TECHNICAL NOTE

Optimized preconcentration method using magnetic dispersive solid-phase microextraction with GO–γFe₂O₃ nanoparticles for the determination of Se in fish samples by FIA-HG-AAS†

Mayara Cristina Leal do Nascimento,ab Fernanda Nunes Ferreira,a Ana Paula Benevides,a Danilo de Castro da Silva,a Deborah Vargas Cesar,ab Aderval S. Luna,ab and Jefferson S. de Gois,a,b

This work describes the development of an analytical method for the preconcentration of Se in digested fish samples using magnetic nanoparticles of graphene oxide GO–γFe₂O₃ with detection by hydride generation atomic absorption spectrometry and flow injection analysis (FIA-HG-AAS). The magnetic nanoparticles (MNPs) were synthesized and their structural properties were characterized by Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), and X-ray diffraction (XRD). The domain of the factors pH, MNP mass, and adsorption time was optimized using a central composite design, as well as the carrier gas flow, the concentrations of hydrochloric acid (HCl) and sodium borohydride (NaBH₄) for the hydride generation system. The optimized experimental conditions were obtained to be pH 2, 60 mg of MNPs, and 30 min for the adsorption process, and 4.5% (v/v) HCl, 0.19% (m/v) NaBH₄, and 273.5 mL min⁻¹ carrier gas flow for hydride generation. Under the optimized conditions, the enrichment factor (EF), the limits of detection (LOD), and quantification (LOQ) were 70, 30 ng g⁻¹, and 90 ng g⁻¹, respectively. The short-term precision of the method, defined by the relative standard deviation (RSD) (n = 10), was 7.54%. The accuracy of the method was assessed through recovery tests, as well as the analysis of certified reference materials (CRMs). The recovery values ranged from 103.8 to 117.3%, while the Se concentration determined in the CRMs were in agreement with the certified values obtained through a t-test at a 95% confidence level.

1. Introduction

Selenium is an essential micronutrient for human beings, and it has multiple biological functions for the life, like preventing cardiovascular and cancer diseases as well as anti-inflammatory and antiviral properties.¹ However, in high amounts, Se can lead to toxic effects like hair loss, problems in the nails and irritation to both skin and eyes, or even death.²–⁴

Most protein-rich foods contain Se, such as fish, vegetables, different types of meat, and Brazilian nuts. Among them, fish is a potential source of Se for the human diet, the concentration of which can vary depending on the fish species, age, size, and availability of Se in the environment.⁵–⁷

Government organizations around the world have established maximum levels for Se in food samples, and furthermore, recommended a daily intake of Se of 34 μg for adults, 17 μg for children between 1 and 3 years old, and 21 μg for children between 4 and 10 years old.⁸–⁹

Selenium determination in fish samples can be performed by numerous instrumental analytical techniques; among them, hydride generation atomic absorption spectrometry (HG-AAS) stands out⁰–¹² given its relatively low cost, high sensitivity, separation of the analyte from the sample matrix, and limit of detection in the order of ppb.¹³–¹⁵ The HG-AAS technique combined with a flow injection analysis (FIA-HG-AAS) system can also be used, providing an automated analytical system that enables the analysis of small aliquots of samples.

Although direct solid sample analysis may be applied with the need for special apparatus and studies for sample analysis, normally, solid food samples require at least a sample preparation step (when solid sample accessories are not available), in general, aiming to transfer the analyte to an acceptable medium for determination. The most common methods involve sample

---

¹Department of Analytical Chemistry, Rio de Janeiro State University, Rua São Francisco Xavier 524 – Maracanã, Rio de Janeiro, 20550-013, Brazil. E-mail: jefferson.gois@uerj.br
²Graduate Program in Chemical Engineering, Rio de Janeiro State University, Rua São Francisco Xavier 524 – Maracanã, Rio de Janeiro, 20550-013, Brazil
†Electronic supplementary information (ESI) available. See DOI: 10.1039/d1ja00091h
dissolution using inorganic in a microwave oven or a heating plate.16–18

One disadvantage of sample digestion is the inherent sample dilution, which jeopardizes the limit of detection (LOD) of the analytical method. Even with the advances of modern analytical techniques, separation and preconcentration methods are often necessary to improve the LOD and enable Se determination in fish samples.17

Preconcentration methods that were already applied for Se determination include liquid-phase microextraction (LPME),18 liquid–liquid microextraction (LLME),19 dispersive liquid–liquid microextraction (DLLME),20 dual-cloud point extraction (d-CPE),21 ultrasound-assisted extraction (UAE),22 solid-phase extraction (SPE),3 and magnetic solid-phase microextraction (MSPME).23

