriamine-penta(methylphosphonic) acid (DTPMP), whose molecular formula is $C_9H_{29}N_3O_{15}P_5$, has in its structure several phosphate groups and three amino groups. It is considered an aminophosphonate compound. The DTPMP chemical structure is shown in figure 3. Diethylenetriamine-penta(methylphosphonic) acid has been used as a chelating reagent in the textile industry (CARDIANO et al., 2017; CIGALA et al., 2014).

Figure 3: Chemical structure of DTPMP



Source: Author himself

As can be seen in figure 3, the DTPMP compound has several phosphate groups and a chemical structure similar to EDTA, which guarantees good chelating characteristics.

This molecule has 10 acidic hydrogens; however, studies show that one of the hydrogens is strongly bound to the structure even at extreme pH, being therefore represented as $HDTPMP^{-9}$ as the ligand structure (CIGALA et al., 2014). Thus, hydrogen complexation

constant values of this molecule are calculated using the expression:

$$L^{-9} + iH^{+} \leftrightarrows H_iL^{(9-i)}$$

The values for log β are: β_1 = 12.15; β_2 = 23.49, β_3 = 32.25, β_4 = 39.62, β_5 = 45.21, β_6 = 50.49, β_7 = 53.91 using 25°C and I = 0.1 mol L^{-1}.

1.7 Shrimp

As the global demand for seafood increases, the use of aquaculture systems dramatically expands to become an alternative to fishery products. (TWOMEY, 2017). The growth of aquaculture systems is set to increase 5.3 percent per year producing 240 million tons until 2030.

Shrimp represents around 18% of total fish trade with values estimated in USD 28 billion per year. The Pacific white legs shrimp (*Litopenaeus vannamei*) is the most important specie in aquaculture being Asia and American the biggest producers.

Despite its economic importance, there is evidence that shrimp farming causes environmental contamination due its intensive production (RIBEIRO et al., 2016). The use of aquaculture systems for shrimp production increases the concentration of trace elements in sediment and water (MIOLA et al., 2016). Contamination of aquatic ecosystems is of particular concern because marine animals are known to have the ability to bioaccumulate inorganic elements (SCHMIDT et al., 2017), often toxic to humans. Thus, elemental trace analysis in seafood samples is essential, as they can be a route for contamination by potentially toxic elements due to their ability to bioaccumulate these contaminants.

Arsenic is commonly analyzed in seafood, since a considerable portion of As consumed by human beings comes from this type of product, as shown by Borak & Hosgood, 2007. These authors demonstrated that 90% of the As consumed by the US population comes

from seafood. In recent work, Signes-Pastor et al., 2017, establishes a linear relationship between urinary As species with rice and seafood intake in study with 400 children of 4-years and 7-years old in Spain. An increase of consume of iAs represents bigger quantities of MMA in urine, specially, younger children due to the methylation of As for excretion.

There are still few limitations in the legislation for distinct species of inorganic elements in seafood, but this approach is sorely needed, especially for As. Only China, Australia and New Zealand have legislation for As species (0.5 mg kg^{-1} of iAs) (CHIOCCHETTI et al., 2017).

Mercosul regulations (Southern Common Market) establishes 1.0 mg kg^{-1} as maximum limits for As concentration in crustaceans. An update in Brazilian legislation (ANVISA e Ministério da Saúde, 2021) brought a recommendation to perform speciation analysis when the As concentration found is higher than allowed for crustaceans but does not suggest a safe limit to iAs. European standards do not limit the concentration of As in food; however, there are recommendations for the study of As concentrations in several samples, including seafood with an emphasis on chemical speciation (THE EUROPEAN COMMISSION, 2015). Chinese legislation has limits for iAs in crustaceans set in 0.500 mg kg^{-1} (CHINA, 2013).

It is also important to emphasize that there are few studies about trace elements content in seafood from farms and the distribution of these substances in this type of sample. This kind of research could provide relevant information to update the legislations around the world.

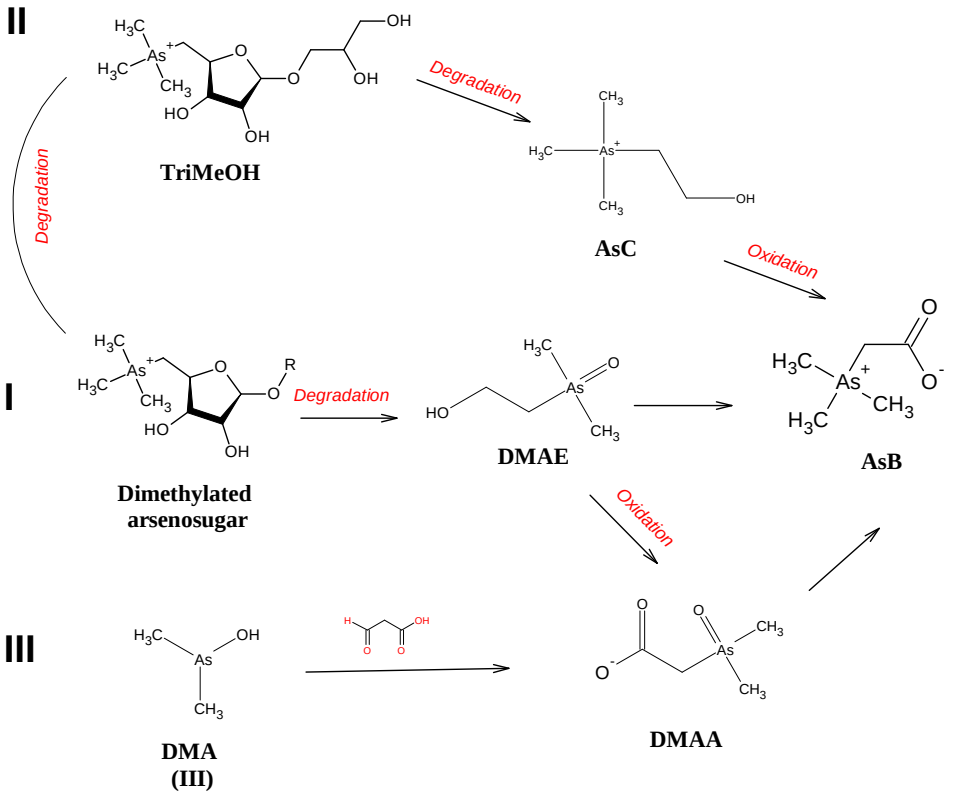
In addition, the metabolism of some metal/metalloid species is not completely known. Recently, the literature reported a new class of organic arsenocompounds known as arsenolipids which have similar toxicity to the inorganic forms of As (PÉTURSDÓTTIR et al., 2018). The identification of new compounds of As classes in shrimp samples does not

exist in the literature.

The chemical speciation of As in seafood is not a trivial task mainly for iAs quantification. The major species found in this type of sample is AsB, non-toxic for humans (AMLUND E BERNTSSEN, 2004; POPOWICH, ZHANG AND CHRIS LE, 2017). In general, less than 1% of As content in seafood is found as inorganic form (SLOTH, LARSEN E JULSHAMN, 2005). Although the low rate of iAs, even the low concentration of As(III) and As(V) may represent a risk for consumers. The difference in concentration of inorganic and organic As forms is the major issue to be solved in analytical methods for speciation analysis.

The mechanisms of absorption of AsB in marine organisms are not a consensus in the scientific community. There are pieces of evidence that AsB could participate in the osmotic regulation of marine invertebrates (AMLUND AND BERNTSSEN, 2004; CLOWES AND FRANCESCONI, 2004; PAPRY et al., 2019; POPOWICH, ZHANG AND CHRIS LE, 2017). Besides that, animals cannot biotransform iAs in AsB (FRANCESCONI & EDMONDS, 1996; WRENCH et al., 1979), thus, food is the main source of AsB. Otherwise, microorganisms are capable to perform the biotransformation of iAs in organic molecules (HUSSAIN et al., 2021). There are three main pathways for the formation of AsB based on intermediates found in marine organisms. Figure 4 shows these possible mechanisms.

Figure 4: Arsenobetaine biotransformation pathway.



(TriMeOH: Trimethylarsonic ribose; DMAE: 2-dimethylarsinoylethanol; DMMA: 2-dimethylarsinoylacetic acid.)

Source: Author based in POPOWICH et al., 2017

The pathway-based on the degradation of arsenosugar with DMAE as intermediated has evidence due to the presence of DMAA and AsC in diverse marine organisms (NAWROCKA et al., 2022)., The conversion of AsC to AsB could occur through the actionof microorganisms in intestine flora or by other unclear mechanisms (POPOWICH et al., 2017; XUE et al., 2021).

Shrimps submitted to feed with high concentrations of arsenosugar accumulate less As than when feeding with high content of TriMeOH (FRANCESCONI et al., 1989) suggesting a mechanism-based in the conversion of this species to AsB, probably by degradation of arsenosugar and oxidation of TriMeOH for AsC. The low concentrations of TriMeOH found in seafood could be evidence of the fast conversion of this species in other organic compounds (XUE et al., 2021).

The possibility of biotransformation and uptake of several arsenic-organic compounds in seafood shows the complexity of this sample. For total As speciation in seafood is necessary powerful techniques for separation and identification. For this purpose, chromatographic methods as LC-ICP-MS is common applied (CHI et al., 2018; HIRATA & TOSHIMITSU, 2007; LARSEN et al., 1997; LORENC et al., 2020; SCHMIDT et al., 2017). However, there is a risk of As (III) co-elute with AsB and other organic species as TMAO (trimethylarsine oxide) when anionic column is used.

Attempts to avoid interferences for iAs quantification in HPLC-ICP-MS, methods have been described in the literature with the use of hydride generation for formation of volatile species of iAs (PÉTURSDÓTTIR, et al., 2012). Trivalent and pentavalent As species have different kinetic rates being the As(III) the most reactive and using high acid concentration this is the unique specie to form hydride (CAVA-MONTESINOS et al., 2003; ESPERANZA et al., 2017; MARSCHNER et al., 2019).

Inorganic arsenic speciation has issues with the validation step (MARSCHNER et al., 2019; PETURSDOTTIR, SLOTH E FELDMANN, 2015), once the certified reference materials (CRMs) available in the market are not validated for this As species. Generally, only tAs and AsB content are certified.

Besides that, recently, the literature reported different quantification of iAs content in the same samples of CRMs submitted to analysis by several techniques. These studies show issues with coelution in chromatographic steps or formation of hydrides of other As species besides As(III), such as DMA or MMA (MARSCHNER et al., 2019; WOLLE AND CONKLIN, 2018).

New methods for speciation of As in these samples are required for more accuracy and lower detection and quantification limits using techniques less expensive and following the guidelines of green chemistry.

1.8 Algae

Algae is a term to designate photoautotrophic organisms that are distributed in aquatic ecosystems. It includes prokaryotic and eukaryotic unicellular microalgae and macroalgae (LEE AND RYU, 2021)

Seaweed is consumed widely worldly for culinary purposes (COELHO, 2019; DÌAZ et al., 2019; REIS & DUARTE, 2018) being a source of minerals. Also, it has therapeutic properties as gastroprotective, anticoagulant, antiplatelet and antithrombotic effects (CHAGAS et al., 2020; PEREIRA JÚNIOR et al., 2021). The employment of this product in the pharmaceutical and cosmetic industry is increasing (ABREU et al., 2018; ALBUQUERQUE et al., 2021; SOUSA et al., 2016)

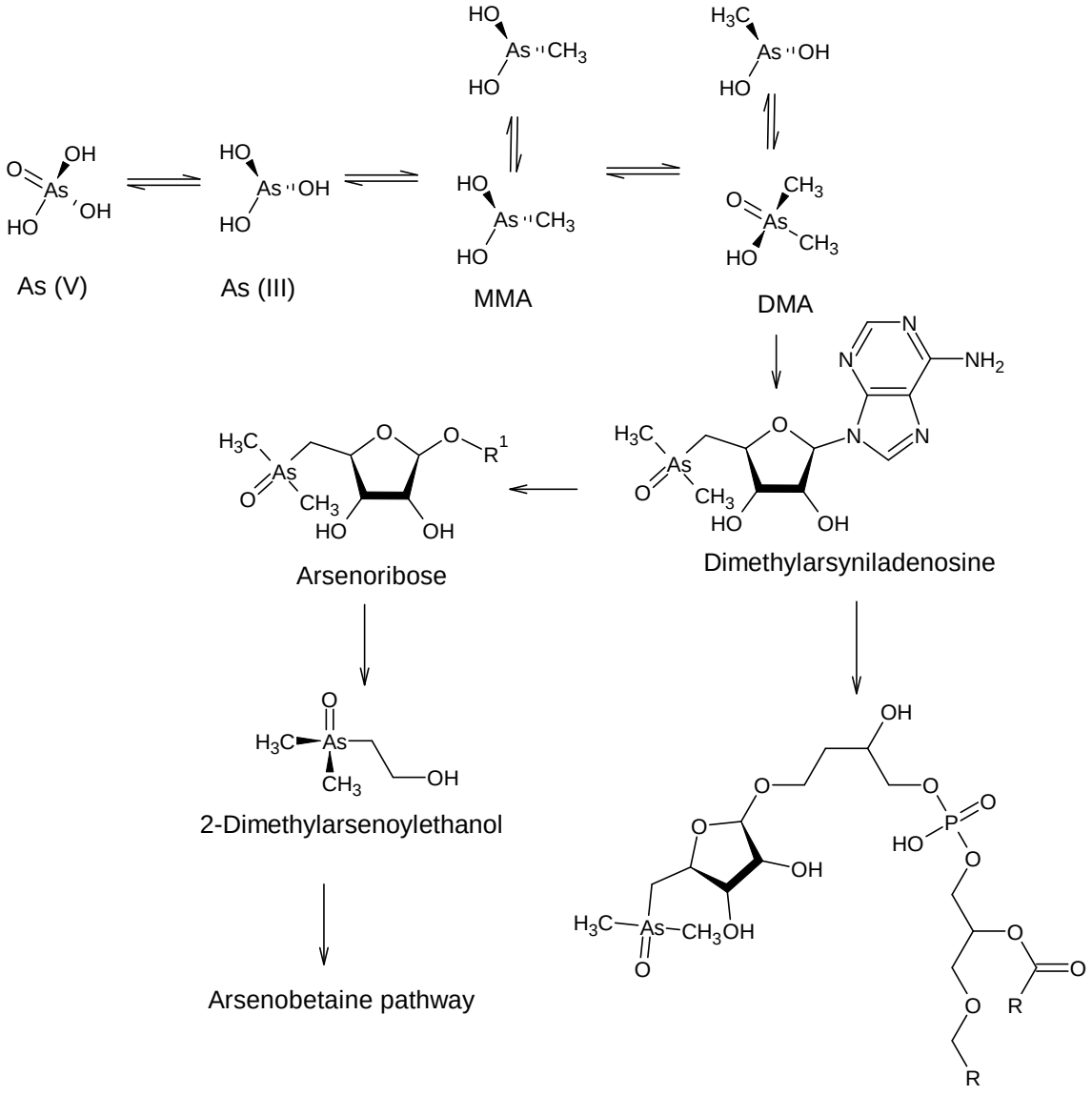
The farming of macroalgae in Brazil has few commercial scales in operation. The process of farming of these products is carried out mainly by producers from traditional riverside communities from the states of the northeast region, such as Rio Grande do Norte and Ceará (VALENTI et al., 2021). The most produced species are Graciliaria, Porphyra, and Hypnea with a production of 30 tons in 2016 (Food and Agriculture Organization of the United Nations Statistics Division (FAOSTAT), 2017).

Marine algae are primary producers and have the capability of bioaccumulate inorganic elements, especially, As from seawater. This capability is around 1000 times higher than other organisms (HUSSAIN et al., 2019). They also have the ability to biotransforme As by microorganisms, thus the highest As content in algae is in arsenosugar form which normally represents >80% of total As and is considered less toxic (FELDMANN AND KRUPP, 2011).

The role of biotransformation of As in algae begins with the absorption of As(V) through phosphate transporter channels, then it is reduced to As (III) and methylated to the organic forms so they are excreted (WANG et al., 2015). The uptake mechanisms of As

remain unclear yet, there is evidence that this element uses the same mechanisms as phosphate inside and outside the cells mostly dependent of the P concentration (LEVY et al., 2005). However, many studies show independence between phosphate and arsenate uptake, suggesting the existence of others uptakes mechanisms for As in algae (DUNCAN et al., 2013). There are evidence that As(III) is absorbed through plasma membrane with the auxilium of aquaglyceroporins and hexose permeases enzymes (CHEN & ZHANG, 2019).

Figure 5: Arsenic pathway biotransformation in algae.



Source: Author based in Chen & Zhang, 2019

With the increasing interest in the use of macroalgae as food or in products for human consumption, the analysis of As content speciation is necessary to guarantee the safety of algae and algae-based products consumers.

The species of As found in this kind sample are numerous and total speciation is not needful to assure the possibility of toxicity. Feldmann & Krupp, 2011, proposed a strategy for chemical speciation in algae samples based in three groups: the iAs forms due to the toxicity known; the AsB form due to the non-toxicity; potentially toxic As species as arsenosugar, arsenolipids, and other organic species.

There are few legislations worldly that limit the As content in seaweed. Brazilian and European legislation has not allowed content of As in this kind of sample. In the past, Brazilian legislation considered seaweed as exotic food and limited the content to 1.0 mg kg^{-1}, but recently, this recommendation was revoked. In Europe, there are As content limits for seaweed when this is used for animal feed. France and China limit the iAs content in algae to 0.3 mg kg^{-1} (CHINA, 2013; FRANCE, 2010).

The speciation analysis in algae sample is not trivial and the standard analytical methods for As speciation as EN 15517:2008 and GBIT 5009 11-2003 do not provide reliable results with biased quantifications when applied in algae samples (LA CALLE et al., 2012)

Extremely high concentrations of As have been reported for European species in the literature with levels between 1 to 150 mg kg^{-1} being 0.4 a 99.9% of iAs (COELHO, 2019). Concentrations higher could be observed in seaweed submitted to As contamination (CHEN AND ZHANG, 2019) with a content of 109.000 mg kg^{-1} in a *Dunaliella salina* species (DUNCAN et al., 2013). In Brazil, there are few studies in algae samples. Coelho et al., 2016, found a concentration of tAs in Brazilian algae species almost 20 times higher than allowed in China (CHINA, 2013) and France (FRANCE, 2010) legislation.

There are no data for algae natives from the Brazilian Northwest farmed and/or those commercialized by small communities producers.

1.9 Rice

Rice (*Oryza sativa*) is one of the main products in the diet of several countries. Most recent data estimate production of rice above 510 million tons in 2018, with Asia responsible for almost half of world production and consumption of this grain (FAO, 2018).

It is well known that rice, unlike other grains, can accumulate As during its growing stage making it a primary source of this element for humans on a seafood-free diet (HUN et al., 2019). Arsenic is found naturally in soil and water, but it can occur at high concentrations in regions of industrial activity, excessive applications of fertilizers and manure, and can also be found in regions with sewage contaminated by some pesticides. Arsenic accumulation in plants depends on several farming factors as wellas the speciation of As species available in the rhizosphere. (SURIYAGODA, DITTERT AND LAMBERS, 2018)

Under anaerobic conditions, as flooded fields, As(V) is reduced to As(III) and it is transported through the silicon uptake pathway (ABEDI AND MOJIRI, 2020). Arsenic (III) is transported by proteins called aquaporins (nobulin 26-like intrinsic proteins (NIP)) (SURIYAGODA, DITTERT AND LAMBERS, 2018). Arsenite is an uncharged molecule with a diameter of 411 nm, approximately, like silicic acid (SHRI et al., 2019; WU et al., 2016). Within the cell, As(III) can be detoxified via reaction with phytochelatins, and the complex formed is sequestered in the vacuoles (Briat, 2010).