A new approach based on the principles of the solid-phase microextraction (SPME) technique called dispersive solid-phase microextraction (DSPME) has been drawing attention for the separation and preconcentration of analytes at trace levels.21 In this case, the extraction occurs by applying vigorous stirring, thus allowing a quick interaction between the analytes and the adsorbents, like magnetic nanoparticles (MNPs), most recently being called magnetic dispersive solid-phase microextraction (MDSPME).22–23 These adsorbents can be separated from the aqueous phase by centrifugation and collected by filtration, or by applying an external magnetic field. The extracted target analytes can be desorbed from the adsorbent using an eluting solution.24

Among the materials that can be applied as adsorbents, graphene oxide (GO) has found wide application due to its mechanical and thermal stability, high surface area, and abundance of functional groups that promote adsorption.25 Graphene oxide is normally difficult to separate from aqueous media by centrifugation, precipitation, or filtration; to overcome this problem, GO can be incorporated onto iron oxides, such as magnetite (Fe3O4) and maghemite (γ-Fe2O3), thus facilitating the separation and improving the adsorption capacity of the material.26–28 These nanomaterials are eco-friendly, presents low toxicity, and do not retain residual magnetism after the removal of an external magnetic field.29 Moreover, the application of GO–γ-Fe2O3 for the preconcentration of Se and determination using FIA-HG-AAS is not presented in the literature; thus its use and optimization of the experimental conditions for method development are new and may present advances in the field of sample preparation techniques.

Therefore, the aim of this work is the synthesis and application of GO–γ-Fe2O3 for the preconcentration and determination of Se in fish samples via FIA-HG-AAS, resulting in an optimized, relatively high throughputs, and simple method that can be applied for food analysis.

2. Experimental

2.1. Instrumentation

The Se measurements were performed using a flame atomic absorption spectrometer model iCE 3000 Series (Thermo Analytica, USA) equipped with a hydride generation system, model VP 100 (Thermo Scientific, USA). A hollow cathode lamp, model Photon Lamps (USA), was used (monitored wavelength, 196.09 nm), and the analytical signal was obtained by integrating the transient signal. Argon with a purity of ≥99.95% (Air Liquide, Brazil) was used as the carrier gas for the hydride generation system, while acetylene (Messer, Brazil) and compressed air were used for the flame. The operating parameters used were set as follows: acetylene flow rate of 800 mL min⁻¹ and carrier gas flow rate of 308 mL min⁻¹. All measurements were carried out in triplicate.

The multielement determination was carried out using an inductively coupled plasma optical emission spectrometer (ICP-OES) model iCAP 6300 equipped with a Babington V-Groove nebulizer and a cyclonic spray chamber (Thermo Scientific, USA). The monitored emission wavelengths were 203.98 nm (Se), 193.09 nm (C), 396.85 nm (Ca), 766.49 nm (K), 280.27 nm (Mg), 213.62 nm (P), and 227.32 nm (Sc – internal standard). The operating parameters were set as follows: radio frequency power of 1300 W, auxiliary gas flow rate of 1.0 L min⁻¹, and nebulizer gas flow rate of 0.39 L min⁻¹.

Microwave-assisted digestion (MAD) was carried out in a Multiwave PRO microwave oven (Anton-Paar, Graz, Austria) equipped with a rotor for twenty-four Teflon® vessels, model 24HVT50, with a capacity of 50.0 mL. To adjust the pH of each digested sample and the analytical curve solutions, measurements were performed using a pH meter, model PM608 (Analion Apparatus and Sensors, Brazil). And for the experiments of adsorption and desorption processes, a horizontal orbital shaking incubator, model Te-4200 (Tecnal, Brazil), was used.

The nanomaterials were chemically characterized by Fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM), and X-ray diffraction (XRD). The FTIR (PerkinElmer Spectrum One, USA) measurements were performed from 4000 to 400 cm⁻¹, with a resolution of 4 cm⁻¹ and 20 scans. FEG-SEM (Jeol-7100F, Japan) was performed using an acceleration voltage of 15 kV, without metallic covering, while the XRD (Rigaku Miniflex II, Japan) measurements were performed using Cu-Kz radiation (λ = 1.5405 Å), in the range of 5° < 2θ < 70° with a step size of 0.05°. Surface area analysis (Quantachrome Nova 1200e, USA) was performed with the adsorption of N2 at 77.3 K. The samples were degassed at 200 °C for 19 h before the analysis.