Arsenic (V) is more phytoavailable in aerobic soils using the phosphate uptake system being carried into xylem systems by phosphate transporter proteins (PTP), then this species is reduced through oxidation of glutathione (BRIAT, 2010).

The organic species, as MMA and DMA, have a much lower rate of absorptivity

by roots than iAs due to inferior affinity to transporters (RAAB et al., 2007; SHRI et al., 2019). Previously, some literature reported the capability of plants to methylate As species producing organic forms, but recent studies found divergent results showing that organic species are absorbed by plants through pathways unclear yet (ABEDI AND MOJIRI, 2020; DOMÍNGUEZ-GONZÁLEZ et al., 2020; FENG MA et al., 2008; RAAB et al., 2007). The order of uptake of As species in rice plants could be demonstrated by As(III) > As(V) > DMA > MMA.

Due to the high consumption of rice in some countries, the daily intake of As exceeds the limit considered acceptable by WHO (World Health Organization) of 10 μg kg^{-1}. Countries like India consume more than what is recommended as safe by the WHO of iAs per day (2.32 μg iAs kg^{-1} body weight per day). The safe value is approximately 2.1 μg As kg^{-1} body weight per day. It is important to mention that the As content in brown rice is disproportionately higher than in polished rice (JACKSON & PUNSHON, 2015).

Another concern is that the main form of As found in rice is the inorganic species and dimethylarsenic (WELNA AND POHL, 2017) which have the highest toxicity for humans. However, the quantitative speciation analysis of As in rice samples is not a trivial task, mainly due to matrix interferences.

Several regulatory bodies limit the total As content in this type of food. The National Health Surveillance Agency of Brazil (ANVISA) determines total As values of 300 μg kg^{-1}. International legislation is a little stricter, the *Codex alimentarius* 2014 and the European Union Regulatory Commission 2015 limit the iAs content in polished or white rice to 200 μg kg^{-1} and, to 100 μg kg^{-1} in rice intended for infant food production. FAO/CODEX 2016 recommends that the iAs limit should not exceed 350 μg kg^{-1} in paddy rice.

Speciation methods in rice are well studied in the literature, mainly using chromatographic systems, such as LC-ICP-MS (BARNET et al., 2021; GRAY et al., 2017;

KARA et al., 2021; NARUKAWA et al., 2008; WEBER et al., 2021) and use of selective hydride generation for iAs analysis (SANTOS et al., 2017; YANG et al., 2016). Bralatei et al., 2015, proposed a method for determination of iAs in rice using field test kits based in Gutzeit reaction of the AsH_3 with $HgBr_2$ forming a colored compound, the method is fast (1h) with precision between 8 and 13%. The method was compared with HPLC-ICP-MS with results statistically similar and the accuracy was evaluated with use of CRM.

Different from seafood, there are certified reference materials available for iAs content in rice, but the low iAs content in this kind of sample is an issue for analysis using less sensitivity techniques. In general, the use of ICP-MS is necessary or coupled system with hydride generation what increases the cost of analysis and the waste production.

The concentration of iAs in rice is very variable for each country (WEBER et al., 2021) because there are several soils and environmental conditions, flooded crops, and distinctive anthropogenic contamination, for this reasons analysis in several countries is required for a study of these variations around the world.

The research developed in this thesis was realized between 2017 and 2022 and part of these results was published in scientific journals. The data were presented and discussed in three chapters identified as the paper published or in the process of publication. A summary of each chapter is described below.

Chapter 01 describes the synthesis and characterization of a new nanomaterial based on Fe_3O_4 functionalized with an organophosphorus compound. This material was submitted to an experimental design for optimization of a procedure to use it in a magnetic solid-phase extraction of iAs. The optimized method was used for the quantification of iAs in shrimp samples by ICP-MS. These results were validated using LC-ICP-MS and certified reference materials. In this work, there was a collaboration with Dra. Ana Rita Araujo Nogueira from "Embrapa Pecuária Sudeste" (São Carlos, SP) and Dr. Luelc S. da Costa from

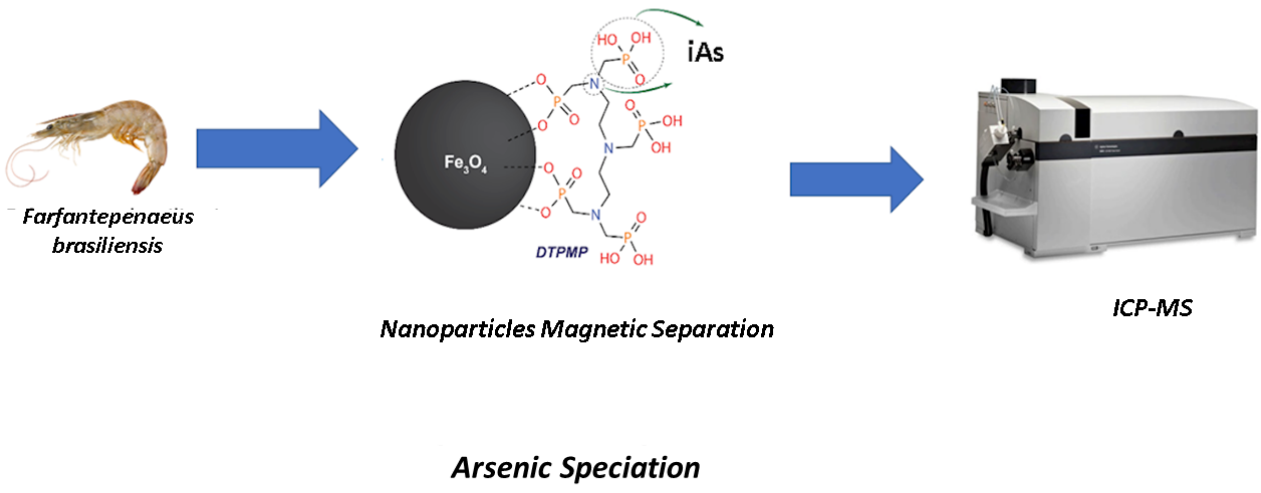
LCNano (Campinas, SP) where part of these results was obtained.

Chapter 02 describes the use of the developed nanoparticles in magnetic solid-phase extraction of arsenic in rice samples by ICP OES analysis, besides that is proposed a possible method of interaction between the analyte and the surface of the material using XPS and FT-IR. The results obtained show the capability of enhancing the signal of As of 20-fold allowing the analysis of this element using a less sensitive technique. It is important to mention the possibility of the use of the developed procedure for total analysis of As in rice samples when total digestion is performed. In this paper, the method was validated using certified reference material and comparison with LC-ICP MS. This manuscript is in the final step of evaluation by the editor of Food Chemistry for publication. There is a collaboration with Dra. Ana Rita de Araujo Nogueira from "Embrapa Pecuária Sudeste" and Professor Dr. Abdullah Akhdhar from the University of Jeddah (Saudi Arabia).

Chapter 03 is a technical note about the feasibility of the use of the developed nanomaterial for As speciation in algae. Besides that, As speciation using LC-ICP-MS/MS was also done. There is no study in the literature about As or mineral content in algae samples from Brazilian Northest. Since the rising use of seaweed in several products of human consumption, As speciation analyses are essential to guarantee safety for consumers. In this work, there was a collaboration with Tesla Group from the University of Graz (Austria) where part of the experiment was performed. This group is coordinated by Professor Dr. Jörg Feldmann that is one of the most prominent researchers of chemical speciation and the chemical of As in the environment.

Chapter 1

Non-chromatographic arsenic speciation analyses in wild shrimp (*Farfantepenaeus brasiliensis)* using functionalized magnetic iron-nanoparticles



Arsenic speciation in shrimp samples using a new functionalized iron-nanoparticles for analysis by ICP-MS

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Non-chromatographic arsenic speciation analyses in wild shrimp (*Farfantepenaeus brasiliensis*) using functionalized magnetic iron-nanoparticles

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ABSTRACT

A new iron-magnetic nanomaterial functionalized with organophosphorus compound was used as solid-phase for arsenic speciation in seafood samples with analyses by ICP-MS. The procedure was optimized using chemometric tools and the variables pH = 4.0, 15 min extraction time, and 20 mg of mass of material were obtained as the optimum point. The inorganic arsenic (iAs) extracted using nanoparticles presented concentrations between 20 and 100 µg kg^{-1} in the evaluated samples. The method was validated for accuracy using CRMs DOLT-5 and DORM-4. It was possible to reuse the same magnetic nanomaterial for 6 successive cycles, and we obtained a detection limit of 16.4 ng kg^{-1}. The proposed method is suitable for the use of inorganic speciation of As, presenting good accuracy, precision, relatively low cost, and acquittance to green chemistry principles.

Keywords: Inorganic arsenic; Shrimp; Arsenic speciation; Magnetic nanoparticles separation; ICP-MS;

1. INTRODUCTION

Arsenic (As) is a metalloid that can be found in various chemical forms, in both environmental and biological systems. Inorganic arsenic species (iAs), As (III), and As (V) are generally more toxic than other As species such as the methylated forms monomethyl arsenic (MMA) and dimethyl arsenic (DMA), as well as arsenobetaine and arsenocholine (Pétursdóttir & Gunnlaugsdóttir, 2019). However, some organic arsenic species with a toxicity similar to inorganic forms, especially the arsenolipids classes, have been studied in recent years (Pétursdóttir et al., 2018; Witt et al., 2017).

Symptoms of arsenic poisoning include muscle pain, weakness, nausea, vomiting, abdominal pain, and diarrhea. Furthermore, iAs species interfere with the cellular energy metabolism, causing oxidation by phosphorylation and inhibiting the synthesis of Adenosine Triphosphate (ATP). There is evidence that iAs contribute to the development of type 2 diabetes and are considered to have carcinogenic potential (Costa, 2019).

The main form of As intake by humans is through the food, mainly by the consumption of seafood and rice. Borak & Hosgood, 2007, show that 90% of As consumed by the US population comes from seafood. Signes-Pastor et al., 2017, established a relationship between arsenic species found in urine along with rice and seafood intake in children from Mexico. They concluded that these two dietary sources are responsible for virtually all of As consumed by that population. Brazil consumes about 14.4 kg per capita year^{-1} of seafood, exceeding the World Health Organization (WHO)'s recommendation (12 kg per capita year^{-1}(World Health Organization – WHO, 2010)). On the Brazilian coast, shrimp fishing is carried out by natives in artisanal fishing, employing, directly or indirectly, around 100,000 people in the Northeast region, the largest producer in Brazil. Additionally, Brazil is one of the largest shrimp producers in the world, with annual production close to 65,000 tons (Carvalho et al.,

2019). The species *Farfantepenaeus brasiliensis* is the most common wild shrimp species in the north and northeast of Brazil, having a great commercial importance for regional fisheries (França et al., 2019).

The As content allowed in crustaceans has different legislation around the world. For example, Southern Common Market (Mercosul) (Ministério Da Saúde RESOLUÇÃO DA DIRETORIA COLEGIADA - RDC No 22 , DE 29 DE ABRIL, 2013) standards set 1.0 mg kg^{-1} as total As (tAs) as maximum limits allowed. European standards do not limit the concentration of this element in crustaceans; however, there are recommendations for the study of As concentrations in several samples, including seafood with an emphasis on chemical speciation analysis (The European Commission, 2015). Chinese legislation requires analysis of As speciation in several samples allowing up to 0.500 mg kg^{-1} of iAs in seafood samples, excluding fish (0.1 mg kg^{-1}) (China, 2013).

The iAs analysis in seafood is not trivial since the concentration of this As species in this matrix is extremely low considering the total element content, once arsenobetaine is the predominant As species in seafood (Pétursdóttir et al., 2012; Schmidt et al., 2017b). Speciation using HPLC-ICP-MS presents coelution problems in complex matrices that may hinder the determination of iAs, requiring the introduction of other steps, such as hydride generation (Marschner et al., 2019; Pétursdóttir et al., 2012). In this context, new methods for As speciation analysis have been developed.

Non-chromatographic speciation methods generally have lower costs, are faster than chromatographic methods, comply with green chemistry guidelines, and can be used for chemical speciation analysis associated with more accessible detection methods such as Atomic Absorption Spectrometry (AAS), Optical Emission (ICP OES). It is easily derivatized with chemical vapor generation (HG) techniques (Cava-Montesinos, Nilles, Cervera, & Guardia, 2005; Esperanza et al., 2017; Gonzalvez, Armenta, Cervera, & de la Guardia, 2010;

Welna & Pohl, 2017).

Nanomaterials can be used for species separation in chemical speciation analysis, showing a tendency for non-chromatographic methods. The great versatility, ease and low cost of producing nanomaterials make them very attractive for this purpose. Also, these materials may be functionalized with complexing agents or may have their pore size-adjusted, making them selective to specific chemical species and less susceptible to interference.

The literature reports the use of magnetic nanomaterials in chemical speciation. Among the elements under study, there is an interest in As species. In a review, (Karadjova, Dakova, Yordanova, & Vasileva, 2016) cites 4 works that use magnetic nanoparticles for As speciation using techniques such as inductively coupled plasma mass spectrometry (ICP-MS), photoluminescence spectrophotometry, and electrothermal induction atomic absorption spectrometry (ETAAS). Otherwise, most of the methods are applied for water samples.

Recently, (Montoro Leal et al., 2018a)used magnetic Fe_3O_4 nanoparticles modified with a chelator ([1,5-bis (2-pyridyl) 3-sulfophenylmethylene] thiocarbon- hydrazide) to analyze As(III) and As(V) in water samples using an on-line system of preconcentration and determination by ICP-MS. Pourghazi, Amoli-Diva, & Beiraghi, 2015, also proposed a method using modified Fe_3O_4 on a silica surface employing ETTAS for quantification of iAs in water and rice samples.

The anionic characteristics of As in aqueous media make it difficult to remove this element from an aqueous sample using nanoparticles. Studies in the literature report the use of phosphoric acid to assist As removal (ZENG et al., 2008). In this work, the chelating agent used to functionalize the nanoparticles contains phosphoric groups that help in this extraction.

The development of procedures exploring the use of magnetic nanoparticles, particularly for speciation in biological and environmental samples, is a trend in the area. The

present work aims to develop a method using an organophosphate compound functionalized magnetic material for iAs speciation in wild shrimp by ICP-MS.

2. Materials and Methods

Instrumentation

For the total decomposition of the samples, a microwave oven Multiwave GO (Anton Paar, Austria) with a 12HTV50 rotor and PTFE-TFM tubes was used.

The extraction procedures were performed on a digester block (SOLARB, Piracicaba, São Paulo) equipped with PFA tubes and lids. After the extraction step, the samples were filtered before ICP-MS quantifications using a cellulose filter (30mm, 0.45 µm).

An ICP-MS Agilent 7800 Quadrupole (Agilent Technologies, Tokyo, Japan) was used in all experiments. The sample was introduced in the ICP using a concentric nebulizer and a cyclonic spray chamber. parameters were: 1.55 kW radio-frequency power; 15 L min^{-1} argon plasma flow rate1.00 L min^{-1} auxiliary argon flow rate1.02 L min^{-1} carrier argon flow rate; and 1.4 L min^{-1} sample flow rate and ^{75}As was the monitored isotope.

An 1100 series LC (Agilent Technologies) and a column Zorbax SB-C18 4.6 x 250 mm were used for the separation of As species. An isocratic elution with 50 mmol L^{-1} $(NH_4)_3PO_4$, pH =7.0, as eluent, and a sample flow rate of 1.2 mL min^{-1} and injection volume of 20 µL was used in the chromatographic step.

A super magnet of neodymium with 20 x14 x 4 cm was used for magnetic separations.

For characterization of material, the following techniques were used: a

PANalytical X-ray diffractometer MPD using cobalt $K_{\alpha 1}$ radiation (λ = 1.78890100 Å) with 2 θ angle scanning from 10° to 100° at a 0.013° pitch for 70 s. The program used to identify crystalline phases was HighScore Plus. A Shimadzu IRTracer-100 infrared spectrometer (China) in transmittance mode in the 4000 - 400 cm^{-1} range was used for FTIR analysis. Transmission Electronic Micrography (TEM) was carried out on a MSC JEOL TEM-2100 200 kV microscope, equipped with a CCD (TVip–16 MP) and TV (Gatan ES500W). The size distribution curves were obtained using the software ImageJ (U.S. National Institutes of Health, Bethesda, MD) for manually measure the particles size on the micrographs.

Sample and reagents

Wild shrimp (*Farfantepenaeus brasiliensis* (Latreille,1817)) samples were purchased from a local market in Fortaleza, Ceará, Brazil, in two periods: January 2019 and August 2019. The samples were frozen with liquid nitrogen, freeze-dried (Liobras L 108, São Carlos, SP, Brazil), ground using a coffee grinder fitted with stainless steel blades, and stored in previously decontaminated vials. It should be mentioned that the mill was cleaned before its usage by grinding a disposable subsample of shrimp and discarding it before processing the sample used for analysis.

Certified reference material samples DORM-4 (Fish protein, National Research Council of Canada, Canada) and DOLT-5 (Dogfish liver, National Research Council of Canada, Canada) were used for sample preparation accuracy test.

All glassware was immersed in 10% v v^{-1} nitric acid, HNO_3, (Vetec, Rio de Janeiro, Brazil) for 24 h and thoroughly rinsed with ultrapure water.

Solutions were prepared using ultrapure water (18.2 MΩ cm resistivity) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Sample preparation procedures were performed using HNO_3 (Synth, Diadema, SP,

Brazil) purified in a sub-boiling distillation apparatus Distillacid™ BSB-939-IR (Berghof, Eningen, Germany). Hydrogen peroxide, H_2O_2, (30% w w^{-1}) (Sigma Aldrich, Germany) was used in the total decomposition procedure. Sodium arsenite (As(III)) > 95% w w^{-1} (Sigma Aldrich, Germany) and Potassium arsenate monobasic(As(V)) >90% w w^{-1} (Sigma Aldrich, Germany) were used as arsenic standards.

Acetic acid, CH_3COOH, (99% w w^{-1}), boric acid, H_3BO_3, (90 % w w^{-1}) and phosphoric acid, H_3PO_4, (98% w w^{-1}) from Vetec, Rio de Janeiro, Brazil, were used for buffer solution preparation.

For nanoparticle synthesis, ≥99 % w w^{-1} iron(II) sulfate heptahydrate, $FeSO_4.7H_2O$ (Sigma Aldrich, Germany), 98 % w w^{-1} iron(III) chloride hexahydrate, $FeCl_3.6H_2O$ (Vetec, Brazil), 50% w v^{-1} dimethyl triamine-pentamethylene phosphonic acid (DTPMP, $C_9H_{29}N_3O_{15}P_5$) (Sigma, Germany) and 27 % w w^{-1} ammonium hydroxide, NH_4OH (Vetec, Brazil) were used.

Synthesis and characterization of nanoparticles

Magnetic iron oxide nanoparticles (Fe_3O_4) non-functionalized and functionalized with DTPMP were obtained by the hydrothermal route, as shown in Fig. 1a. Briefly, 2.09 g (7.5 mmol) of $FeSO_4 \cdot 7H_2O$ and 3.88 g (14 mmol) of $FeCl_3 \cdot 6H_2O$ were solubilized in 38 mL of deionized water in a Teflon chamber. After that, 20 mL of 27% w w^{-1} NH_4OH was added (0.33 mL s^{-1}) to the resulting solution under mechanical stirring (180 rpm) for 1 min. To obtain DTPMP-functionalized Fe_3O_4 (named as Fe_3O_4@DTPMP in this study), 1.2 mL of 50% w w^{-1} DTPMP solution was dripped into the resulting mixture. Subsequently, the Teflon vessel was sealed in a stainless-steel autoclave and placed in an oven at 150 °C for 210 min. To remove the exceeding chemicals, non-functionalized Fe_3O_4 nanoparticles were washed with deionized water until they reached neutral pH and lyophilized. Fe_3O_4@DTPMP

nanoparticles were purified with Spectra/Por®6 dialysis membrane with a size of 1 kDa molar mass in deionized water, then dispersed in water, centrifuged for 10 min at 3000 rpm to remove large aggregates and lyophilized. The representative structure for DTPMP-coated Fe_3O_4 MNPs is shown in Figure 1b, where the available phosphate groups for speciate iAs species can be seen.

X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) analysis were performed to study the structure of both Fe_3O_4 and Fe_3O_4@DTPMP nanoparticles. Tramission Electronic Micrography was used to investigate the Fe_3O_4@DTPMP NPs morphology

Total decomposition of samples

For shrimp samples decomposition, approximately 200 mg of sample was weighed and then 6 mL of 50% v v^{-1} HNO_3 and 2 mL of 30% w w^{-1} H_2O_2 were added. The microwave-assisted digestion was performed following the next heating program: 30-180 °C (10 min), 180 °C (25 min), 180-30 °C (10 min). The resultant solutions were then diluted with ultrapure water to 15 mL. The DORM-4 and DOLT-5 CRM samples were also decomposed following the same procedure.

Arsenic extraction procedure

For extraction of As species, the method proposed by Pétursdóttir, Gunnlaugsdóttir, Krupp, & Feldmann, 2014, was adapted. To this, 500 mg of shrimp sample was weighed in a PTFA tube and 10 mL of 2% v v^{-1} HNO_3 was added. The sample solution was then heated in a digester block for 1h at 90ºC. After this step, the extract was diluted to 20 mL with ultrapure water, centrifuged, and filtered using a cellulose resin filter (0.45 µm). This solution was labeled as extract 1A. CRMs samples were subjected to the same procedure;

however, a concentration of 200 µg L^{-1} of iAs was added to CRM extract for spike/recovery accuracy test.

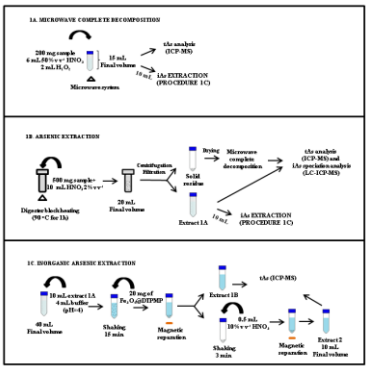
The solid residue of extraction procedure was dried at room temperature in a desiccator until constant weight and microwave-assisted digested following the same procedure for total As decomposition (section 2.4) and analyzed by ICP-MS for As mass balance. Total As content was also quantified in extract 1A, and LC-ICP-MS was used for inorganic arsenic speciation as compared methodology.

Inorganic arsenic extraction procedure

For extraction of iAs, an aliquot of 10 mL of extract 1A was taken and 4 mL of Britton-Robinson (BR) buffer solution (pH 4.0) was added. This mixture was diluted with ultrapure water to 20 mL, and 20 mg of nanoparticle (Fe_3O_4@DTPMP) was added. The mixture was stirred for 15 min, after which the sample was magnetically separated from the nanoparticles. The supernatant (extract 1B) was reserved for total As analysis by ICP-MS. To desorb the iAs from the nanoparticles, 0.5 mL of 10% v v^{-1} HNO_3 was added to them and vortexed for 3 min. After this step, the nanoparticles were magnetically separated from the sample, and the resulting solution was diluted with ultrapure water to 10 mL (extract 2) and As species analyzed by ICP-MS. The arsenic concentration quantified in extract 2 was considered iAs.

The same procedure for iAs extraction was repeated in digested shrimp, DORM-4 and DOLT-5 CRM samples. A scheme of the procedures related to sections 2.4, 2.5, and 2.6 can be observed in Figure 1.

Figure 1: Scheme of experimental procedures



Experimental design

To optimize the iAs extraction process using Fe_3O_4@DTPMP, a central composite design (CCD) was used. The variables pH (4 – 8), nanomaterial mass (10 – 30 mg) and extraction time (15 – 25 min) were monitored in experimental design, and As recovery was used as response. An iAs 200 µg kg^{-1} standard solution was used for all experiments. The supplementary material S1 shows the experimental matrix used.

Study of Fe_3O_4@DTPMP reuse

Successive iAs extractions using the same aliquot of material were performed to evaluate the possibility of nanomaterial reuse. For this purpose, after the first iAs extraction procedure in a known concentration solution, the same nanomaterial particles were again dispersed in a second 200 µg kg^{-1} iAs standard solution. This process was repeated 10 times, with 10 different iAs standard solution samples.

3. RESULTS AND DISCUSSION

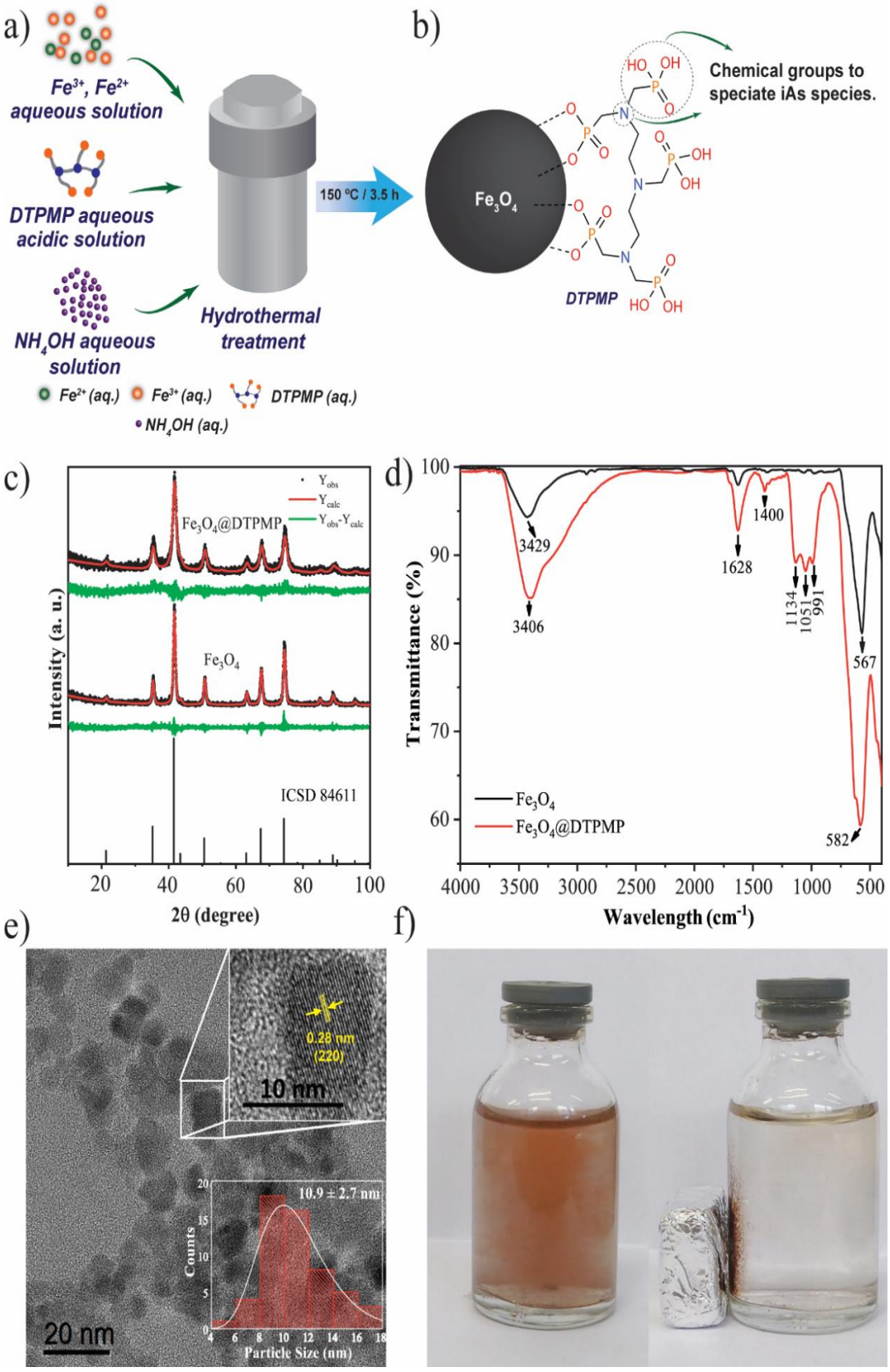
Characterization of nanomaterial

In this work, we synthesized DTPMP-functionalized Fe_3O_4 NPs through hydrothermal methodology under a temperature of 150 ºC (Figure 2a). Under the

hydrothermal conditions employed herein, a relatively high temperature was achieved by the high pressure of the sealed reactor. This temperature provides a higher crystallinity to the nanomaterials in comparison to those synthetic methodologies performed at smaller temperatures (25-100 ºC), which enhance the magnetic susceptibility and saturation magnetization of the magnetic nanoparticles (W. Wu et al., 2015). These features are essential in separation application, once the particles could be separated faster from the liquid containing the analyte, increasing the efficiency of the analytical process.

It was expected for the magnetic nanoparticles to be constituted of an inorganic core, composed by Fe_3O_4 (or partially oxidized to γ-Fe_2O_3), and organic shell made of DTPMP. In this case, part of the phosphoric acid groups would be anchored to Fe atoms of the core surface and the rest would be projected out from NPs surface, in order for them to interact and absorb iAs species (Figure 2b). Aiming to confirm the success of the obtaining of DTPMP-functionalized magnetic nanoparticles, XRD and FTIR characterizations were performed.

Figure 2: a) Chemical route to synthesize Fe3O4@DTPMP MNPs; b) Structure of DTPMP-coated Fe3O4 MNPs; c) XRD patterns of the synthesized Fe3O4@DTPMP, Fe3O4 MNPs, and reference pattern ICSD 084611, where Yobs and Ycalc are the observed and calculated intensities, respectively; d) FTIR spectra of Fe3O4@DTPMP and Fe3O4 NPs; e) TEM micrograph and particle size distribution for Fe3O4@DTPMP MNPs; f) Fe3O4@DTPMP MNPs dispersed in the analytical matrix before and 60 s after the application of a magnetic field.



The lattice structure and phase composition of the synthesized nanoparticles were confirmed by XRD, as shown in Figure 1c. Both samples showed peaks at 21.4, 35.4, 41.7, 50.8, 63.4, 67.7, 74.6, 85.2, 89.2 and 95.8° which are assigned to (111), (220), (311), (400),

(422), (511), (440), (620), (533) and (444) planes of Fe_3O_4 cubic spinel (ICSD 84611), respectively. Rietveld analysis was performed to the samples XRD patterns and the weighted profile R-factor (R_{WP}) and goodness of fitting (χ^2), as shown in Supplementary material S2. R_{WP} and χ^2 are statistical indicators, which indicates, respectively, the convergence of theoretical and experimental XRD patterns and the ratio between Rwp and its expected limit value for the obtained diffraction data. The results confirmed a good agreement with the reference pattern. Interestingly, a smaller average crystallite size (7.9 nm) was observed by Williamson-Hall fitting to functionalized nanoparticles. Non-functionalized Fe_3O_4 NPs showed size of 12.5 nm and also larger cubic cell lattice parameter (a = 8.380 Å, versus 8.365 Å for Fe_3O_4@DTPMP). This behavior was reported in the literature when the functionalizing agent is added to the reaction before the particle formation (Andrade Neto et al., 2017). The phosphonate groups in DTPMP have a great ability to chelate Fe ions, inhibiting the growth of Fe_3O_4 nanocrystal.

Figure 2 d shows the FTIR spectra of Fe_3O_4@DTPMP and Fe_3O_4 NPs. Both samples showed bands around 3400 and 1630 cm^{-1} assigned to stretching vibrations of O–H and bending vibrations of adsorbed H_2O on the surface of the nanoparticles, respectively (Karimzadeh et al., 2017; S. Yang et al., 2015). The bands observed at 567 cm^{-1} for Fe_3O_4 sample and at 582 cm^{-1} for Fe_3O_4@DTPMP sample are characteristic of Fe–O vibration (stretching), indicating that the synthesized NPs consist of Fe_3O_4, as evidenced in XRD (Andrade Neto et al., 2017). The efficacy of functionalization was evidenced by the presence of the bands at 1400, 1134, 1051, and 991 cm^{-1} in the spectra for the sample Fe_3O_4@DTPMP, assigned, respectively, to C–N stretching [29], P=O stretching (Zhu et al., 2014) and asymmetrical and symmetrical P–O–Fe stretching (Barja et al., 1999; Basly et al., 2013).

The morphology of Fe_3O_4@DTPMP NPs were also evaluated by Transmission Electronic Microscopy (TEM). The micrograph and particle size distribution curve are shown

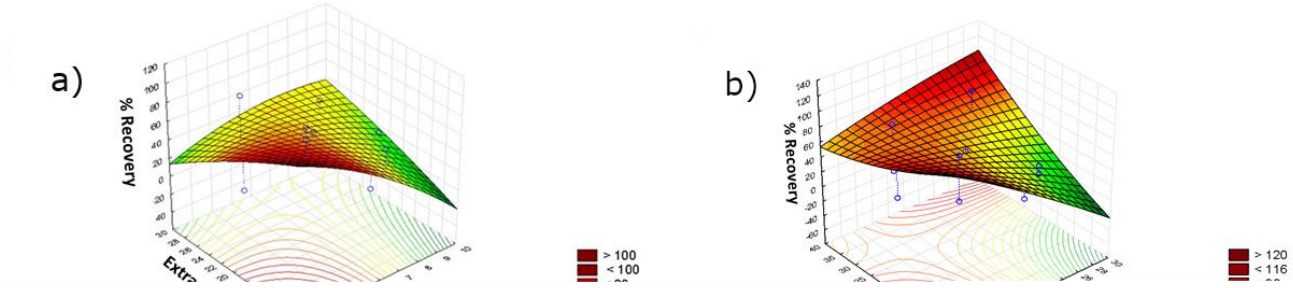
in Figure 1e. The NPs showed sphere-like morphology with a medium diameter of 10.9 ± 2.7 nm, higher than the crystallite size obtained by XRD. The higher value obtained by TEM can be related to the non-crystalline core formed by DTPMP on the NPs surface (Andrade Neto et al., 2017). The inset on the upper right size of the Figure 2e shows an enlarged image of a NP, which presents high crystallinity and lattice spacing of 0.28 nm related to the (220) plane of Fe_3O_4. TEM and XRD analysis corroborated the occurrence of Fe_3O_4 particles smaller than 25-30 nm, which exhibits typically superparamagnetic behavior, interesting for adsorption applications, since the particles can disperse well after the removal of the magnetic field, facilitating their reuse (Li et al., 2017).

The Energy Dispersive X-Ray Spectroscopy (EDS) graph for the Fe_3O_4@DTPMP sample is shown in Figure S3, where can be seen the characteristic peaks of elements that compose the sample (Fe, O, C and P). In addition, Cu and Si peaks are observed, which is attributed, respectively, to the transmission grid and the window EDS detector. The intensity of the peak assigned to carbon can also be related to the carbon sheet, which compounds the transmission grid.

1. Experimental design

A central composite design (CCD) was used to optimize the iAs extraction process using Fe_3O_4@DTPMP. Figure 3 shows the response surface methodology (RSM).

Figure 3: RSM for As extraction using Fe3O4@DTPMP (a) pH x extraction time; (b) extraction time x mass)



Arsenic is found in inorganic form, mainly as arsenous acid (H_3AsO_3) and arsenic acid (H_3AsO_4) (Baranik et al., 2018). In the experimental design, it can be observed that the pH range around 4-5 presented the best recovery of As. In that pH range, the species H_3AsO_3 and $H_2AsO_4^-$ are predominant; and, due to the low dissociation constant of arsenous acid (pKa_1 = 9.3) (Bissen & Frimmel, 2003) in practically any pH extension studied (4-5), this compound is found in a protonated form.

Studies in the literature report that this pH range (4-5) is ideal for the extraction of both iAs species, requiring a pH greater than 9 for the separation of As(III) and As(V) (Liang & Liu, 2007), thus the pH dependence in this study is related to the As(V) forms in solution.

Liang & Liu, 2007; Montoro Leal et al., 2018 observed that, at a pH higher than 7.0, extractions of As(V) using nanomaterials become less effective. The dissociation constants of arsenic acid (pKa_1 = 2.2 and pKa_2 = 7.0) suggest that from pH 7.0 the $HAsO_4^{-2}$ form is in greater concentration. Since the formation of anions makes the molecules larger, this may be an indication that there are steric impediments that make this extraction less effective using nanostructured materials. Even in functionalized materials, this tendency is observed. It occurs probably due to the more anionic characteristic of the ion, thus having no interaction with the ligand used in functionalization.

The DTPMP has nine protonated hydrogens (H_9L) and data from the literature show that at pH 4.0 the forms H_7L^{-2} and H_6L^{-3} are predominant (Cigala et al., 2014). Some of the binding sites available in the complexing agents were used to bond with the nanomaterial. Consequently, it is expected that at the evaluated pH there will be less deprotonated sites. This structure would be more similar to phosphoric acid and the $H_2PO_4^-$ form, which also proved effective in removing arsenic (ZENG et al., 2008).

Due to the large surface area and the mass/volume ratio, nanomaterials generally have high sorption efficiency and low equilibrium times when compared to materials in the

macro-scale (Bendicho et al., 2016). Thus, it can be noted in figure 3b that, in the evaluated times, there were practically no significant variations in extraction efficiency. However, after 15 min, the highest efficiency can be observed. Besides, small masses can be used with large As recovery, so 20 mg was used as an optimal point for the other experiments.

Total decomposition and As extraction

The amount of As in shrimp samples decomposed by microwave-assisted digestion (section 2.4), subjected to As extraction procedure (extract 1A) (section 2.5), and the residues obtained from the As extraction process, were quantified by ICP-MS. To verify the accuracy of the decomposition method, CRM's, DOLT-5 and DORM-4 were also analyzed. The results can be seen in Table 1.

Table 1: Total and extract mass fraction of As in shrimps and CRMs samples.

Arsenic Mass Fraction (mg kg^{-1}, mean ± SD, n=3)					
Sample	Total* (Recovery)	Extraction			
		Extract 1A**	Solid Residue*	Mass balance (%)	Extracted (%)
DOLT-5	34.3 ± 2.0 (99)	22.8 ± 2.3	11.0 ± 0.1	98	67
DORM-4	6.73 ± 0.23 (98)	3.91 ± 0.44	2.76 ± 0.22	99	58
Shrimp 1	4.61 ± 0.43	1.68 ± 0.04	2.91 ± 0.16	99	36
Shrimp 2	4.41 ± 0.43	1.96 ± 0.07	2.44 ± 0.22	100	44

*Microwave-assisted digestion; **2% v v^{-1} HNO_3 extraction

It can be observed that the total decomposition method presented satisfactory accuracy since the As concentrations in the decomposed CRM samples are statistically similar to the certified values (34.6 ± 1.2 and 6.87 ± 0.22 mg kg^{-1} for DOLT-5 and DORM-4,

respectively). Besides, the results show relative standard deviations below 10%, also guaranteeing good precision of both the decomposition and the extraction method.

For both CRM samples, 100% of recovery was obtained through spyke/recovery test, indicating that losses for volatilization do not occur.