2.2. Reagents and solutions

All reagents used were of analytical grade or better, and the dilutions were performed using ultrapure water (with a resistivity ≥ 18.2 MΩ) obtained from an ultra-purifier system (Gehaka, Brazil). The reagents used were: HNO3 (Vetec, Brazil) to adjust the pH of all the solutions, ethylendiaminetetraacetic acid (EDTA) (Quimibrás, Brazil), HCl (9 mol L⁻¹ (Quimis, Brazil) for the hydride generation system. For the synthesis of GO, the reagents used were: graphite (Grafine 996100, kindly provided by Nacional de Grafite, Brazil),

This journal is © The Royal Society of Chemistry 2021

H$_2$SO$_4$ ≈ 95% w/w (Vetec, Brazil), KMnO$_4$, NaNO$_3$, and H$_2$O$_2$ ≈ 30% w/w (Isolar, Brazil). For the synthesis of the MNPs, FeSO$_4$·7H$_2$O 99% w/w (Sigma-Aldrich, Germany), Fe(NO$_3$)$_3$·9H$_2$O 98% w/w, and NH$_4$OH 28% w/w (Vetec, Brazil) were used.

Post-digestion sample matrix simulation was performed using KI, Na$_2$CO$_3$, MgCl$_2$·6H$_2$O, Ca(NO$_3$)$_2$·4H$_2$O, and NH$_4$H$_2$PO$_4$ (Vetec, Brazil). Individual standard solutions containing 1000 mg L$^{-1}$ of Se, Sc, Ca, Mg, P, K, and Na (Specsol, Brazil) were used for the analytical calibration curve, recovery tests, and interference studies.

2.3. Samples and sample preparation

Two fish samples were obtained from a local fish market in Baia de Guanabara, from different beaches of Ilha do Governador, Rio de Janeiro, Brazil. The samples include sample 1 – “Atlantic bigeye” (Priacanthus arenatus Cuv.) and sample 2 – “Mullet” (Mugil brasiliensis). After sampling, the muscle tissue of the fish was separated from the other parts and stored under refrigeration in a freezer until their use. These samples were used for optimization and studies.

The accuracy of the method was assessed by analyzing four certified reference materials (CRMs): (1) lobster hepatopancreas - TORT-2 (National Research Council, Canada), (2) fish muscle - ERM® BB422 (Institute for Reference Materials and Measurements), (3) bovine liver - SRM 1577A (National Institute of Standards and Technology), and (4) bovine liver - SRM 1577B (National Institute of Standards and Technology).

The microwave-assisted digestion of the samples was carried out by measuring about 600 mg of each sample directly inside Teflon® vessels and adding 3.0 mL of concentrated HNO$_3$ to the sample. The vessels were closed, fixed in a rotor, and submitted to the heating program as follows: heating to 190 °C for 20 min, staying for 10 min, and cooling down to 70 °C for 20 min. The safety parameters were: maximum pressure increase rate, 0.5 bar s$^{-1}$; maximum microwave power, 1000 W; maximum internal temperature, 200 °C; maximum pressure, 20 bar. The samples were transferred to a 50.0 mL polyethylene flask, ultrapure water was added up to 30.0 mL and the pH was adjusted to 2 with the aid of 10 mol L$^{-1}$ NaOH solution, and finally the volume was made up to 40.0 mL.

2.4. Synthesis of the GO–γFe$_2$O$_3$ nanocomposites

Graphene oxide was prepared by a modified Hummers’ method.$^{22}$ Graphite was previously treated with HCl (1.37 mol L$^{-1}$) to remove impurities. This graphite (0.2 g) was mixed with NaNO$_3$ (0.25 g), and H$_2$SO$_4$, 95% w/w (11.5 mL) was slowly added to this mixture under magnetic stirring, at 0 °C. Then, KMnO$_4$ (1.5 g) was added in small portions, keeping the temperature under 10 °C. After, the temperature was kept at 35 °C for 2 h. Distilled water (80.0 mL) was added in small portions, and the temperature was increased to 90 °C and (which was kept for 30 min). The suspension was cooled to 20 °C, followed by the addition of 60 mL of H$_2$O$_2$ (2.94 mol L$^{-1}$), and stirred for 60 min; the GO was separated by centrifugation and washed three times with H$_2$O$_2$ (2.94 mol L$^{-1}$) and H$_2$SO$_4$ (0.51 mol L$^{-1}$), followed by washing with distilled water until the pH reached 6. The resultant solution was filtered using a 0.45 μm nylon membrane and dried at 50 °C.$^{29}$

The GO–γFe$_2$O$_3$ MNPs were prepared by the method reported by Luo et al., 2017. To obtain an Fe-oxide/GO mass ratio of 9, GO (0.2 g) was dispersed in deionized water (100.0 mL) for 15 min followed by sonication for 30 min. This dispersion was transferred to a round-bottom flask and stirred and purged with nitrogen for 30 min. 2.1 g of FeSO$_4$·7H$_2$O and 6.1 g of Fe(NO$_3$)$_3$·9H$_2$O were added to this mixture, and then 10.0 mL of NH$_4$OH was added dropwise, followed by additional stirring for 30 min. The MNPs were separated through centrifugation and washed with water until reaching neutral pH, and dried at 50 °C.$^{13}$

2.5. Magnetic dispersive solid-phase microextraction procedure

The preconcentration and MDSPME experiments were performed in a 50.0 mL polyethylene flask containing 40.0 mL of digested sample solution with pH adjusted to 2 with 10 mol L$^{-1}$ NaOH, where 60 mg of MNPs was added. The mixture was stirred at room temperature (at 25 °C) on a shaker (at 270 rpm) for 30 min. After the adsorption step, the solid phase was separated, with an external super magnet, and the supernatant was discarded. Subsequently, the analyte was eluted from the MNPs with 500 μL of a 0.1 mol L$^{-1}$ EDTA solution (pH 12 adjusted with NaOH) at 200 rpm, for 5 min at room temperature. The solid phase was separated again, with an external magnet, and the supernatant was collected for analysis by FIA-HG-AAS.

2.6. Determination of Se and FIA-HG-AAS operation

An amount of 500 μL of the eluted solution obtained in Section 2.5 was inserted into the FIA system using a syringe, which was coupled to the switching valve connected to the hydride generation accessory. The gaseous hydrides were then carried to the atomization cell of the AAS instrument, a T-shaped quartz cell placed over the flame so the analytical signal was obtained. Fig. 1 represents the scheme of the FIA system used.

The FIA system used is composed of a valve where the reagents HCl (4.5% v/v) and NaBH$_4$ (0.19% w/v) were pumped through the system. These two solutions merged with the sample in the switch valve (V), with the mixture pumped directly to the cell (Z) and gas–liquid separator (D). The loop was filled with ultrapure water up to 0.4 mL and 10 μL of the reagents were added directly to the working solution, with washing three times with ultrapure water. The FIA system used is composed of a valve where the reagents HCl (4.5% v/v) and NaBH$_4$ (0.39% w/v) were pumped through the system. These two solutions merged with the sample in the switch valve (V), with the mixture pumped directly to the cell (Z) and gas–liquid separator (D). The loop was filled with ultrapure water up to 0.4 mL and 10 μL of the reagents were added directly to the working solution, with washing three times with ultrapure water.

![Fig. 1](image-url) The flow injection analysis system used in this study. P is the peristaltic pump; V is the switch valve; S is the sample syringe; W is the waste; Z is the reaction cell; D is the gas–liquid separator.
with 500 µL of the sample, and then the valve was rotated allowing it to be carried to the point of confluence. The mixture reaches the reaction cell where volatile hydrides of the analyte are formed and carried to the atomizer, producing a transient signal. Although the pre-reduction of Se(VI) to Se(IV) is advised, it are formed and carried to the atomizer, producing a transient signal. Therefore, obtained for Se(IV) only, and related to the total Se concentration through the analytical calibration curve.

2.7. Statistical analysis

All experimental procedures and statistical treatment were performed using the “rsmDesign [4]” function available in the “qualityTools” package of software R. The significance of the effects of each factor and their interaction was assessed by the analysis of variance (ANOVA) and using the p-value (when the p-value < 0.05, the effect of the factor is significant). The residues of the models were evaluated using the Shapiro–Wilk test (α = 0.05) to confirm the normal distribution. For multi-response optimization, the desirability (D) was used, where its range is from 0 (unacceptable value) to 1 (the most desirable value).31

3. Results and discussion

3.1. Chemical characterization of the GO–γFe₂O₃ MNPs

The X-ray diffraction pattern of GO (Fig. 2a) shows a peak at 2θ = 10.85°, which characterizes the oxidation process of graphite, and an interlayer distance of 0.814 nm.35,36 Both pure γFe₂O₃ and GO–γFe₂O₃ present diffraction peaks characteristic of the iron oxide in the maghemite phase (PDF 39-1346) as shown in Fig. 2b and c, respectively.35,37

As mentioned by Yang et al., 2012, the peak from GO cannot be observed in the composite sample, and one reason for this may be the fact that the strong signs of iron oxides tend to overlap with the carbon peaks. Besides, the lower amount of GO in the sample compared with γFe₂O₃ could be another reason for this fact. The broad diffraction peaks suggest that MNPs have a very small size,37 with a crystallite size of 11 nm (2θ = 35.5°) for GO–γFe₂O₃ and 12 nm for pure γFe₂O₃, calculated using Scherrer’s equation.