A range between 36 and 67% of As could be extracted from the samples. (Pétursdóttir, Gunnlaugsdóttir, et al., 2014), by using microwave-assisted acid extraction (MAE) for As extraction, obtained recovery of 66% for DOLT-4 sample. We obtained similar recovery for CRM DOLT-5 using the digestion block in the extraction procedure. It is necessary to emphasize that the extractions were performed using PFA flasks with lids, which avoided losses due to volatilization. In a recent work, recoveries higher than 80% for DOLT-5 CRM samples were obtained using a similar extraction method but employing MAE (Marschner et al., 2019). However, the intent of the present work is the extraction of iAs species. It is known that these species are water-soluble (Ronan et al., 2017; Wolle & Conklin, 2018), therefore it is expected that iAs was completely extracted by the proposed extraction procedure. In an attempt to make the As mass balance, the solid residue of the extraction was decomposed using microwave-assisted digestion (section 2.4). The results are presented in Table 1. The As in extract plus the As in solid residue was approximately 100% of the total As for all the samples, indicating that there had been no loss of As during the extraction process.

iAs quantification using MNP's

For iAs analysis, all the As extracted by the nanoparticles was considered to be iAs. As explained in section 2.5, extractions of iAs species were carried out from extract 1A obtained using acid media and digester block heating. In order to check the As mass balance, besides the iAs extracted by nanoparticles, the As content in preconcentration discard (extract 1B) was also analyzed.

The As mass fractions quantified by ICP-MS in shrimp and CRMs samples after iAs extraction using Fe_3O_4@DTPMP are presented in Table 2. The LC-ICP-MS was used for comparison with the proposed method.

Table 2: iAs mass fraction in shrimps and CRMs samples after iAs extraction, using Fe3O4@DTPMP compared to the iAs quantified by LC-ICP-MS.

	As mass fraction (mg kg^{-1}, mean ± SD, n=3)				**iAs LC-ICP-MS (mg kg^{-1}, mean ± SD, n=3)**
Sample	**iAs (extract 2)**	**As (extract 1B)**	**Mass Balance**	**% Recovery**	**iAs (extract 1A)**
DOLT-5	0.023 ± 0.001	21.422 ± 0.751	21.443 ± 0.751	94	0.057 ± 0.005
DORM- 4	0.204 ± 0.013	3.885 ± 0.160	4.001 ± 0.160	102	0.218 ± 0.013
ırimp1	0.054 ± 0.002	1.600 ± 0.069	1.654 ± 0.070	98	0.058 ± 0.006
Shrimp 2	0.060 ± 0.002	1.889 ± 0.078	1.949 ± 0.078	99	0.065 ± 0.007

LOD: 16.4 ng kg^{-1}; LOQ: 54.7 ng kg^{-1} (proposed method)
LOD: 7.2 µg kg^{-1}; LOQ: 24.0 µg kg^{-1} (LC-ICP-MS method)

It can be observed that the iAs content in all samples in the study is much smaller than the total As. This is expected since most of the As in seafood is in the form of organic arsenic, especially as arsenobetaine (Marschner et al., 2019; Pétursdóttir et al., 2012; Schmidt

et al., 2017b). The small concentration of iAs compared to total As makes iAs analysis difficult even for sensible methods as LC-ICP-MS. Shrimp samples presented a ratio of iAs/tAs between 1.17 and 2.39%. Even with low analyte content, the proposed procedure showed relative standard deviations (RSD) <10%.

The CRMs used do not have iAs certified value, and few studies in the literature report iAs content in these CRMs. In a recent study, (Marschner et al., 2019), performed the analysis of several CRMs using different techniques and determined the concentrations of iAs by HPLC-HG-AFS in DORM-4 and DOLT-5 samples, finding 0.205 ± 0.010 and 0.029 ± 0.007 mg kg^{-1} iAs, respectively. (Wolle & Conklin, 2018), also analyzed iAs content in DORM-4 by LC-ICP-MS, reporting 0.214 ± 0.021 mg kg^{-1} iAs. These values are statistically similar to those found in this study (0.023 ± 0.001 and 0.204 ± 0.013 for DOLT-5 and DORM-4, respectively). Thus, the method presents compatible results with the literature.

The DORM-4 sample also presented similar iAs values to those found by (Wolle & Conklin, 2018), using the LC-ICP-MS method. With the use of this technique, only the DOLT-5 sample had higher iAs levels than that found when using the method proposed in this work. Most likely, this occurred due to the possible coelution of As species. This phenomenon was also reported by (Marschner et al., 2019; Pétursdóttir et al., 2012).

The supernatant solutions (extract 1B) from the proposed nanoparticle procedure were directly analyzed by ICP-MS to assess whether the loss of As was occurring during the iAs extraction procedure or if the element was not being wholly desorbed from the nanoparticles. As it can be seen (Table 2), the As mass balance represented by As concentration extracted by Fe_3O_4@DTPMP designated as “extract 2” plus As concentration in disposal extract, supernatant solution of Fe_3O_4@DTPMP extraction named “extract 1B”, ranged between 98 and 102%, representing analytically representative recoveries.

The levels of iAs in shrimp samples showed values below that allowed by

Chinese legislation (0.500 mg kg^{-1}). It is important to note, however, that the Mercosul legislation when demanding the maximum limit for As, does not consider the chemical speciation of this element. Thus, the samples under study would be disapproved by Mercosul requirements (1 mg kg^{-1}), even though most of the As in these samples has little or no toxicity for a human being. It is important to mention that arsenolipids, another class of As species, can be as toxic as inorganic species (Pétursdóttir et al., 2018). Otherwise, there is no study reporting the presence of arsenolipids in shrimp samples.

The iAs extraction was also applied to the solution samples obtained after microwave-assisted digestion (section 2.4). It is expected, after total decomposition, for all the As contained in the final solution to be in the inorganic form. In the same way as the previous experiment, As mass fraction in the extraction disposal was also analyzed by ICP-MS for As mass balance calculations. The results can be observed in Table 3. It can be seen that the recovery of As was between 98 and 103%, ensuring that there was no loss of As during the process. However, in all experiments, there was As content in the disposal solutions. The CRM sample DOLT-5 showed the highest concentration of As in the disposal solution, likely due to this reference material having the highest mass fraction of arsenobetaine (24.2 ± 0.8 mg kg^{-1}), about 70% of all As in the sample. Even though it is expected that all As is present in inorganic form after decomposition in HNO_3 + H_2O_2 media, the literature reports the difficulty of complete decomposition of arsenobetaine. In most decomposition methods that use less than 300 ^oC, this As species is only partially decomposed (Šlejkovec et al., 2001). The decomposition method applied in this work used a maximum temperature of 180 ^oC. Because of that, it is probable that part of the arsenobetaine is not decomposed and, therefore As was still found in the disposal solutions. These results give an indication of the selectivity of the nanomaterial for iAs species.

Table 3: Inorganic As mass fraction in shrimps and CRMs samples after

microwave-assisted digestion followed by iAs extraction, using Fe3O4@DTPMP

Arsenic mass fraction mg kg^{-1} (mean ± SD, n=3)				
Sample	**iAs Digested**	**As Disposal**	**tAs**	**Mass balance (%)**
DOLT-5	10.58 ± 0.86	23.17 ± 1.56	33.74 ± 1.78	98
DORM-4	5.169 ± 0.048	1.856 ± 0.187	7.02 ± 0.24	103
Shrimp 1	4.40 ± 0.10	0.16 ± 0.01	4.56 ± 0.10	99
Shrimp 2	4.34 ± 0.12	0.12 ± 0.01	4.46 ± 0.12	101

The limits of quantification (LOQ) were calculated according to IUPAC. The LOQ obtained with the LC-ICP-MS (24.0 µg kg^{-1}) was higher than the LOQ obtained with the proposed procedure (54.7 ng kg^{-1}). The LC-ICP-MS needed an excessive dilution (Gonzalvez et al., 2010). In contrast, the proposed procedure allows a preconcentration of iAs during nanomaterial extraction process, which can explain its lower LOQ.

The most works in the literature use water samples or herbal infusions (Ahmad et al., 2017; Huang et al., 2011; López-garcía et al., 2018; Montoro Leal et al., 2018a; Pourghazi et al., 2015) the present work used solid samples with complex composition of As and a lower concentration of iAs than organic arsenic species. The detection limits varied between 0.21 and 20 ng L^{-1}, and the proposed method obtained LOD value of 16.4 ng kg^{-1}.

3.5 The reuse of Fe_3O_4@DTPMP for several extraction procedures

The possibility of reusing the same nanomaterial for iAs extractions was evaluated. Since the particles are insoluble in water and easily captured by a magnetic field, it is expected to perform more than one extraction with the same material.

The recovery data shows that the successive recovery of iAs in the solutions remains statistically equal until the 6^{th} cycle of use, from the 7^{rh} cycle onwards the recoveries

fall below 85%. Thus, it can be said that the same mass of nanomaterial (20 mg) can be used up to 6 times while maintaining analytical recoveries (85-110%). A bar graph can be seen in figure S4 in the supplementary material.

The iron nanoparticles can agglutinate and lose magnetization (Qiao et al., 2019), which reduces their extraction capacity; and due to the low mass used (20 mg), a little of the material may be lost during magnetic separation.

4. CONCLUSIONS

The proposed method of using Fe_3O_4@DTPMP for iAs analysis in shrimp samples presented good accuracy and precision, with adequate LOD and LOQ for this type of analysis.

The analysis of iAs content in the shrimp samples showed that the organic As forms are the main component in the samples. Thus, the legislation must adapt to estimate the quantities of harmful chemical species.

The use of the non-chromatographic method, developed in this study, improved around 1000 times the limits of detection/quantification when compared with the chromatographic method.

The group continues the work in an attempt to understand the mechanisms that allow speciation of iAs using this material, in addition to the possibility of speciation of As (III) and As (V) in other types of samples.

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Supplementary Material

Table S1: Levels and factors established for the experimental design

	Levels				
Variable	**α-**	**-**	**0**	**+**	**α +**
pH	2.6	4	6	8	9.4
Mass (mg)	3	10	20	30	37
Extraction time (min)	11.6	15	20	25	28

Table S2 – Structural parameters of Fe_3O_4@DTPMP and Fe_3O_4 NPs obtained through Rietveld refinement.

Sample	Lattice parameters (Å)	R_{WP} (%)	$\chi 2$	Average crystallite size (nm)
Fe_3O_4@DTPMP	8.365	19.81	1.09	7.9
Fe_3O_4	8.380	21.64	0.99	12.5

R_{WP} = weighted profile R-factor, which indicates the convergence of refinement; Å=angstrom; χ^2= goodness of fit, which should be close to 1.0.

Figure S3: EDS spectra for Fe_3O_4@DTPMP NPs.

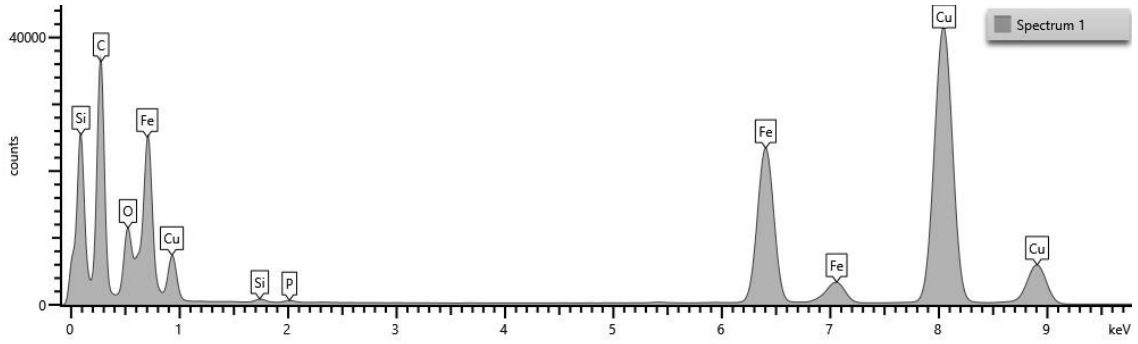
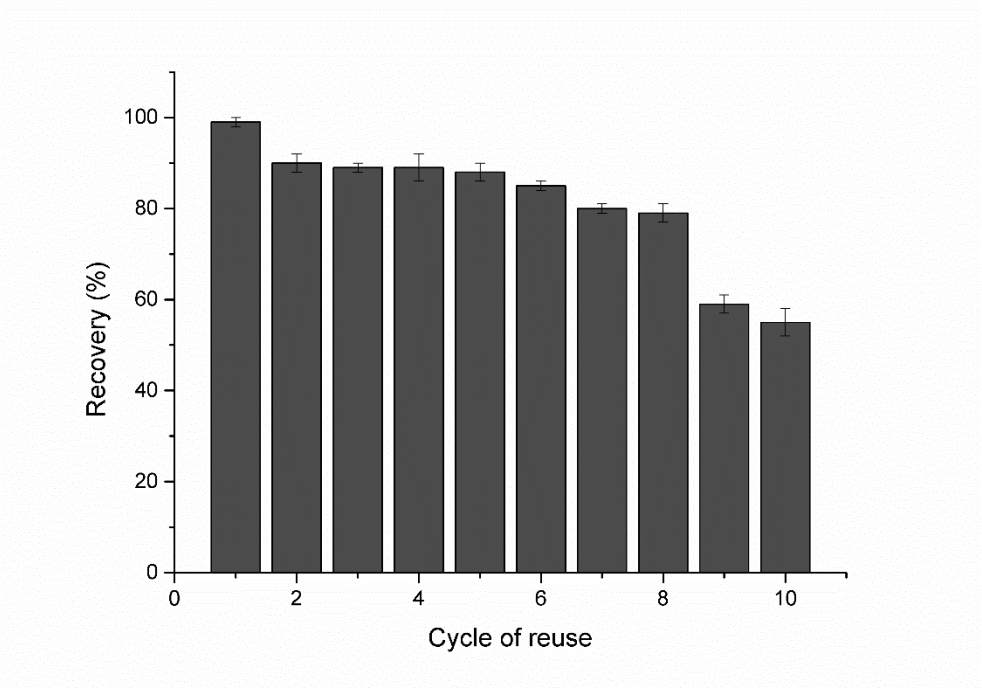
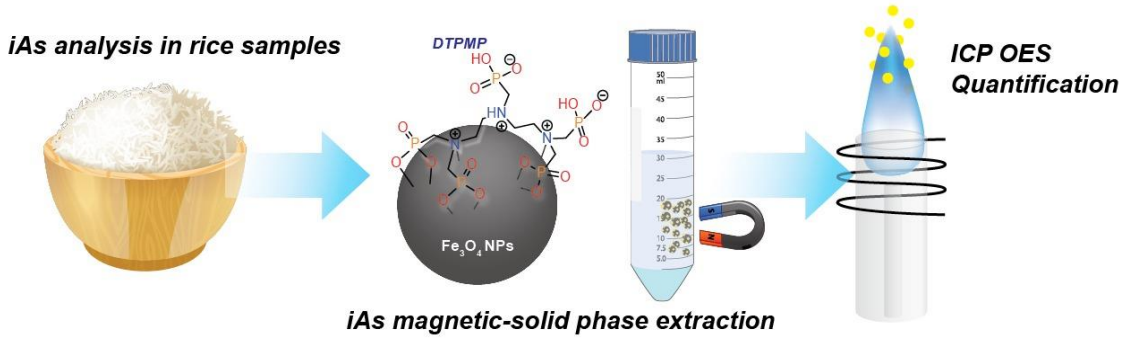


Figure S4: Cycle of reuse of Fe_3O_4@DTPMP in successive iAs extractions (mean ± SD, n=3)



Chapter 2

Magnetic solid phase extraction as non-chromatographic method for ultra-trace inorganic arsenic quantification in rice samples by ICP OES



Food Chemistry

Magnetic solid phase extraction as non-chromatographic method for ultra-trace inorganic arsenic quantification in rice samples by ICP OES

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ABSTRACT

An alternative analytical method for speciation of inorganic arsenic (iAs) in rice by ICP OES was developed, using iron nanoparticles modified with an organophosphorus compound as the solid phase for extraction/pre-concentration of iAs. This procedure was performed employing the following experimental conditions: 4 mL buffer solution pH 4.0, 20 mg nanomaterial, and 15 min of extraction. The accuracy for total arsenic (tAs) and iAs quantification was verified using CRM NIST 1568b (showing 97% and 101% recovery, respectively). The proposed method shows satisfactory precision (RSD less than 15% for all samples) as well as LOD and LOQ (1.08 and 3.70 µg kg^{-1}, respectively). Furthermore, samples present tAs content between 0.090 and 0.295 mg kg^{-1} and iAs mass fraction between 0.055 and 0.109 mg kg^{-1}. These values are compliant with food legislation in force. The developed method is a low-cost, simple, greener alternative for ultra-trace As and iAs analysis.

KEYWORDS: Rice, arsenic speciation, nanomaterials, non-chromatographic methods, ICP OES, magnetic-solid-phase extraction.

1. Introduction

Rice (Oryza Sativa L.) is a crop that grows on all continents except Antarctica and has been a part of the cultural identity of a number of countries (Singh et al., 2017). Economically, rice is the third most consumed agricultural commodity, behind wheat and corn. Further, it has the second market variability, as rice is a high-demand food product with a worldwide per capita consumption of about 50 kg per year (Prasad et al., 2017). Asia is the largest producer (about 90% of global production) and consumer (110-120 kg per person a year) of rice and Brazil is the sixth-largest exporter (exporting 1.1 million tons a year and consuming about 25 kg per person a year) (Moreno García et al., 2021).

Despite its economic and food importance, rice is considered the primary source of arsenic (As) for humans, apart from seafood. Arsenic is a toxic metalloid, and different species of this element exhibit distinct toxicities. The As species commonly present in rice are inorganic and organic species, such as dimethylarsinic acid (DMA), methylarsonate (MMA), and, to a lesser extent, tetramethylarsonium (TMA) (Hansen et al., 2011; Pétursdóttir, Friedrich, et al., 2014). The inorganic As (iAs) (As(III) and As(V)) is from 67 to 271-fold more toxic than the methylated species (Costa, 2019). Rice is more affected by As contamination than other crops due to the flooding conditions required for its cultivation (Suriyagoda et al., 2018). Arsenic(V) uptake by rice is through phosphate channels, and As(III) is carried with Si in membrane channel proteins (Abedi & Mojiri, 2020).

Major food legislations around the world focus only on the iAs toxicity. The US Food and Drugs Administration has set an action level of 100 μg kg^{-1} for iAs in rice (FAO, 2018), whereas Europe has set between 100-250 μg kg^{-1} as the maximum limit for iAs, depending on the type or use of rice (The European Commission, 2015). The Codex Alimentarius reports only total As values for this commodity in the range of 200-350 μg kg^{-1} but recommends speciation analysis on samples showing As concentration higher than 200 μg

kg^{-1} (FAO, 2018). The recommendation of the Agência Nacional de Vigilância Sanitária (ANVISA) is a maximum of 300 µg kg^{-1} of total As (tAs). However, if the As concentration is above the allowed, speciation analysis is required (Brazil, Anvisa, N° 88, DE 26 DE MARÇO DE 2021, 2021) However, for the production of infant food, the iAs content is limited to 150 µg kg^{-1}.

Because the tAs concentration does not provide analytical information regarding food safety, speciation analysis is required. Therefore, methods of analysis of As speciation in rice and rice-derived products are needed. The analytical challenge, in this case, is the determination of inorganic species of As in the presence of organic species of this element, such as DMA, MMA, and TMA. However, because it involves the quantification of elements at low concentrations and the discrimination of species of these elements in trace amounts, chemical speciation analysis is not trivial because it requires methods with low sensitivity and high selectivity. The technique most employed for this kind of analysis is Inductively Coupled Plasma Mass Spectrometry (ICP-MS) hyphenated with High Performance Liquid Chromatography (HPLC). Although high performance, HPLC-ICP-MS is expensive and complex, requiring highly trained analysts. Thus, the best procedures developed for speciation analysis are restricted to research laboratories and are not feasible for implementation in routine laboratories (Gonzalvez et al., 2010). Furthermore, it requires the use of large volumes of solvents that are not following the principles of green chemistry (Wojnowski et al., 2022). Therefore, in recent years, simpler non-chromatographic speciation strategies have been developed (Bendicho et al., 2016).

Solid phase extraction (SPE) is a non-chromatographic strategy used for speciation analysis that can promote pre-concentration and sorption of chemical species. Recently, nanoparticles have been used as solid phases to pre-concentrate elemental species for detection. Compared to conventional sorbents, nanomaterials provide high surface area,

specific sorption capacity, and fast sorption kinetics (Tseng et al., 2015). Although a wide variety of nanomaterials have already been applied for separation and pre-concentration for the total analysis of trace elements, their use as a selective phase for speciation analysis has been little explored (Bendicho et al., 2016).

Although still in an early stage of development, nanomaterial-based chemical speciation is facilitated by the rapid fabrication, high selectivity, and low cost of nanomaterials (Fechine, 2020). Nanomaterials may be functionalized with complexing agents, which makes them more selective sorbents and less susceptible to interferences. Magnetic nanoparticles (MNPs) may provide effective separation of analytes from the sample matrix under a magnetic field, without the need for filtration or centrifugation (Keçili et al., 2021). This type of separation is known as magnetic solid phase extraction (MSPE).

Recently, a new nanomaterial (Fe_3O_4@DTPMP, where DTPMP is dimethyl triamine-pentamethylene phosphonic acid) was used for As speciation in seafood samples by ICP-MS, presenting LOD and LOQ values better than HPLC-ICP-MS (Silva et al., 2021). Iron magnetic nanoparticles modified with a chelator (Montoro Leal et al., 2018) and Ni0.5Zn0.5Fe2O4 modified nanomaterial (Afkhami et al., 2020) were used for online analysis of As(III) and As(V) in water samples by ICP-MS. The As content in rice and water analysis could be quantified, using a Fe_3O_4 nanomaterial on a silica surface (Pourghazi et al., 2015) and pure Fe_3O_4 with controlled pH (López-García et al., 2018) by ETTAS.

The rice sample has a lower As content than that found in seafood samples. Although most of the As content is in the inorganic form, the lower content is a challenge for trace analysis. Pre-concentration is necessary to use low-cost and less sensitive methods in routine analysis.

The aim of this study is to evaluate the feasibility of the use of Fe3O4@DTPMP as a solid phase for pre-concentration of tAs and iAs analysis by ICP OES in rice samples. A

simple, rapid, sensitive, and low-cost method was developed and evaluated that, using Fe_3O_4@DTPMP nanomaterial as MSPE sorbent, is easily implementable in routine laboratories for the enrichment of iAs in rice followed by ICP OES quantification.

2. Materials and Methods

2.1 Instrumentation

For digestion and extraction of As species, it was used a Tecnal TE-007D block digester (Piracicaba, São Paulo, Brazil) equipped with 15 Teflon® tubes with lids.

An inductively coupled plasma optical emission spectrometer (ICP OES) dual view iCap 6000 (Thermo Scientific) was used for As quantification. The sample was introduced in the ICP using a concentric nebulizer and a cyclonic spray chamber. The ICP OES operating parameters were: 1.55 kW radio-frequency power; 12 L min-1 argon plasma flow rate; 1.00 L min-1 auxiliary argon flow rate; 1.02 L min-1 carrier argon flow rate; and 1.4 L min-1 sample flow rate. Arsenic and Fe were measured employing axial view at 193.7 nm and 259.9 nm, respectively.

An ICP-MS Agilent 7800 Quadrupole (Agilent Technologies, Tokyo, Japan) equipped with a collision/reaction cell operated using He gas (99.999%, White Martins-Praxair, Brazil). Argon (99.999%, White Martins-Praxair, Brazil) was used as the plasma generator gas, and for sample introduction and nebulization, was used for method comparison. An 1100 series HPLC (Agilent Technologies) coupled to a UV-Vis detector (Diode Array Detector, Agilent Technologies) and a column Zorbax SB-C18 4.6 x 250 mm were used for the separation of As species. The instrumental parameters are provided in the supplementary material (S1).

A shaker table (Tecnal, Piracicaba, SP) was used for the As extraction. A pH meter calibrated with pH 7.0 and 4.0 standard solutions (Sigma Aldrich, Germany) was used to measure the pH of the solutions. A 20 × 14 × 4 cm NdFeB super magnet was used for

magnetic nanoparticles separations.

A K-Alpha X-ray photoelectron spectrometer (XPS) (Thermo Fisher Scientific, UK) fitted with a hemispherical electron analyzer and an aluminum anode (Kα = 1486.6 eV) as the X-ray source was used in chemical surface analysis. The spectra were performed using charge compensation during the analyses, and the chamber pressure was kept below 2 × 10-8 mbar. Survey (i.e., full-range) and high-resolution spectra were recorded using pass energies of 1 and 0.1 eV, respectively. The spectrum fitting was performed assuming a mixed Gaussian/Lorentzian peak shape (the ratio of the Gaussian to the Lorentzian shape was 0.4).

For FTIR analysis, we used a Shimadzu IRTracer-100 infrared spectrometer (China) in transmittance mode in the 4000-400 cm^{-1} range.

2.2 Sample and reagents

Rice samples were purchased in Fortaleza, state of Ceará, Brazil (between April and May 2019) and Jeddah, Saudi Arabia (between August and September 2021). All samples were ground in a coffee grinder and stored in decontaminated vials until analysis.

The certified reference material SRM – 1568b Rice Flour (NIST, USA) and the reference material (RM) CRM-AGRO Ar_01/2015 (Embrapa-USP, São Carlos, São Paulo, Brazil) were used for the test of tAs trueness. The certificate reference material SRM – 1568b Rice Flour (NIST, USA) was used for the test of iAs trueness.

Solutions were prepared using ultrapure water (18.2 MΩ cm resistivity) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All glassware was immersed in 10% v v^{-1} HNO_3 (Sigma-Aldrich, Germany) for 24 h and thoroughly rinsed with ultrapure water. Extraction and digestion procedures were performed using 65% w w^{-1} HNO_3 (Sigma-Aldrich).

A Britton-Robinson buffer solution (pH 4.0) was prepared using an equimolar mixture of 65% w w^{-1} CH_3COOH (Neon, Suzano, SP, Brazil), 98% w w^{-1} H_3PO_4 (Sigma-Aldrich, Germany), and 35% w w^{-1} H_3BO_4 (Vetec, Rio de Janeiro, RJ, Brazil). The pH of the buffer solution was adjusted using 0.1 mol L^{-1} NaOH (Vetec) and/or 0.1 mol L^{-1} HCl (Vetec).

For nanoparticle synthesis, ≥ 99% w w^{-1} $FeSO_4 \cdot 7H_2O$ (Sigma Aldrich, Germany), 98% w w^{-1} $FeCl_3 \cdot 6H_2O$ (Vetec), 50% w v^{-1} dimethyl triamine-pentamethylene phosphonic acid (DTPMP, $C_9H_{29}N_3O_{15}P_5$) (Sigma, Germany) and 27% w w^{-1} NH4OH (Vetec) were used.

Sodium arsenite (As(III)) > 95% w w^{-1} (Sigma Aldrich, Germany) and potassium arsenate monobasic(As(V)) >90% w w^{-1} (Sigma Aldrich, Germany) were used as arsenic standards. Standard calibration solutions were prepared using successive dilutions of 1000 mg L^{-1} As (Acros Organics, Belgium) stock solution with 1% v v^{-1} HNO_3.

2.3 Synthesis of nanoparticles

Iron nanoparticles were synthesized following the experimental procedure proposed by Silva et al., 2021. Approximately 2.09 g of FeSO4.7H2O and 2.88g of $FeCl_3.6H_2O$ were solubilized and taken into a Teflon® chamber. Subsequently, 20 mL of 27% w w^{-1} NH_4OH was added, and the mixture was mechanically shaken for approximately 1 min. At the end of this process, Fe_3O_4 nanoparticles were formed. To obtain the functionalized magnetic nanoparticle (Fe_3O_4@DTPMP), 1.2 mL of 50% w w^{-1} DTPMP was added dropwise to the solution of Fe nanoparticles. In addition, the chamber was sealed in an autoclave and kept at 150 °C for 210 min. Fe_3O_4@DTPMP nanoparticles were purified by dialysis on Spectra/Por®6 equipment using 1 kDa membranes in deionized water to eliminate the excess functionalizing agent. Then, the Fe_3O_4@DTPMP nanoparticles were dispersed in water and centrifuged for 10 min at 3000 rpm to remove the aggregates and were then lyophilized. Fe_3O_4 nanoparticles and the Fe_3O_4@DTPMP were tested for iAs pre-concentration. The characterization of the material is described by Silva et al. 2021.

2.4 Evaluation of the need for functionalization of Fe_3O_4 nanoparticles for iAs pre-concentration

The functionalization requirement for iAs pre-concentration performance was verified through a preliminary test. To do this, it was added 2 mL of Britton-Robinson buffer solution (pH 4.0) into a Falcon® tube and added an aliquot of iAs (As(III)) standard solution so that in 40 mL of solution a concentration of 150 µg L^{-1} of iAs was obtained. A total of 20 mg of Fe_3O_4@DTPMP were dispersed in this solution. The obtained mixture was mechanically shaken for 15 min, and the nanoparticles were separated using a neodymium super magnet. The supernatant solution was discarded, and 2 mL of 10% v v^{-1} HNO_3 was added to the nanoparticles and mechanically shaken for 3 min. Once again, the nanoparticles were magnetically separated, and the solution was taken for As analysis by ICP OES.

For comparison purposes, the same procedure was performed with the non-functionalized nanoparticle (Fe_3O_4).

2.5 Evaluation of the reproducibility of Fe_3O_4@DTPMP nanoparticles synthesis, their lifetime, and mechanism of As adsorption

Three different batches of Fe_3O_4@DTPMP nanoparticles production were evaluated for iAs pre-concentration according to the procedure described in section 2.6.3 (MSPE extraction/pre-concentration for iAs analysis). For all batches, the experimental procedure for nanoparticle synthesis was the same.

Fe_3O_4@DTPMP nanoparticles were produced in July 2019 (Batch 1), March 2020 (Batch 2), and September 2021 (Batch 3). Approximately 2.0 g of nanomaterial was produced in each batch. Batch 1 was evaluated for how long it might be stored, and the experiments were conducted in July 2019, September 2019, and February 2022.

The stability of the nanomaterial after the pre-concentration procedure was evaluated using FT-IR.

It was evaluated the possible structural changes in the nanomaterial when subjected to arsenic extraction from the solution. To investigate this, it was dispersed 20 mg of nanoparticles into a solution (pH 4.0) containing 200 mg kg^{-1} of As. The solution was mechanically shaken for 15 min, and then the solution was removed. The nanomaterial was taken then to lyophilization to constant weight. The dried solid residue was analyzed by Fourier-transform infrared spectroscopy (FT-IR) and X-ray photoelectron spectroscopy (XPS).

2.6 Sample preparation

2.6.1 Total decomposition

Rice samples were decomposed by wet digestion using a block digester. Approximately 0.250 g of sample was weighed into Teflon® tubes and 5 mL of 65% w w^{-1} HNO_3 was added. The tubes were placed in the block digester for 3h at 120 °C. After the tubes had cooled to room temperature, the samples were diluted by 25 mL with ultrapure water. Total As was analyzed in these sample solutions using ICP OES. These sample solutions were also subjected to iAs pre-concentration using Fe3O4@DTPMP nanoparticles, and As was then analyzed by ICP OES.

2.6.2 Arsenic species extraction

For As species extraction from rice samples, the modified procedure proposed by Pétursdóttir et al., 2014, was used. Approximately 0.500 g of sample was weighed into a Teflon® tube with a cap, and 10 mL of 2% v v^{-1} HNO_3 was added to the sample. The tubes were then capped and placed in the block digester for 1h at 90 °C. After that, the vials were cooled to room temperature, and samples were diluted to 40 mL with ultrapure water and

filtered using a quantitative filter (15 μm). The extract (extract 1B) was subjected to MSPE iAs extraction and the solid residue was oven dried at 60 °C to constant weight. The solid extraction residue was digested by applying the procedure described in section 2.6.1. The As from the solution resulting from the solid residue digestion was pre-concentrated by applying MSPE for As analysis by ICP OES and mass balance calculations.

2.6.3 MSPE extraction/pre-concentration for iAs analyses

The variable used in the system was optimized using chemometric tools. The response surface is found in the supplementary material (S2) showing that the method is not lacking in fit and the optimal point is chosen by the signal derivate from the surfaces.

To extract and pre-concentrate iAs, a 40 mL aliquot of extract 1B was added into Falcon® tubes as 4 mL of Britton-Robinson buffer solution (pH 4.0). The mixture was diluted to 50 mL with ultrapure water. Approximately 20 mg of Fe_3O_4@DTPMP were dispersed in the solution and mechanically shaken for 15 min. Then, the nanoparticles were magnetically separated and the supernatant (supernatant 1C) was reserved for total decomposition. Two milliliters of 10% v v^{-1} HNO_3 were added to the vial containing the nanoparticles to elute the iAs. The mixture was shaken using a vortex for 3 min and the nanoparticles were again separated by magnetic action. The As content of the resulting solution (solution 1C) was analyzed by ICP OES.

Supernatant 1C was decomposed in a block digester using 65% w w^{-1} HNO_3 to convert the extracted species to the inorganic form. The procedure used was the same for preparing rice samples for tAs analysis (2.6.1). These samples were subjected to the same iAs pre-concentration procedure using Fe_3O_4@DTPMP nanoparticles, and the As content analyzed by ICP OES to obtain the mass balance of As.

The sample preparation methods are schematically summarized in the supplementary material (S3).

2.7 Quality control

The accuracy of tAs concentration in rice samples was assessed by NIST 1568b certified reference material (Rice flour) analysis and CRM-AGRO Ar-01/2015. For the accuracy test of iAs, only NIST 1568b was used. The As (III) standard addition/recovery test was also performed to check any analyte losses. The spike was performed prior to decomposition/extraction in three levels (0.03, 0.10, and 0.15 mg kg^{-1}).

The certified reference material sample (NIST 1568b), the reference material (Ar_01/2015), and the rice flour sample (BR) were subjected to LC-ICP-MS analysis for comparison of results.

The limits of detection (LOD) and quantification (LOQ) were calculated following the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) using the background emission correction (BEC) as shown in the equations below:

$$LOD = 3 \times RSD \times BEC$$

$$LOQ = 10 \times RSD \times BEC$$

$$BEC = I_{blanck} / s$$

where RSD is the relative standard deviation of 10 independent blanks, Iblanck is the intensity of emission of the blank sample and s is the slope of the analytical curve without MSPE treatment. The blank solution was prepared using 2% w w^{-1} HNO_3 solution, subjected to the same MSPE procedure applied for the rice samples.

3. Results and discussion

3.1 Fe_3O_4 nanoparticles functionalization test for iAs pre-concentration

To verify the need for functionalization of Fe_3O_4 nanoparticles for iAs pre-concentration, a comparison was performed between iAs pre-concentration with functionalized magnetic nanoparticles (Fe_3O_4@DTPMP) and non-functionalized

nanoparticles (Fe_3O_4). The As analysis were performed by ICP OES. In addition to As, the Fe concentrations in the solution were also determined to obtain information regarding the leaching of Fe from the nanomaterial into the sample solution (Table 1). The iAs concentration of the standard solution was 0.150 mg L^{-1}. Since for this test the pre-concentration factor was 20 (from 40 mL of sample 2 mL of extract was obtained), the maximum concentration to be found would be 3.0 mg L^{-1}, assuming a quantitative pre-concentration. The two nanomaterials produced recoveries greater than 85%, which may be considered quantitative recoveries (Table 1).

Table 1: Quantification of As and Fe after iAs preconcentration from a 0.150 mg L^{-1} iAs standard solution using Fe_3O_4 ¬and Fe_3O_4@DTPMP as solid phase. (Mean ± SD, n=3)

Samples	**As (mg L^{-1})**	**% Recovery**	**Fe (mg L^{-1})**
Fe_3O_4	2.67 ± 0.04	89	5.68 ± 0.04
Fe_3O_4@DTPMP	2.98 ± 0.05	100	0.16 ± 0.02

The use of Fe magnetic nanomaterials to remove As from water samples has been widely used in the literature (Wong et al., 2017), as well as the application of this type of nanomaterial for As species extraction (Montoro Leal et al., 2018; Pandey et al., 2021; Xu et al., 2017). However, most of these methods are only applicable to the water sample matrices.

The concentrations of Fe in solution when using the non-functionalized nanoparticle are significantly higher compared to the functionalized nanomaterial. The release of Fe from the non-functionalized nanoparticles into the sample solution may cause some quantification difficulties, especially when using more alkaline media due to Fe precipitation. Furthermore, probably the Fe_3O_4 nanoparticles could not be reused many times due to the loss

of Fe in the solution, which represents an increase in the cost of the analysis.

The nanomaterial before and after pre-concentration was subjected to FT-IR to evaluate its stability after the procedure. These spectra were shown in the supplementary material (S4). It can be seen that there are no significant changes in the structure of the nanomaterial after the MSPE procedure, which could indicate the stability of the nanomaterial.

Three different batches of Fe_3O_4@DTPMP produced at different times were used for iAs pre-concentration following the exact same procedure to investigate whether the reproducibility of nanoparticle synthesis could interfere with iAs pre-concentration performance. Batch 1 was analyzed at three different time slots to check

the lifetime of the nanoparticles. Table 2 displays the result of iAs recovery for pre-concentration using three different batches of Fe_3O_4@DTPMP.

Table 2: Recovery values for iAs preconcentration of 0.150 mg L^{-1} iAs standard solution using three different batches of Fe_3O_4@DTPMP nanoparticle synthesis. (Mean ± SD, n=3)

Fe_3O_4@DTPMP batches	**iAs (mg L^{-1})***	**% Recovery**
1 (July 2019)	2.98 ± 0.05	99
1 (September 2019)	3.02 ± 0.02	101
1 (February 2021)	3.05 ± 0.06	102
2 (March 2020)	2.89 ± 0.04	96
3 (September 2021)	2.95 ± 0,08	98

*Enrichment factor = 20 times

The same batch of nanomaterials (batch 1) was used for iAs pre-concentration at different time periods, and the results did not show a significant variable for iAs concentration recovery. This demonstrates that the same batch of nanoparticle synthesis might be used for a long time – in the case of the current study, up to 3 years. Additional experiments are needed to see this long-term stability.

It can be observed in Table 2 that there was no statistically different (two-way t-test, $p < 0.05$) in the recovery of iAs among nanomaterial batches, which shows that the Fe_3O_4@DTPMP synthetic route used is reproducible and no brings significant differences for its use as a solid phase for the procedure described in the manuscript.

In an attempt to evaluate the possible adsorption mechanism, XPS and FT-IR analyses of As adsorbed in nanomaterials were analyzed. These data are presented in Figure 1.

The dry nanomaterial (Fe_3O_4@DTPMP) subjected to the MSPE procedure with and without iAs was analyzed by FT-IR. Additionally, the material with iAs was also taken for XPS analysis, the result was compared with XPS of Fe_3O_4@DTPMP without arsenic provided by Neto et al., 2021.