Fourier transform infrared spectroscopy was applied for the characterization of functional groups and to analyze the interaction between GO and γFe₂O₃. Fig. 3 shows the spectra referring to GO, γFe₂O₃, and GO–γFe₂O₃. The identification of vibrational bands related to oxygen-containing groups in the GO spectrum, such as C=O (1719 cm⁻¹), C–OH (1417 cm⁻¹), and C–O (1055 cm⁻¹), confirms the oxidation process of graphite.37–39 The 628, 580, and 447 cm⁻¹ bands in the γFe₂O₃ sample are associated with Fe–O bonds.25,40 In both of them, it is possible to observe the existence of a broad and intense band around 3474 cm⁻¹, due to the stretching vibration of O–H bonds of water molecules. In 1630 cm⁻¹, it is observed a peak related to the angular deformation mode of these molecules.37,41 The GO–γFe₂O₃ spectrum shows the disappearance of the C=O band of GO at 1719 cm⁻¹, suggesting the interaction between GO and γFe₂O₃ through this group.25

The BET surface area was found to be 106 m² g⁻¹ for pure γFe₂O₃ and 177 m² g⁻¹ for GO/γFe₂O₃, which is consistent with the values of GO-MNPs reported in the literature (142 to 409 m² g⁻¹). The higher surface area value of GO–γFe₂O₃ compared to pure γFe₂O₃ favors the adsorption process.37,42,43

The SEM technique (Fig. 4) was used to evaluate the morphology of GO, pure γFe₂O₃, and GO–γFe₂O₃ materials. SEM of GO (Fig. 4A) shows a waveform, similar to a sheet with wrinkles and folds. Such characteristics can be attributed to the interaction of groups containing oxygen present in the sheets of GO.29,37,45 SEM of γFe₂O₃ (Fig. 4B) shows the presence of aggregated particles, which is common in nanomaterials. In Fig. 4C, from the SEM image of GO–γFe₂O₃, it is possible to observe the γFe₂O₃ phase over the GO sheets’ surface, indicating that GO acts as a deposition structure25,46 and confirming the interaction between GO and γFe₂O₃ as observed by FTIR analyses.
3.2. Microwave-assisted digestion method optimization

The optimization of the sample preparation step is crucial to achieving the conditions under which a given sample mass can be used promoting the complete analyte extraction and/or promoting the degradation of the organic matrix of the sample. The domain of the factors the volume of HNO₃ (mL) and sample mass (g) was optimized using a central composite design (CCD) consisting of four factorial points (2²), four axial points, and three replicates of the central point (Table S1†). The analytical signals of Se and C were monitored for all experiments. The selenium signal was monitored to evaluate the maximum analyte extraction, while C was monitored to obtain the conditions under which the organic matrix is decomposed; therefore, this analysis was performed by ICP-OES rather than AAS.

For Se extraction, the linear terms of the volume of concentrated HNO₃ and sample mass, as well as the interaction of both, were statistically significant (p < 0.05) for the mathematical model, and the R-squared obtained was 0.97 (adjusted R-squared = 0.96). On the other hand, for the mathematical model monitoring the signal for C, the linear and quadratic terms of the volume of concentrated HNO₃ as well as the linear term of the mass of the sample and the interaction of both were statistically significant, and the R-squared obtained was 0.98 (adjusted R-squared = 0.97). The residuals of both models are normally distributed, according to the Shapiro–Wilk test (α = 0.05). Fig. S1† presents the contour plot for both models.

The optimal conditions were obtained to be 3.00 mL of 14 mol L⁻¹ HNO₃ and 600 mg of sample mass. These values were obtained using the desirability function where the surface response obtained is presented in Fig. S2.† The overall desirability obtained was 0.51, which is an acceptable value; however it demonstrated that the maximum Se extraction did not occur under the same conditions where the whole organic matrix of the sample is destroyed.

3.3. Selenium preconcentration using GO–γFe₂O₃, MNPs

To reveal the matrix composition and, thus, investigate the potential presence of ions that may jeopardize the adsorption process, the determination of Ca, K, Mg and P was performed by ICP-OES in two fish samples after microwave-assisted digestion. The average content (mg kg⁻¹) obtained in each sample was about: sample 1 – Ca (1953), K (22 173), Mg (913) and P (11 487); sample 2 – Ca (2180), K (26 664), Mg (900) and P (10 407).