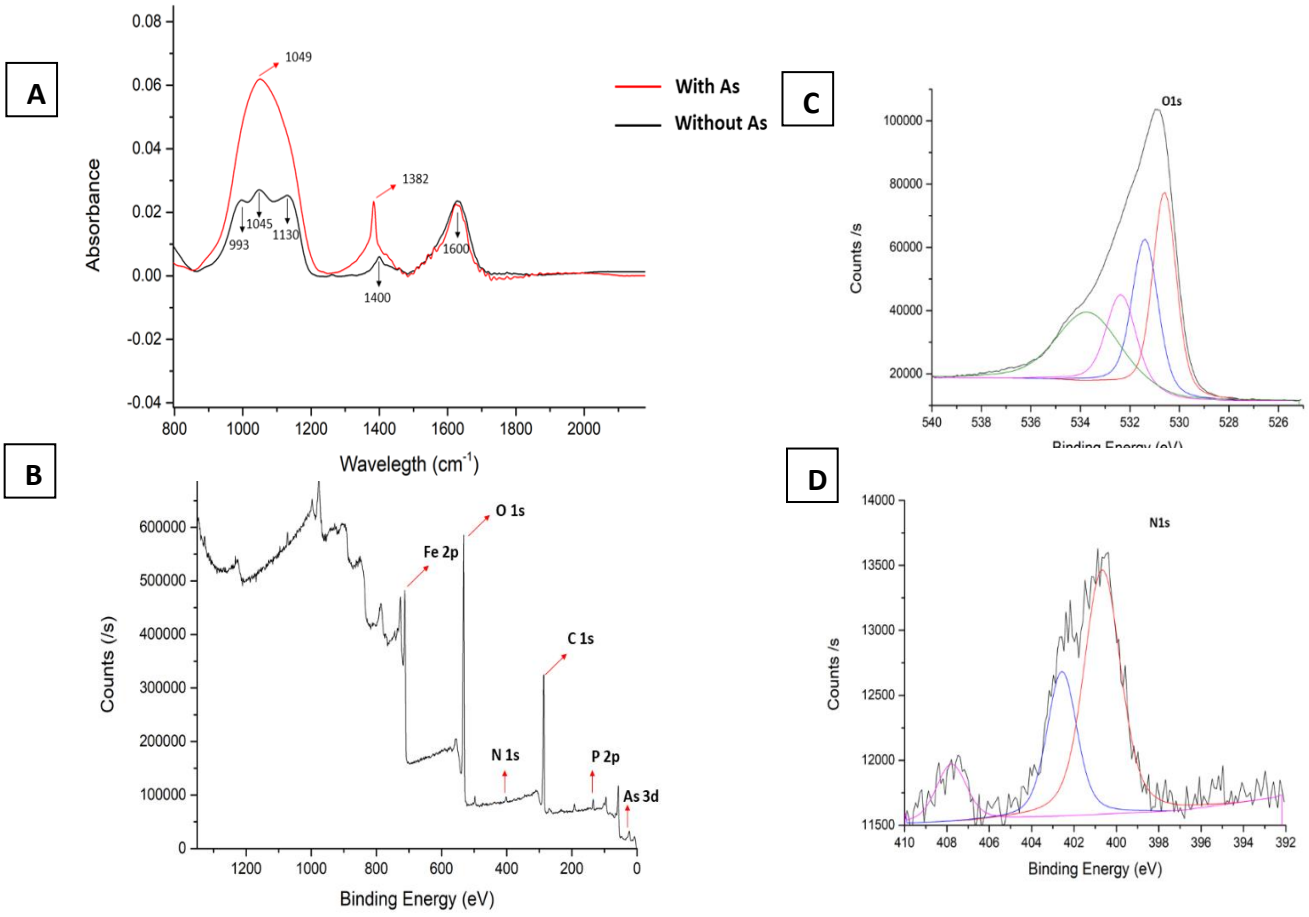
In Figure 1A, the region between 800 and 2000 cm^{-1} was used because of the differences found in this area. For material without As, it was possible to distinguish 3 bands related to phosphate bonds at 993, 1045, and 1130 cm^{-1}, in addition to a band at 1400 cm^{-1} related to the CN bond vibration. There is a noticeable difference when the material is used in a solution containing arsenic. In this case, only a band at about 1049 cm^{-1} is observed, besides the shift of the CN band to 1382 cm^{-1}, which evidences the presence of iAs on the nanomaterial surface.

Ilczyszyo et al., 1992, has studied the infrared spectra of urea crystals (H_2N-CO-NH_2) with arsenic acid. In this experiment, a band at around 1382 cm^{-1} was identified as

related to the CN bond. Probably, this shift may occur due to the influence of As on the nitrogen bond. This behavior is similar to that in the present study.

X-ray photoelectron spectrometry (XPS) was used to observe the bonds found in the nanomaterial. The complete spectra for nanomaterial (1B) and the high-resolution O1s (1C) and N1s (1D) deconvoluted spectra are displayed in Figure 1.

Figure 1: (A) FT-IR for Fe_3O_4@DTPMP before and after iAs extraction, (B) XPS spectra and deconvoluted spectra of O1s (C) and N1s (D)



It was possible to observe in the scanning spectra that there is arsenic adsorbed on the nanoparticle surface. Furthermore, it was possible to identify the presence of C, Fe, N, O, and P, which are constituents of the functionalized nanomaterial (Fe_3O_4@DTPMP). The spectra were deconvoluted, and it was possible to identify four components for C 1s, five doublets for Fe, three components for N, four components for O, and two components for P.

These spectra are available in the supplementary material (S5).

Figures 1C and 1D display the deconvolution for spectra related to O1s and N1s when compared to XPS of nanomaterial without As. Andrade-Neto et al., 2021, identify three components for O1s at 530.08, 531.29, and 533.94 eV for Fe3O4@DTPMP nanoparticles. In the XPS of nanoparticles with adsorbed As (Figure 1C), there is a new component centered at 532.09 eV. Lee et al., 2021, produced an iron-based nanotube with nitrogen mediation for removing As in water samples. The author identifies a peak centered around 531.7 eV related to the As-O bond, similar to the new peak found in the present study.

Deconvoluted nitrogen spectra in nanomaterial without As show two components centered at 399.7 and 401.7 eV. However, the material with adsorbed As shows a new peak centered at 407.8 eV, which is related to NO_3^- (Beard, 1990). These bonds in Fe3O4@DTPMP are consistent with an R_3-N^+H-Fe formation, which demonstrates that the N atoms of DTPMP are bound to the Fe of magnetite. This is important to explain As extraction due to aqueous solution, as it is found in the anionic form, in this case, H2AsO4-.

Further experiments should be performed to clarify the whole mechanism. Probably, the O atom of the arsenic anion is binding to the N of DTPMP, which explains the change in FT-IR and the formation of a high-energy N bond in XPS. Nonetheless, it can be stated that As is removed from the solution when this nanomaterial is used, probably in pH-dependent region (protonated N).

3.2 Total arsenic in rice samples

Rice samples were digested, and tAs level was analyzed by ICP OES. Solutions of digested samples were also subjected to MSPE iAs pre-concentration using Fe_3O_4@DTPMP to evaluate the ability of the solid phase to increase the sensitivity of tAs analysis in rice samples. To verify the accuracy of tAs analyses, we used SRM NIST 1568b (rice flour) and reference material CRM-Agro Ar_01/2015 (USP-EMBRAPA). Table 3 displays the results of

tAs analyses on rice samples.

Table 3: Determination of As in rice samples by ICP OES after only wet digestion and associated with MSPE using Fe_3O_4@DTPMP nanoparticle. (Mean ± CI, n=3, 95% of confidence)

Sample	**tAs (mg kg^{-1}) Without MSPE**	**tAs (mg kg^{-1}) With MSPE**	**ertified Value* (mg kg^{-1})**	**% Recovery**
Rice 1 (SA)	< 0.53	0.295 ± 0.048	-	-
Rice 2 (SA)	< 0.53	0.268 ± 0.056	-	-
Rice 3 (SA)	< 0.53	0.245 ± 0.065	-	-
Rice 4 (BR)	< 0.53	0.199 ± 0.086	-	-
ice Flour (BR)	< 0.53	0.090 ± 0.017	-	-
CRM-AGRO r_01/2015 (BR)	< 0.53	0.110 ± 0.086	0.112 ± 0.015	98
NIST 1568b	< 0.53	0.276 ± 0.086	0.285 ± 0.014	97

* Mean ± U, k =2, n=3. SA = Saudi Arabia; BR = Brazil

It was observed that without magnetic solid phase extraction, it was not possible to determine the As content in any rice sample by ICP OES since the As content in these samples are under the limit of quantification (LOQ) of the technique (0.53 mg kg^{-1}). The As content in these samples is too low for ICP OES quantification, and the sample preparation requires excessive dilutions to decompose the samples and obtain a solution with residual acidity compatible with the analytical technique. Nevertheless, samples submitted to the

MSPE process using Fe3O4@DTPMP nanoparticles showed satisfactory As levels with adequate precision (RSD less than 15% for all samples), even at low concentrations. These results show that the pre-concentration procedure improves the sensitivity of the method, enabling the As analyses in rice by ICP OES.

The analysis of the reference material and certified reference material showed recoveries of 98 and 97%, respectively. Furthermore, these analyses showed statistically similar values to the reference and certified value by Student's t-test with 95% confidence interval.

The rice matrix is composed mainly of carbohydrates (Barnet et al., 2021) and the As species found are mainly simple organic As compounds such as MMA and DMA, as well as inorganic arsenic (Wang et al., 2015). Silva et al., 2021 applied the same pre-concentration procedure and nanoparticles for iAs analysis in shrimp samples. However, in this case, it would not be possible to use the nanoparticles for tAs analysis after sample digestion due to the high thermostability of arsenobetaine, the predominant species in shrimp. This As species does not decompose in the inorganic form, even applying temperatures higher than 300 °C (Šlejkovec et al., 2001). The data presented in the manuscript proved that, unlike in the case of shrimp, it is possible to use nanomaterial to pre-concentrate tAs in rice for ICP OES analysis.

3.3 Inorganic arsenic in rice samples

The As species were extracted from rice samples in a dilute acid medium, and the extract was subjected to the MSPE procedure using Fe_3O_4@DTPMP nanoparticles for iAs extraction/pre-concentration. It is expected that extraction 1B is selective for iAs. Supernatant 1C, the residual solution from the MSPE procedure, was subjected to complete decomposition in a block digester to convert the organic As species to the inorganic As form. After that, the MSPE procedure was applied to the digested solution, and the As was analyzed by ICP OES

for mass balance calculations. The results of iAs analysis in rice and mass balance are shown in Table 4.

Table 4: Inorganic arsenic content in rice samples after preconcentration by MSPE using Fe_3O_4@DTPMP as solid phase. (Mean ± SD, n=3)

Sample	iAs in extract 1B (mg kg^{-1})	As content in residual solution (supernatant 1C) (mg kg^{-1}) (solid residue)	Arsenic Mass Balance ¥*(% Recovery)	Certified Value (mg kg^{-1})	% Recovery
Rice 1 (SA)	0.108 ± 0.005	0.180 ± 0.004	0.288 ± 0.005 (98)	-	-
Rice 2 (SA)	0.100 ± 0.004	0.167 ± 0.005	0.267 ± 0.005 (100)	-	-
Rice 3 (SA)	0.109 ± 0.009	0.132 ± 0.008	0.241 ± 0.009 (98)	-	-
Rice 4 (BR)	0.055 ± 0.004	0.143 ± 0.002	0.198 ± 0.004 (99)	-	-

Rice Flour (BR)	0.058 ± 0.002	0.032 ± 0.002	0.090 ± 0.002 (100)	-	-
CRM-AGRO Ar_01/2015 (BR)	0.059 ± 0.003	0.048 ± 0.002	0.117 ± 0.004 (106)	-	-
NIST 1568b	0.093 ± 0.016	0.178 ± 0.018	0.271 ± 0.082 (98)	0.092 ± 0.010**	101

[¥] Representing the sum of iAs and As in residual solution

*Propagation of error ($\sqrt{e_1^2 + e_2^2 + \ldots + e_n^2}$)

** Mean ± U, k =2, n=3

The certified reference material analysis showed good recovery for iAs (101%), and the resulting iAs concentration agreed statistically with the certified value using Student's t-test with a 95% confidence interval. The results presented in Table 4 reveal that there is still As in the supernatant of the MSPE iAs extraction (supernatant 1C), which represents the As species that were not adsorbed by the nanoparticles, such as MMA and DMA. This result is an important indicator of the selectivity of the nanomaterial used in the present study for the pre-concentration of iAs species. After the decomposition of supernatant 1C, iAs extraction/pre-concentration using Fe_3O_4@DTPMP nanoparticles was possible because this procedure changed all organic species from As to iAs. This change occurred due to oxidation of the sample with concentrated nitric acid since the decomposition temperature is sufficient to decompose the carbohydrate, the main component in rice samples (Krug, 2019).

The arsenic mass balance showed satisfactory recoveries of As content in rice samples (98-110%). This result demonstrated that in addition to iAs analysis, it is also possible to apply MSPE to tAs-in-rice determination by ICP OES using Fe3O4@DTPMP nanoparticles. The solid residues from the As species extractions were also decomposed. However, the arsenic content was found to be below the LOQ, which indicates that all arsenic species were extracted in an acidic medium from the rice sample since they are water-soluble.

To verify the accuracy of the test, analysis using LC-ICP-MS was performed, as well as the spike of three levels in the samples. These results are shown in Table 5.

		Spike						
		0.03 (mg kg^{-1})		0.10 (mg kg^{-1})		0.15 (mg kg^{-1})		LC-ICP-MS (mg kg^{-1})
Sample			Recovery (%)		Recovery (%)		Recovery (%)	
Ar_01/2015	0.059 ± 0.016	0.091 ± 0.020	107	0.160 ± 0.014	101	0.198 ± 0.023	93	0.059 ± 0.022
NIST 1568b	0.093 ± 0.003	0.120 ±0.012	90	0.194 ± 0.013	101	0.240 ± 0.026	98	0.095 ± 0.008

Rice Flour (BR)	0.058 ± 0.002	0.089 ± 0.010	103	0.159 ± 0.017	92	0.210 ± 0.023	101	0.057 ± 0.004	Table 5:

Spike /recovery in NIST 1568b, Ar_01/2015 after preconcentration by MSPE using Fe_3O_4@DTPMP as solid phase and LC-ICP-MS quantification. (Mean ± SD, n=3)

The LC-ICP-MS data show that the method is statistically similar to the method proposed in this paper, as well as the standard recoveries show the recoverability at the established levels, showing the accuracy of the method. The tAs concentration in all rice samples (Table 4) is below the recommended by Brazilian legislation (0.300 mg kg^{-1} tAs). However, when considering the confidence interval, only two samples (4 and 5) are in accordance with the legislation (Brazil, Anvisa, N° 88, DE 26 DE MARÇO DE 2021, 2021)). Likewise, the samples have iAs content below the European and FAO recommendations (0.200 mg kg^{-1} iAs) (The European Commission, 2015). However, according to FAO recommendations, none of these samples could be used as infant formulations, except sample 4. Furthermore, European legislation established 0.100 mg kg^{-1} as the maximum limit of tAs for this type of sample (The European Commission, 2015). Nonetheless, the tAs content in the samples complies with the Brazilian limits (0.150 mg kg^{-1} of tAs) (Brazil, Anvisa, N° 88, DE 26 DE MARÇO DE 2021, 2021).

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In rice samples, the tAs and iAs ratio is between 36-44%. These values are below rice samples from Aberdeen, UK (26-85% iAs) (Bralatei et al., 2015). Chen et al., 2018 analyzed rice samples from China and found a ratio between 50-61% iAs.

In the rice flour sample, more than half of the As concentration consists of iAs (~67%). The values found in this work, as well as the percentage of iAs in rice flour, agree with the findings of found by dos Santos et al., 2017, 54.3 ± 1.4 µg kg^{-1} iAs, 60% iAs in parboiled rice flour samples.

3.4 Evaluation of LOD and LOQ for the proposed method

For the proposed methodology, the calculated LOD and LOQ are 1.08 and 3.60 µg kg^{-1} As, respectively, assuming a 20-fold enrichment factor for iAs and the mass of the sample used in the decomposition/extraction. These LOD and LOQ values are low due to the high

pre-concentration factor (around 20 times) achieved with Fe_3O_4@DTPMP, which occurs because nanomaterials present a high superficial area. Further, the easy separation of the nanoparticles from the sample matrix using a magnet also reduces sample handling (Corps Ricardo et al., 2020)

The literature reports LOD of 10 ng L_{-1} for As analysis using Fe_3O_4 nanoparticles and quantification by electrothermal atomic absorption spectrometry (ETAAS) (Pourghazi et al., 2015). This value is lower than the one found in this work, but the pre-concentration factor for ETAAS is about 200-fold since this technique does not require nebulization steps for sample introduction. Barnet et al., 2021 recently found LOD and LOQ values of 1.87 and 5.85 µg L^{-1} for As(III), and 3.0 and 9.10 µg L^{-1} for As(V), respectively. The method used by these authors uses LC-ICP-MS, a more sensitive technique than ICP OES. However, due to the chromatographic step, a decrease in sensitivity was observed.

The proposed method shows lower LOD and LOQ when comparable to chromatographic methods, and features a pre-concentration factor suitable for ICP OES analysis. Further, this method decreases the cost of analysis due to the use of a less expensive detection technique and eliminates chromatographic steps that represent high instrumentation costs and the requirement of high-purity solvents.

Non-chromatographic methods are becoming an important alternative for trace analysis and speciation. Hydride generation (HG) has also been employed as an alternative to chromatographic methods for iAs speciation. It is possible to produce selective hydride for the determination of inorganic and methylated species as MMA e DMA (dos Santos et al., 2017; Gonzalvez et al., 2010; Karadjova et al., 2005). This technique achieves detection limits in µg kg^{-1} with good accuracy based on the addition of spikes and recovery from certified reference materials (Welna & Pohl, 2017). However, HG requires the use of expensive and unstable reagents such as $NaBH_4$. In addition, HG requires the use of very concentrated acid solutions

at a rate of 4 mL min^{-1} (dos Santos et al., 2017) in analysis systems that produce a large volume of residue.

The present method achieves LOD and LOQ comparable to those found in the HG method and has the advantage of not requiring the use of expensive and unstable reagents, allowing less waste to be disposed of.