The adsorption process was, therefore, studied in two situations: (1) – with an aqueous solution containing Se without these matrix ions; (2) adding these ions in a test solution containing Se, as follows: Ca (40), K (400), Mg (20) and P (200) mg L⁻¹ (values estimated for the resulting solution where a sample mass of 600 mg is prepared for a final volume of 40.0 mL). The domain of the factors agitation time, pH, and mass of MNPs was optimized to investigate the influence of the three variables on the Se adsorption process in an aqueous solution and a solution containing the ions from the matrix of the sample. The factors were optimized using a CCD and the two experimental procedures are presented in Tables S2 and S3.† All experiments were performed with a Se final concentration of 40 µg L⁻¹.

Fig. 5 shows all contour plots for different experimental conditions: for the Se adsorption procedure in a solution containing the ions from the matrix of the sample (A, B, and C) and for the Se adsorption procedure in aqueous media (D, E, and F). For the Se adsorption procedure in the aqueous medium, all terms presented statistical significance, including all interactions and quadratic terms. The R-squared obtained was 0.99 (adjusted R-squared = 0.97). The optimum adsorption conditions obtained for Se were 39 min of agitation, pH 4, and 11 mg of MNPs. Meanwhile, for the Se adsorption procedure in a solution containing the ions from the matrix of the sample, only the quadratic terms did not present statistical significance and the R-squared obtained was 0.96 (adjusted R-squared = 0.94). The optimum adsorption value for Se in the presence of foreign ions was reached in 30 min of agitation, pH 2, and 60 mg of MNPs. There is a decrease in both the agitation time and pH; however, the major difference is observed for the MNP mass, perhaps due to the increased ion loading in the solutions requiring a higher mass of the adsorbent. Lu et al., 2017, have found similar results when adsorbing Se in wastewater with Fe₃O₄ MNPs in the presence of other ions.⁴⁷

As can be noticed the adsorption processes occurred in acidic media, which was also shown by Li et al., 2014, where the phosphate adsorption process in an Fe–Cu binary oxide was favorable at low pH. Lu et al., 2017, also found high efficiency of the Se adsorption process with magnetic carbon microspheres.

---

Fig. 4 Scanning electron microscopy images of (A) GO, (B) γFe₂O₃, and (C) GO–γFe₂O₃ MNPs.
at low pH. However, in our study, the process was favored at lower pH than the ones obtained by those authors. This can be explained by ligand exchange as one of the mechanisms of adsorption, which is facilitated at lower pH since there is a greater release of OH\(^-\). Concomitantly, at lower pH values, the protonation of the MNPs' surface may occur, increasing the number of active sites with a positive charge and the force of attraction between the surface of the MNPs and the existing anions (considering HSeO\(_3\)\(^-\) the anionic form of Se predominant at low pH values).\(^{47,48}\)

The optimized values obtained for the adsorption of Se in the presence of the salts of Ca, K, Mg, and P can be compared to the conditions of methods already developed. He et al., 2019, preconcentrated Se in river, lake, and seawater samples using 10 mg of NPMs and a pH range between 2 and 4. Acikkapi et al., 2019, determined Se in water and food samples using 100 mg of PoleS-PEG copolymer for the preconcentration step, with a stirring time of 20 min at 1200 rpm and pH 6. Kumar et al., 2015, used 1 mg of Pd nanoparticles (NPs) to preconcentrate Se in water samples, using pH 3 with a 10 min stirring time at 3500 rpm. Thus, it is possible to observe that the pH ranges of the methods are very close, and the amount of adsorbent as well as the agitation time vary due to the nature of the sample studied and the material chosen as an adsorbent.\(^{20,30,49}\)

3.4. Hydride generation conditions
For this study, the domain of the factors carrier gas flow, the concentration of HCl, and concentration of NaBH\(_4\) was optimized using a CCD with eight factorial points (\(2^3\)), six axial points, and six replicates of the central point. The optimization was performed using a solution of Se 40 \(\mu\)g L\(^{-1}\), in EDTA/NaOH (pH 12) (due to the composition of the eluting solution). Table S4† presents the experimental procedures with their real and coded values, in addition to the absorbance signal response for Se.

The only factor that did not present statistical significance was the linear parameter of concentration of NaBH\(_4\), and all the others, linear, quadratic, and interactions, presented statistical significance. The value of \(R\)-squared obtained was 0.94 (adjusted \(R\)-squared = 0.88), and the Shapiro–Wilk test indicated that the residues of the model follow a normal distribution. The contour plot is presented in Fig. S3,† and the optimal conditions were obtained to be 4.5% (v/v) HCl, 0.19% (w/v) NaBH\(_4\), and 273.5 mL min\(^{-1}\) argon carrier gas flow.