4. Conclusion

The feasibility of Fe_3O_4@DTPMP nanoparticles as MSPE sorbent for iAs pre-concentration was proven in this work, allowing the quantification of tAs and iAs in rice by ICP OES. The functionalization of the nanoparticles was critical to protect the Fe from leaching into the sample solution. Furthermore, different synthesis batches of Fe_3O_4@DTPMP nanoparticles were evaluated, and no difference was observed, which indicates the reproducibility of the nanoparticle synthesis method. Since this nanomaterial has a lifetime of at least three years, longer lifetimes need to be studied. The presence of As in rice samples complies with the Brazilian legislation, but not with the European regulation for infant products. The proposed method has low LOD and LOQ suitable for regulatory analysis, achieve satisfactory precision and accuracy, and is low cost.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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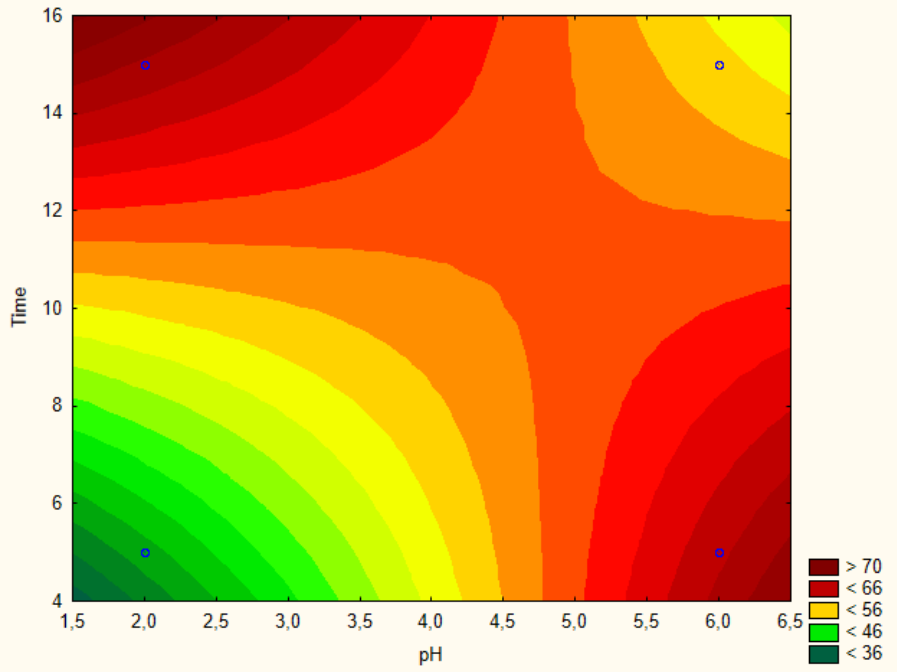
Supplementary Material

S1: Instrumental parameters used in LC-ICP-MS

ICP-MS settings		
RF power (W)	1600	
Carrier gas flow (L min^{-1})	1.18	
Plasma gas flow (L min^{-1})	15	
Nebuliser	Cross Flow	
Auxiliary gas flow (L min^{-1})	1	
Spray chamber	Double-pass	
Interface cones	Nickel	
Lens voltage (V)	3.5–4.0	
Mass resolution (u)	0.8	
Integration time (ms)	1000	
HPLC settings		
Flow rate (mL min^{-1})	1.0	
Injection volume (µL)	20	
Elution gradient:	Anion exchange chromatography	
Time (min)	Water (%)	$(NH_4)_2CO_3$ buffer (%)
2.00	99.90	0.10
15.00	95.00	5.00
30.00	75.00	25.00

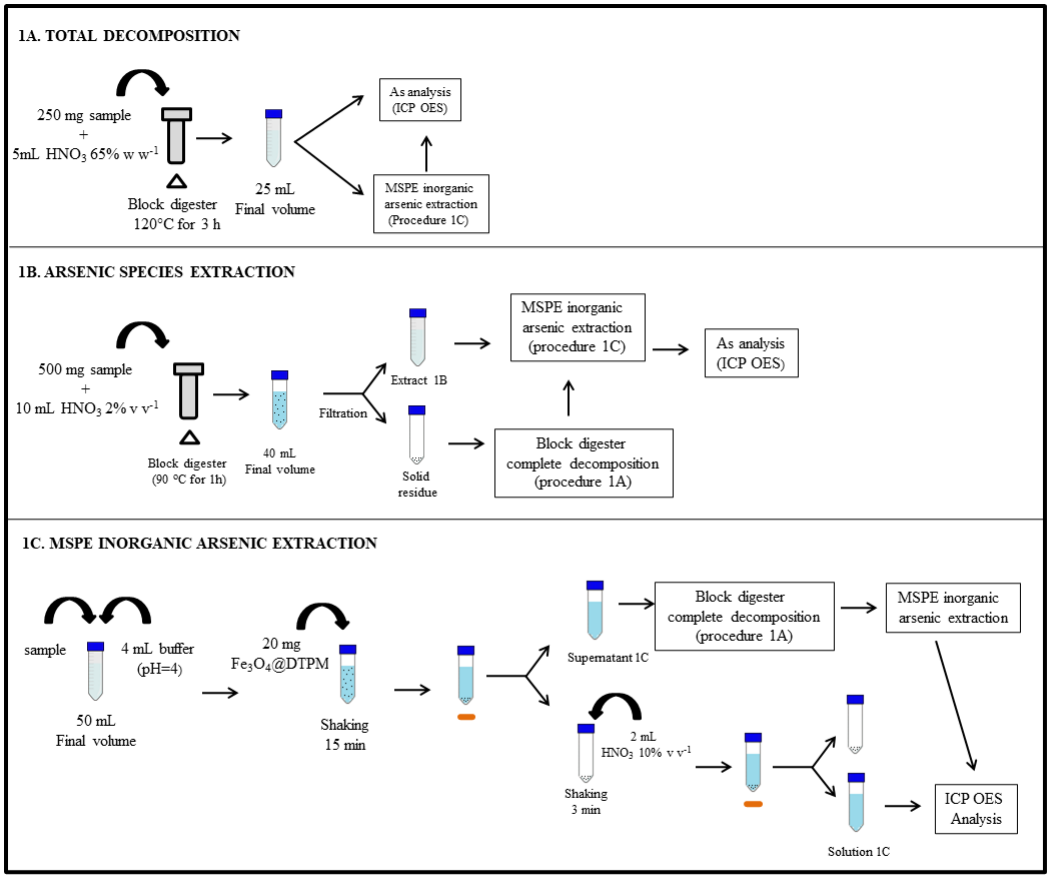
35.00	75.00	25.00
35.01	99.90	0.10

S2: Response surface for full factorial design using %recovery of As as response. (pH (2-8); Time of extraction (5-15 min); Mass of nanomaterial (10-30 mg).

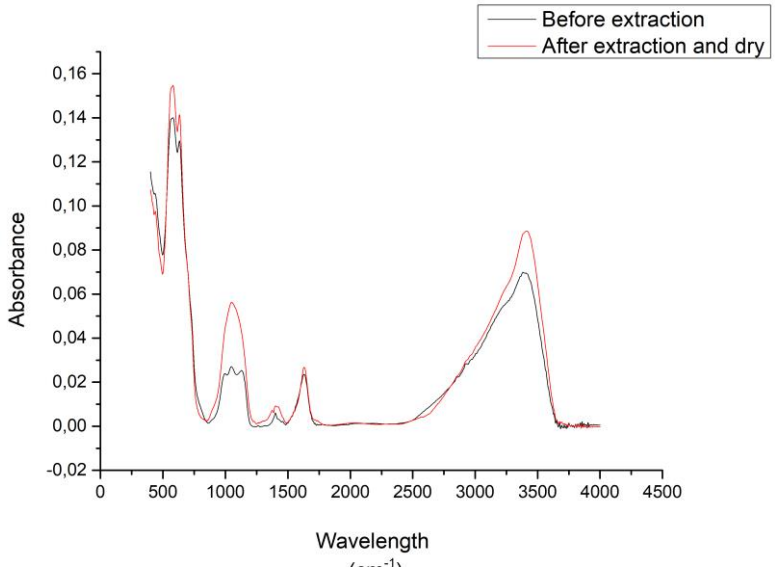


Mass of nanomaterial (30mg)

S3: Scheme of sample preparation procedures

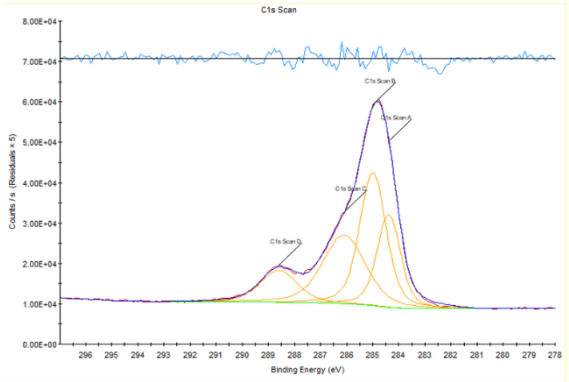


S4: FT-IR before and after MSPE procedure

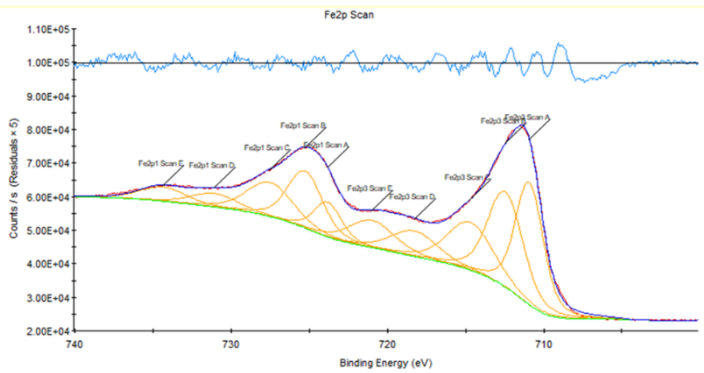


S5: Deconvoluted spectra from (A) C1s; (B) Fe2p; (C) P2p.

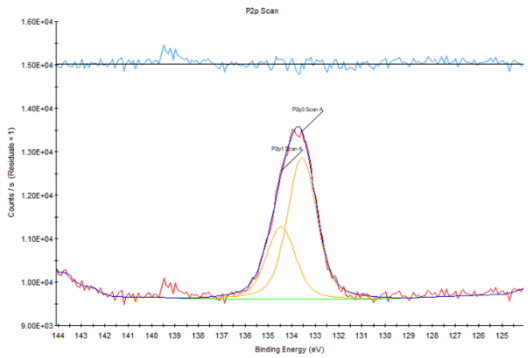
A



B

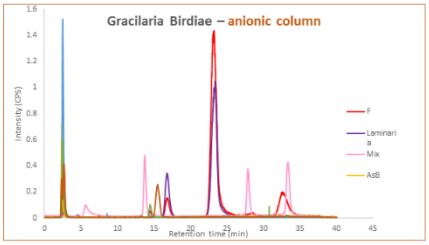


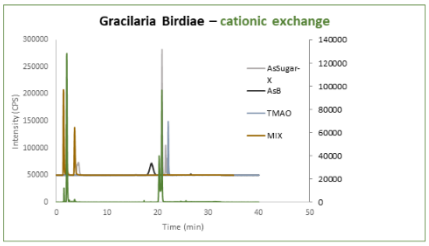
C



Chapter 3

Strategies for arsenic speciation in seaweed from Brazilian northwest using ICP OES





ABSTRACT

Seaweed is known as primary producer in their ecosystems and have capability to bioaccumulate trace elements, especially arsenic (As). Algae can biotransform As forming several arsenocompounds, as arsenosugar that is precursor of arsenobetaine. Due to the increase in seaweed consume for food and cosmetic purpose, As analysis is required. There is a lack in the literature about the As content and As speciation in algae from Brazilian Northest, the mainly producer in country. In this work, a procedure using iron-based nanoparticles was used for iAs speciation in algae sample and HPLC-ICP-MS was used for total As speciation analysis. The content of iAs found was 0.383 μg kg^{-1} and 0.332 μg kg^{-1} for Gracilaria and Solifformis algae samples. The HPLC-ICP-MS show that the major species found in the samples studied are arsenosugar (44%). The high recovery of chromatographic step shows the high content of water-soluble species in algae. However, a more detailed study with a greater variety of species of algae and taking into account seasonality should have been carried out to achieve a more faithful characterization of As content of the algae from Brazilian Northest. Though these preliminary results demonstrated the presence of iAs and arsenosugar in samples that are As compounds that required attention related to food security.

KEYWORDS: Seaweed, Arsenic Speciation, MSPE, HPLC-ICP-MS, ICP OES.

1. INTRODUCTION

In recent years, the consumption of algae has increased mainly for culinary purposes [1,2] but also for pharmaceutical and cosmetic finality due to its therapeutic properties [3-6].

Brazil has few commercial-scale in operation with traditional coastal communities producers in the Northeast region (Rio Grande do Norte and Ceará states) [7]. Gracilaria, Porphyra, and Hypnea are the most produced algae species in aquaculture systems (30 tons in 2016) in this Brazilian region [8]. There are no data in literature about trace elements in algae from Brazilian Northeast.

Marine algae are considered primary producers and have the capability of bioaccumulate inorganic elements, especially, arsenic (As) from seawater. They can preconcentrate 1000 times higher As than other organisms [9]. Algae are able to biotransform As, so several organic and inorganic As forms can be found in these matrices. However, the predominant As species in algae is arsenosugars, which normally represents >80% of total As. Arsenosugars are considered to have low toxicity [10]

The pathway of As biotransformation in algae begins with the absorption of As(V) through phosphate transporter channels, then it is reduced to As (III) and methylated to organic As forms, so they are excreted [11]. The uptake mechanisms of As remain unclear yet. There is evidence that this element uses the same mechanisms as phosphate inside and outside the cells mostly dependent of the phosphor concentration outside cells [12]. However, many studies show independence between phosphate and arsenate uptake, suggesting the existence of other uptake mechanisms for As in algae [13]. There are evidence that As(III) is absorbed across the plasma membrane with the aid of aquaglyceroporins and hexose permeases enzymes [14].

The As speciation analysis in algae and, algae-based products is required to ensure consumer safety. This subject is still scarce in the literature and little attention has been given

to the possibility of As consumption through algae and derivatives. For this reason, there are few laws that include limits for the As content in this matrix, still considered exotic. Brazilian and European legislation did not allow As content in seaweeds. In the past, Brazilian legislation considered seaweed as exotic food and limited the As content to 1.0 mg kg^{-1}, but recently, this recommendation was revoked[15]. In Europe, there are limits to the use of seaweed when it is intended for animal feed (0.200 mg kg^{-1}) [16]. France limit the tAs content in algae to 3.0 mg kg^{-1} [17], Oceania Australia and New Zealand limits to 1.0 mg kg^{-1} [18] and Hong Kong limits to 1.4 mg kg^{-1} [19].

High concentrations of As have been reported in the literature for several algae species with levels between 1 to 150 mg kg^{-1} iAs, representing about 0.4 to 99.9% of tAs in European species [1]. Concentrations higher could be observed in seaweed submitted to As contamination [14] with a content of 109.000 mg kg^{-1} in a Dunaliella salina species [13]. In samples available in USA, tAs content was found between 3 and 105 mg kg^{-1} with iAs fraction lower than 0.350 mg kg^{-1} for the most samples[20]. In Brazil, there are few studies of iAs content in algae samples, Coelho et al., 2016[21], found a concentration almost 20 times higher (tAs) than allowed by international law [17–19].

Different As species have different toxicities; therefore, the total content of this element does not always provide reliable analytical information about risk of toxicity. For this, it is necessary a study of As speciation in this kind of matrix. Feldmann & Krupp, 2011[10], proposed a strategy for As speciation in algae samples based in three groups: the inorganic As (iAs) forms due to the toxicity known; the arsenobetaine (AsB) form due to the non-toxicity; and the potentially toxic As forms, as arsenosugar, arsenolipids, and other organic species.

The speciation analysis in algae sample is not an easy task. The standard analytical methods for As speciation as EN 15517:2008 and GBIT 5009 11-2003 do not provide reliable results with biased quantifications when applied in algae samples [22].

Chromatographic methods are the most common for As speciation analysis in algae, especially HPLC-ICP-MS[9,20,23,24]. However, this method presents problems such as high cost, high waste generation and the need for highly trained analysts. In recent years, options for non-chromatographic speciation have been studied with a special focus on the use of nanomaterials[25–28]. Since the iAs form is generally the most toxic, the determination of this As form is enough to assess the safety of consumption[29] of algae and algae-based products.

Recently, a method for speciation of iAs in shrimp samples was proposed using a new magnetic material for extraction of iAs and analysis by ICP-MS without chromatographic step. This proposed method has accuracy similar to HPLC-ICP-MS with RSD less than 15% [29]. However, this extraction method was not applied to other types of matrices and it was not studied the possibility of using it with ICP-OES, a technique with a lower cost than the ICP-MS.

Thus, this research aims to study the feasibility of analyzing iAs in algae by ICP OES after extraction using Fe_3O_4@DTPMP. Furthermore, it is intended to characterize the As species in samples of algae from Northeastern Brazil, still without data in the literature.

2. EXPERIMENTAL

2.1 Samples

About 1kg of two species of wild red algae (*Gracilaria Birdiae* and *Solirae folliformis)* were collected in Flecheiras beach, Trairi, Ceará, Brazil (3.21668° S, 39.26728° O) in April 2019. The samples were stored in decontaminated airtight bags and carried to laboratory. All the samples were freeze-dried (Liobras L 108, São Carlos, SP, Brazil), ground using a coffee grinder fitted with stainless steel blades. The grinded samples were stored in bags at -20ºC

until the analysis. The samples were homogenized so that pooled samples were analyzed in triplicates for tAs and As speciation including iAs.

The European reference material CD-200 – Seaweed was used for accuracy tests.

2.2 Instrumentation

A CEM Mars 5 non-pressurized system was used for sample digestions before total As quantification. Arsenic analysis was carried out using an ICP-MS/MS (Agilent 8800 ICP Triple Quad, ICP-QQQ).

For liquid chromatographic separations an Agilent 1100 series quaternary HPLC pump, degasser and autosampler (Agilent Technologies, Waldbronn, Germany) were used. The outlet of the HPLC column was connected to the nebulizer of the ICP-MS instrument by a short length of polyethylene tubing. For anion and cation exchange chromatography, the HPLC columns Hamilton PRP X-100, 10 µm, 250 x 4.1 mm and Varian Ionosphere C (100 x 3 mm id), respectively, were used. The ICP-MS and HPLC settings are given in Table 1.

Table 1: Instrumental parameters used to total arsenic analysis by ICP-MS and arsenic speciation analysis by HPLC-ICP-MS.

ICP-MS/MS settings	
RF power (W)	1600
Carrier gas flow (L min^{-1})	1.18
Plasma gas flow (L min^{-1})	15
Nebuliser	Cross Flow
Auxiliary gas flow (L min^{-1})	1
Spray chamber	Double-pass
Interface cones	Nickel

Lens voltage (V)	3.5–4.0
Mass resolution (u)	0.8
Integration time (ms)	1000

HPLC settings		
Flow rate (mL min^{-1})	1.0	
Injection volume (µL)	20	
Elution gradient:	Anion exchange chromatography	
Time (min)	Water (%)	$(NH_4)_2CO_3$ buffer (%)
2.00	99.90	0.10
15.00	95.00	5.00
30.00	75.00	25.00
35.00	75.00	25.00
35.01	99.90	0.10
Elution gradient:	Cation exchange chromatography	
Time (min)	Water (%)	Pyridine buffer (%)
0.00	99.00	0.10
4.00	99.00	0.10
16.00	90.00	10.00
20.00	50.00	50.00
24.00	25.00	75.00
30.00	0.00	100.00
30.01	1.00	99.00

A Tecnal TE-007D block digester (Piracicaba, São Paulo, Brazil), equipped with 15

Teflon® tubes with lids, was used for sample decomposition and As species extraction procedures before MSPE extraction.

An Inductively coupled plasma optical emission spectrometer (ICP OES) dual view iCap 6000 (Thermo Scientific) was used for As quantification. The sample was introduced into the ICP using a concentric nebulizer and a cyclonic spray chamber. The ICP OES operating parameters were: 1.55 kW radio-frequency power; 12 L min^{-1} argon plasma flow rate; 1.00 L min^{-1} auxiliary argon flow rate; 1.02 L min^{-1} carrier argon flow rate; and 1.4 L min^{-1} sample flow rate. Arsenic was measured employing axial view and using the wavelengths of 193.7 nm.

A shaker table (Tecnal, Piracicaba, SP) was used to As species extraction procedure. A pH meter, calibrated with pH 7.0 and 4.0 standard solutions (Sigma Aldrich, Germany), was used to measure the pH of the solutions. A 20 ×14 × 4 cm NdFeB super magnet was used for magnetic nanoparticles separations.

2.3 Materials and Reagents

Solutions were prepared using ultrapure water with a resistivity of 18.2 MΩ cm (Smart2 Pure, Thermo Fisher Scientific, Loughborough, UK). All plasticware was immersed in 10% v v^{-1} nitric acid (HNO_3) for 24 h and thoroughly rinsed with ultrapure water. The digestion of the samples was accomplished using 70% w w^{-1} HNO_3, (p.a., Fisher Scientific, UK) and 30% w w^{-1} hydrogen peroxide (H_2O_2) (p.a., Fisher Scientific, UK). Standard calibration solutions were prepared using successive dilutions of 1000 mg L^{-1} As (Inorganic Venture, USA) stock solution with 1% v v^{-1} HNO_3. Rhodium (Rh) (Inorganic Venture, USA) was used as internal standard for ICP-MS measurements.

For quantification of As-species, sodium dimethylarsinic acid (DMA, Chemservice, USA) was used for calibration since this is stable and validated to work for all eluting As species using ICP-MS detection. Identification of As species was aided by the following

species standard solutions: sodium arsenite (As(III)) > 95% w w^{-1}, potassium arsenate monobasic (As(V)) >90% w w^{-1} (both Sigma Aldrich, Germany), disodium methylarsenate (MA) 98% w w^{-1}, sodium dimethylarsinic acid (DMA) trihydrate >98% w w^{-1}, trimethylarsine oxide (TMAO) (all Chemservice, USA), arsenobetaine (AsB) (BCR-626, Joint Research Centre, Belgium), arsenosugar-OH (a gift from K.A. Francesconi, Austria), and water extracts of the algae species *Fucus vesiculosus* L. (FV) and *Laminaria hyperborea* (LH) which provided an in-house standard for other arsenosugars compounds.