3.5. Influence of sample matrix in the Se adsorption
To investigate the influence of the major sample composition on the Se adsorption process, a solution containing 12.5 \(\mu\)g L\(^{-1}\) Se with a final volume of 40 mL was used adding different concentrations of the coexisting ions; the concentration of Ca,
Mg, P, and K (mg L\(^{-1}\)) was, respectively, for each experiment. (1) 0, 0, 0, and 0; (2) 10, 3, 25, and 10; (3) 25, 5, 50, and 25; (4) 50, 10, 100, and 50; (5) 100, 15, 200, and 100; (6) 200, 30, 300, and 200; and (7) 300, 50, 400, and 300. These experiments were carried out using two adsorption conditions: (1) applying all optimized parameters in an aqueous solution without coexisting ions (39 min of agitation, pH 4 and 11 mg of MNPs) and (2) applying optimized parameters with these ions (30 min of agitation, pH 2, and 60 mg of MNPs). The elution process was performed using 500 µL of EDTA solution with pH 12, and the concentration of Se was determined by FIA-HG-AAS (about 875 µg L\(^{-1}\) Se in the eluted solution considering the enrichment factor of 70).

Fig. 6 presents the eluted concentration for all the experiments, where it is possible to notice that under the optimized conditions without the sample matrix (Fig. 6A) the adsorption process was highly affected by the addition of other ions, while when the adsorption process is optimized considering the sample matrix (Fig. 6B), little influence from other ions was observed. Thus, it is crucial to consider the sample matrix while optimizing the adsorption process. This can be explained by the higher adsorbent mass required when other ions are present, ensuring that the nanoparticles were not overloaded with sample matrix ions (competition for active sites); in other words, in this situation the method presents good tolerance to potential interference. Therefore, the optimized conditions obtained in the presence of Ca, K, Mg, and P were set for the method proposed.

These results are supported by other observations, such as Lu et al., 2017, who reported a high influence of PO\(_4^{3-}\) on the efficiency of Se removal in wastewater using carbon microspheres with MNPs of Fe\(_2\)O\(_4\). Zhang et al., 2008, also observed strong influence and competition of PO\(_4^{3-}\) for active sites in activated carbon and coated with Fe in the process of removing Se from water, and Huang et al., 2012, when using silica-Fe\(_2\)O\(_4\) NPs to preconcentrate Se in water and study the effect of coexisting ions, such as K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Cl\(^-\), and SO\(_4^{2-}\), reported a good tolerance of method developed against interference.\(^\text{47,50}\)

### 3.6. Analytical figures of merit

Table 1 presents the main analytical figures of merit obtained; the LOD of the method was calculated as 3.29 times the standard deviation (SD) of the analytical signal of ten measurements of a blank solution divided by the slope of the analytical curve, and the result was adjusted taking into account the final volume of the solutions and the mass of the samples. Meanwhile the LOQ was calculated as 3 times the LOD. The analytical curve was submitted to the preconcentration procedure in the presence of Ca, K, Mg, and P at the concentrations of 40, 400, 20, and 200 mg L\(^{-1}\), respectively, to simulate the sample matrix and presented a good linearly within the working range. The enrichment factor (EF) was calculated by dividing the slope of the analytical curve using the proposed preconcentration method and the analytical curve without preconcentration.

Table 2 presents the enrichment factor obtained in our article and other already published studies in the literature; all values obtained by the authors are in agreement with the EF obtained in our work (EF = 70). Ali et al., 2019, developed a preconcentration method of Se in different foods and soft drinks, followed by determination using HG-AAS, and obtained an EF of 85.5, while Acikkapi et al., 2019, developed a method of preconcentration of Se in food and beverage samples using magnetic solid-phase microextraction coupled to electrothermal atomic absorption spectrometry (MSPME/ETAAS) and obtained an EF of 50.\(^\text{17}\) Shahid et al., 2020, used ultrasound-assisted dispersive liquid–liquid microextraction (US-DLLmE) and vortex assisted-emulsification liquid–liquid microextraction (VA-ELLmE); both methods were coupled to graphite furnace atomic absorption spectrometry (GFAAS) for the determination of Se in different water samples. The values for EF were 90.8 and 81.8 for US-DLLmE and VA-ELLmE, respectively.\(^\text{52}\)

Meanwhile, Naemullah et al., 2016, developed a novel switchable hydrophobic–hydrophilic transition dispersive solid–liquid microextraction (SHT-DLSME) coupled with GFAAS method for the removal of Se from water samples, obtaining an EF of 72.\(^\text{53}\)

The accuracy of the method developed was assessed by the analysis of a CRM sample and recovery tests. The recovery tests were performed at the concentrations of 7.5, 12.5, and 17.5 µg L\(^{-1}\) of Se using a mullet fish sample previously described in Section 2.3. The two optimized conditions were tested in the recovery tests: (1) obtained in an aqueous solution (39 min of agitation, pH 4, and 11 mg of MNPs) and (2) obtained using the sample matrix (30 min of agitation, pH 2, and 60 mg of MNPs).

![Fig. 6 Effect of coexisting ions on the adsorption of Se (A) using GO/γ-Fe\(_2\)O\(_4\) MNPs under optimized conditions without Ca, K, Mg, and P, and (B) using MNPs under optimized conditions in the presence of Ca, K, Mg, and P.](image-url)
Table 2  Enrichment factor obtained from this work and other articles already published in the literature

<table>
<thead>
<tr>
<th>Analytical technique</th>
<th>Sample</th>
<th>EF</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDSPME</td>
<td>Fish samples</td>
<td>70</td>
<td>This work</td>
</tr>
<tr>
<td>LLME/ HG-AAS</td>
<td>Food and soft drink</td>
<td>85.5</td>
<td>Ali et al., 2019</td>
</tr>
<tr>
<td>MSEPME/ETAAS</td>
<td>Food and water samples</td>
<td>50</td>
<td>Acikkapi et al., 2019</td>
</tr>
<tr>
<td>US-DLLµE</td>
<td>Water</td>
<td>90.8</td>
<td>Shahid et al., 2020</td>
</tr>
<tr>
<td>VA-ELLµE</td>
<td>Water</td>
<td>81.8</td>
<td>Shahid et al., 2020</td>
</tr>
<tr>
<td>SHT-DSLME</td>
<td>Water</td>
<td>72</td>
<td>Naeemullah et al., 2016</td>
</tr>
</tbody>
</table>


Table 3  Quantification of Se by the preconcentration method using magnetic dispersive solid-phase microextraction with GO–γ-Fe_3O_3 nanoparticles and determination by FIA-HG-AAS (n = 3, means ± confidence interval)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Found</th>
<th>Certified</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SRM 1577A</td>
<td>0.78 ± 0.05</td>
<td>0.71 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>SRM 1577B</td>
<td>0.68 ± 0.11</td>
<td>0.73 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>ERM®-BB422</td>
<td>1.01 ± 0.38</td>
<td>1.33 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>TORT-2</td>
<td>5.37 ± 1.70</td>
<td>5.63 ± 0.67</td>
<td></td>
</tr>
<tr>
<td>Mullet sample</td>
<td>2.28 ± 0.73</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Atlantic bigeye</td>
<td>1.75 ± 0.46</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

The recoveries using the conditions obtained without the sample matrix ranged from 39.8 to 69.5%, and the EF obtained was 40; however, when using the conditions obtained with the sample matrix, the recovery values ranged from 103.8 to 117.3% and the EF was 70. The optimization of the method in the presence of potential interfering ions allowed recoveries within the acceptable range of 80 to 120% according to the Association of Official Agricultural Chemists, besides improving the EF.

The CRM analysis was performed, in triplicate, submitting this sample to the MAD and preconcentration process. The results obtained (Table 3) demonstrated the accuracy of the developed method according to a two-tailed *t*-test, with a 95% confidence level. The short-term precision was evaluated in terms of the repeatability of the analytical results using 10 measurements of a spiked sample (4 µg L^{-1} of Se) (RSD ≤ 7.54 (n = 10)) and demonstrated the good precision of the method.

4. Conclusion

The preconcentration of Se using GO–γ-Fe_3O_3 MNPs and determination by FIA-HG-AAS has been presented as a very attractive alternative for determining Se at ng g^{-1} levels. The method was sensitive and presented a high sample throughput and it is relatively simple compared to the conventional preconcentration methods available. The optimization of the factors using solutions containing the major inorganic matrix constituents was crucial for the robustness of the method, in which the presence of the sample matrix did not affect the accuracy. The limit of detection, short-term precision, EF, and accuracy of the methods were evaluated and the results were comparable to or better than those reported in the literature.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

The authors are thankful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES), Fundação de Amparo a Pesquisa no Rio de Janeiro (FAPERJ, E-26/202.755/2019 and E-26/010.002212/2019), and Universidade do Estado do Rio de Janeiro (Programa Pro-Ciência, PIBIC,PROATEC, and InovUERJ) for their financial support. The authors would like to thank NANOFAUBERJ for FEG-SEM analyses and the Laboratory of Instrumental Characterization/UERJ for the infrared spectroscopy analyses, and Prof. Tatiana Dillenburg Saint’Pierre for the CRMs for analysis. ASL and F. JSG received a research grant from CNPq. ASL, DVC, and F. JSG received a research grant from UERJ (Programa Pró-Ciência). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

References