Ammonium carbonate (p.a., BDH, UK) and pyridine (masspec quality, Fluka, UK) were used to prepare the mobile phase for anion exchange chromatography and cation exchange chromatography, respectively. Formic acid (masspec quality, Fluka, UK) was used to adjust pH of the pyridine buffer.

A Britton-Robbinson buffer solution (pH 4.0) was prepared using an equimolar mixture of 65% w w^{-1} CH_3COOH (Neon, Suzano, SP, Brazil), 98% w w^{-1} H_3PO_4 (Sigma-Aldrich, Germany), and 35% w w^{-1} H_3BO_4 (Vetec, Rio de Janeiro, RJ, Brazil). The pH of buffer solution was adjusted using 0.1 mol L^{-1} NaOH (Vetec) and/or 0.1 mol L^{-1} HCl (Vetec).

For nanoparticle synthesis, ≥99% w w^{-1} $FeSO_4 \cdot 7H_2O$ (Sigma Aldrich, Germany), 98% w w^{-1} $FeCl_3 \cdot 6H_2O$ (Vetec), 50% w v^{-1} dimethyl triamine-pentamethylene phosphonic acid (DTPMP, $C_9H_{29}N_3O_{15}P_5$) (Sigma, Germany) and 27% w w^{-1} NH_4OH (Vetec) were used.

2.4 Quality controls

The accuracy of the tAs concentration results were verified by the analysis of European reference material (CD-200 - Seaweed) and the iAs mass fraction accuracy was verify using spike recovery tests.

2.5 Synthesis and characterization of iron-based nanomaterial

Iron nanoparticles were synthesized following the experimental procedure proposed by

Silva et al., 2021. About 7.5 mmol of $FeSO_4.7H_2O$ and 14 mmol of $FeCl_3.6H_2O$ were solubilized and carried to a Teflon® chamber. For the precipitation of nanoparticles, 20 mL of 27% w w^{-1} NH_4OH was added, and this mixture was mechanically shaken. After this, 1.2 mL of 50% w w^{-1} DTPMP was added in drops to the Fe nanoparticles solution, the chamber was sealed in an autoclave and kept at 150°C for 210 min. Fe_3O_4@DTPMP nanoparticles were purified by dialysis procedure in the Spectra/Por®6 equipment using 1 kDa membranes in deionized water. The solution was lyophilized and used in iAs speciation method.

2.6 Analytical method for total arsenic (tAs) analysis

For the digestion of algae samples, about 100 mg of the samples were weighed in 50 mL Falcon® vials and 2 mL of 70% w w^{-1} HNO_3 was added. The mixture was left overnight, then 2 mL 30% w w^{-1} H_2O_2 was added, and the samples were submitted to the following heating program using a microwave oven system (Mars-5, CEM instrument, U.K.): 2 min at 50 ºC and 800 W (2 min temperature ramp), 2 min at 75 ºC and 800 W (2 min temperature ramp), 5 min at 95 ºC and 800 W (5 min temperature ramp). The final volume was adjusted to 50.0 g with ultrapure water.

The total arsenic concentrations in the digested samples were determined by ICP-MS/MS, using hydrogen mode, m/z 75 was monitored and a solution of 10 µg L^{-1} Rh (m/z 103) in 1% v v^{-1} HNO_3 was used as internal standard for ICP-MS measurements. Quantification was carried out against standard solutions of sodium arsenate (Inorganic Venture, USA) in the calibration range of 0-100 µg L^{-1} in 1% v v^{-1} HNO_3.

For digester block sample preparation, around 200 mg of sample was weighted in Teflon® tubes and 5.0 mL of 65 % w w^{-1} HNO_3 was added. The mixture was carried to block digester for 3h at 120 ºC, after this 2.0 mL of H_2O_2 was added and the digestion continued for 1h at 120ºC. The final solution was diluted to 25 mL with ultrapure water and As quantified by ICP OES.

The accuracy of the method was evaluated for the analysis of the European Reference Material – CD 200 – Seaweed.

2.7 Extraction of water-soluble arsenic species

An amount of 100 mg of seaweed samples were mixed with 5 mL of ultrapure water to extract the water-soluble As species. The mixture was shaken overnight, and the supernatant was separated from the solid residue by centrifugation (10 min, 4000 rpm). An aliquot of 1.0 mL of ultrapure water was added in solid residue and shaken using vortex, the mixture was centrifuged (10 min, 4000 rpm) and the supernatant combined with the previous one. This procedure was repeated twice. Total As and As species were quantified in the water extract.

The arsenic concentrations in the extracts (iAs) and total digestion (tAs) were determined by ICP-MS/MS using hydrogen as the reaction gas, As was monitored at m/z 75 and Rh (m/z 103) was used as internal standard for ICP-MS measurements. Quantification was carried out against standard solutions of sodium arsenate in the calibration range of 0-100 $\mu g\ L^{-1}$.

In the ICP OES was quantified iAs after MSPE procedure, the quantification was carried out against standard solutions of As (Acros Organic) 1000 mg kg^{-1} and the calibration range of 1-30 mg L^{-1} was used after successive dilutions.

2.8 Quantification of iAs using Fe_3O_4@DTPMP

To extract and preconcentrate iAs, an aliquot of 40 mL of the extract was added into Falcon® tubes as 4 mL of Britton-Robbinson pH 4.0 buffer solution. Approximately 20 mg of Fe_3O_4@DTPMP was dispersed in the solution and mechanically stirred during 15 min. Then, the nanoparticles were magnetically separated, and the supernatant was discarded. Two milliliters of 10% v v^{-1} HNO_3 was added to the vial containing the nanoparticles in order to elute the analyte. The mixture was shaken using vortex for 3 min and the nanoparticles were separated again by magnetic action. The As content in the resulting solution was analyzed by

ICP OES. The accuracy of the procedure was verified using spike recovery test in three levels: 0.05, 0.10 and 0.150 mg L^{-1} of iAs (standard for ICP OES). Besides that, the content was compared with the result obtained by HPLC-ICP-MS method.

3. RESULTS AND DISCUSSION

3.1 Total decomposition of algae sample

Wet digestion methodologies using a microwave digestion oven (MW) and block digester (BD) were compared for algae decomposition.

The first methodologies use MW digestion and analysis by ICP-MS and it has their accuracy verified using European Reference Material (ERM) - CD 200 - Seaweed.

Decreasing the cost of analysis, the sample preparation of algae samples was carried out using BD prior to analysis by ICP OES. The results were compared using the t-Student's test with 95% of confidence ($p<0.05$). The results are shown in table 2

Table 2: Arsenic content (mg kg^{-1}) in algae sample using microwave oven digestion with quantification by ICP-MS/MS and block digester decomposition with quantification by ICP OES. (mean ± SD, n=3)

	Methods	
Sample	MW / ICP-MS	BD / ICP OES
	As (mg kg^{-1})	As (mg kg^{-1})
Gracilaria Birdiae	11 ± 1	10 ± 1
Solineria Folifformis	-	9 ± 1
ERM-CD-200	58 ± 1	-

The As content in ERM obtained when MW digestion was applied is statistically similar

to reference content (55± 4 mg kg^{-1}) with 95% of confidence.

Likewise, the As content in *Gracilaria Birdiae* seaweed sample obtained when digestion was performed by BD is statistically similar to that found when MW-digestion was used. So, it is possible to affirm that total As analysis could be performed using ICP OES after sample preparation by block digester. This methodology has an operation cost less expensive than the use of MW with ICP-MS.

There are few studies about trace elements in Brazilian algae[21,34], however, the mineral content in the seaweed of the species studied is still not known. Recent studies[34] report the content of As in algae from state of Bahia as 4.71 ± 0.25 mg kg^{-1} (*U. fasciata*), 10.90 ± 0.18 mg kg^{-1} (*C. corneus*), 82.71 ± 1.68 mg kg^{-1} (*S. vulgare*), besides that, the authors inform a difference in As content in algae collected in the rainy and dry season. Similar content of As was found in *Uva* sp. species (5.60 ± 0.25 mg kg^{-1}) from Rio Grande do Sul, South of Brazil[21].

In a recent review[35], the As content of algae from American countries was investigated. The results for Brazilian algae show As content around 55.03 mg kg^{-1} but with a great standard deviation (38.62) showing the heterogeneity of this sample.

G. Birdiae and *S. Folliformis* are red algae species, the content of As found is similar to other red algae samples from South Atlantic Ocean (9.10 ± 6.23 mg kg^{-1}). It is important to discuss that, As content in seaweed seems to be different according with the group being red algae with less As than brown and green algae.

There is no legislation in Brazil regarding the content of As in algae/seaweed, while France[17] (3 mg kg^{-1}), Australia and New Zeeland (1 mg kg^{-1})[18] and Hong Kong (1.4 mg kg^{-1}) [19] have limitations about this contaminant.

The water-soluble arsenic species were extracted following the procedure proposed by Matos et al, 2021[36] and total As analyzed by ICP-MS and ICP OES. The results are shown

in table 3.

Table 3: Water-soluble arsenic species mass fraction (mg kg^{-1}) quantified by ICP-MS and ICP OES. (mean ± SD, n=3)

	Methods	
Sample	ICP-MS (% extraction)	ICP OES (% extraction)
Graciliaria Birdiae	8.38 ± 0.64 (76)	7.98 ± 0.56 (80)
Solineria Folifformis	-	8.61 ± 0.44 (96)

The most common As compound in algae is arsenosugar[1,10], thus, it was expected that the most As in the samples could be extracted in a water fraction.

The results show again the accordance between the As quantification by ICP OES and ICP-MS with results statistically similar for the same sample (*Graciliaria Birdiae*).

For G. *Birdiae* almost 80% of As species are contained in water-soluble fraction, however, around 2.0 mg kg^{-1} may represent species not extracted with water. On the other hand, S. *Filliformis* have As mass fraction higher than 90% in the water extract.

3.2 Quantification of iAs using Fe_3O_4@DTPMP

Magnetic-solid extraction using Fe_3O_4@DTPMP was employed for the quantification of iAs in aqueous extract of seaweed samples by ICP OES. To verify the accuracy of the procedure for iAs, spike/recovery tests were performed on three levels. The results are summarized in table 4.

Table 4: Inorganic arsenic (iAs) mass fraction (mg kg^{-1}) quantified in algae samples by

ICP OES. (mean ± SD, n=3)

		Spike					
		0.05 (mg kg^{-1})		0.10 (mg kg^{-1})		0.15 (mg kg^{-1})	
Sample	**iAs mg kg^{-1}**		Recovery (%)		Recovery (%)		Recovery (%)
Graciliaria	0.383 ±	0.431 ±	99	0.476 ±	98	0.545 ±	104
Birdiae	0.011	0.022		0.023		0.024	
Solineria	0.332 ±	0.479 ±	98	0.434 ±	101	0.578 ±	99
Folifformis	0.014	0.018		0.022		0.027	

The iAs content in samples represents 1.83% and 1.47% of total As for G. Birdiae and S. Folifformis, respectively. In algae samples commercially available in the USA, the content of iAs is found in a range between 0.1 to 59% of tAs depending on the origin and specie of the seaweed. This data shows the importance of analysis of iAs in seaweed species around the world.

The spike/recovery test shows the good accuracy of the developed method with analytical recoveries (98 - 104%) at all levels.

This method is a low-cost alternative for iAs speciation. The information of this As species gives an important information about food security and possible contamination.

3.3 Arsenic speciation using HPLC-ICP-MS

High-Pressure Liquid Chromatography is a powerful separation technique and coupled with ICP-MS becoming an essential tool in total speciation[37–39].

As discussed above, the major fraction of As in algae is in arsenosugar form, though this specie does not represent apparent toxicity, some authors consider it as a possible toxic specie[10].

There are a lot of As species[40], and they have distinctive chemical characteristics, therefore, it is required to use cationic and anionic columns to perform total As speciation in a sample[41,42].

G. *Birdiae* sample water extract was submitted to total speciation in HPLC-ICP-MS (S1). The quantified species are described in table 5.

Table 5: Arsenic species mass fraction in *Gracilaria Birdiae* algae sample water extract using HPLC-ICP-MS (mean ± SD, n=2)

Specie	**Time of retention (min)**	**Column**	**Concentration (mg kg^{-1})**
Dimethylarsenic acid (DMA)	2.17	Cationic	0.842 ± 0.024
Monomethylarsenic acid (MMA)	3.77	Cationic	0.072 ± 0.002
Y1	20.28	Cationic	1.370 ± 0.090
Arsenosugar-X	20.63	Cationic	1.985 ± 0.017
Arsenobetaine (AsB)	2.33	Anionic	0.887 ± 0.067
Arsenosugar-OH	2.66	Anionic	1.690 ± 0.025
Y2	18.50	Anionic	1.034 ± 0.081
iAs	35.10	Anionic	0.391 ± 0.03
SUM of species			8.27
% Recovery			98.7

Y1 and Y2 are unknown As species

It was possible observe with speciation data that the use of both anionic and cationic column is efficient for total speciation in the algae sample, once, the recovery of species is higher than 98%.

Arsenosugar represents around 44.4% of tAs content in this seaweed sample. While AsB content is around 11% of tAs and DMA slightly higher than 10%. Seaweed sold in USA commerce[20] has a content of arsenosugar reported between 2 and 84% of tAs. Like the tAs content, iAs content is also dependent on species and origin of the sample.

Unknown arsenic species represent almost 30% of tAs in this algae sample, could be represented arsenosugar non-identified yet, or other arsenic species present in secondary metabolism of algae.

The content of iAs found using Fe_3O_4@DTPMP by ICP OES (0.383 ± 0.011 mg kg^{-1}) is statistically similar (t test, 95% of confidence) to the HPLC-ICP-MS (0.391 ± 0.03 mg kg^{-1}) showing the possibility of the use of the less expensive technique in the quantification of iAs.

The use of algae for the culinary purpose has been increasing, as well, as the use for therapeutical objectives. Thus, analysis of iAs mass fraction is essential to guarantee the food security, besides that, more studies about bioavailability of arsenosugar through the digestive tract is required for a full understanding of food security in algae.

4. CONCLUSIONS

It was possible using Fe_3O_4@DTPMP for preconcentration and extraction of iAs from algae samples for analysis by ICP OES. This procedure decreases the cost of analysis and has an accuracy similar to HPLC-ICP-MS method.

Arsenosugar is the predominant As species in the seaweed samples studied. Inorganic arsenic mass fractions found in the samples are in allowance with the existing international legislation; however, it is important mentioning that the most countries do not have even legislation about total arsenic. It is necessary to update food regulations, as its use for food purposes is increasing.

There are no works in literature about arsenic composition in *Gracilaria Birdiae e Solineria Folifformis*

, so more studies are required to verify the iAs and arseno-compounds present in algae from the Brazilian northwest for guarantee the food security of this commodity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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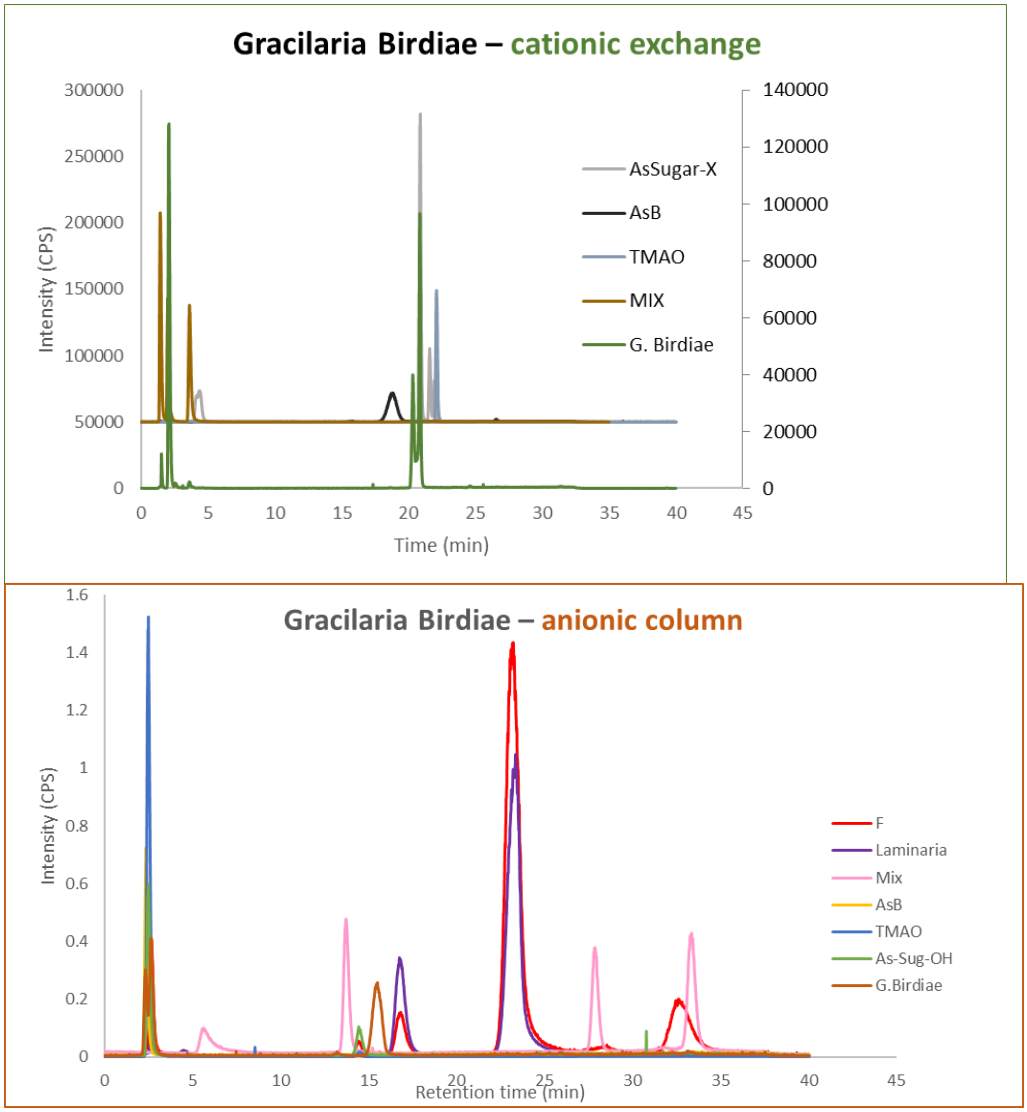
Supplementary Material

S1: Chromatogram in A) cationic and B) anionic column for water-soluble As species.



5. CONCLUSION

Observing the results showed in the chapter 1, 2 and 3 the authors could conclude that it was possible to develop a new ferrous material functionalized with a phosphorous agent as a solid phase for the extraction of inorganic arsenic for samples of algae, shrimp, and rice. The material was characterized and a possible binding site between the nitrogen of the functionalizing molecule and the oxygen of the arsenate/arsenite anion could be observed. However, more experiments should be done to clarify the adsorption mechanism.

The developed nanomaterial proved to be stable and can be reused up to 8 times without loss of analytical efficiency (recoveries above 85%).

A solid phase magnetic extraction method was proposed and proved to be viable for the pre-concentration and speciation of iAs in aqueous extracts and in the solution obtained from the complete digestion of rice, showing the selectivity of the nanomaterial inorganic As species. It was possible to detect arsenic ultra-traces (μg kg^{-1}) using ICP OES.

The proposed method proved to be less-cost, sensitive, accurate and precise in relation to the standard HPLC-ICP-MS method.

The data presented show the need for specific legislation for inorganic arsenic species, with the total content of the element being insufficient for decision making.

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ANNEX A - COPYRIGHT PERMISSION OF PAPERS