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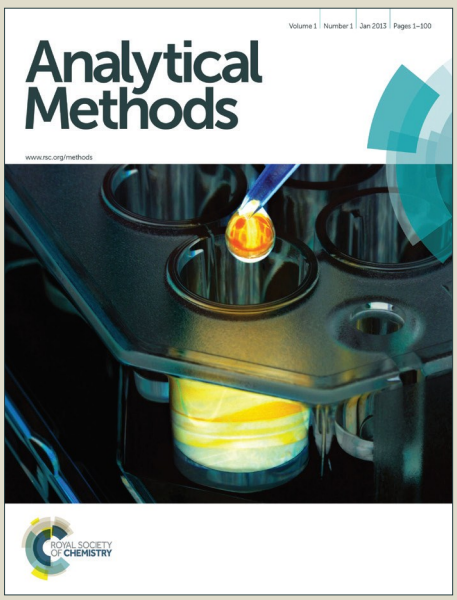

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Assessment of organosilane-functionalized nano-carbon black for interference-free on-line Pb(II) ions enrichment in water, herb medicines and environmental samples

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ABSTRACT

In the present work, commercial nano-carbon black particles (CB) were functionalized with 3-mercaptopropyltrimethoxysilane (3-MPTMS) through silanization reaction to improve their adsorption capacity towards the Pb(II) ions and hence extend their application as an alternative and low-cost nanocarbonaceous material in the field of separation science. The material was characterized by FT-IR, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), Raman spectroscopy, as well as by thermogravimetric analysis and textural data. After the characterization of material (3-MPTMS/CB) a FIA-FAAS preconcentration method for Pb(II) ions was developed, which in turn was performed by loading 20.0 mL of a 200 μg L^{-1} Pb(II) solution at pH 4.76 (buffered with 0.0358 mol L^{-1} acetate buffer) through 100 mg of 3-MPTMS/CB packed into a mini-column at a flow rate of 4.0 mL min^{-1}. The optimized condition of the proposed method has been achieved through a 2^{5-1} fractional factorial and Doehlert matrix design. The improvements on the adsorption capacity of CB towards the Pb(II) after chemical modification with 3-MPTMS was noticed by comparing the sensitivity of analytical curve built with CB and 3-MPTMS/CB. Very satisfactory figures of merit including limit of detection (LOD) of 1.33 μg L^{-1}, limit of quantification (LOQ) of 4.45 μg L^{-1}, preconcentration factor of 28.0, consumptive index of 0.714 mL, preconcentration efficiency of 5.6 min^{-1} and sample throughput of 12 h^{-1} were obtained. The precision of the method was assessed as relative standard deviation (RSD) (%) for 10 measures of 10.0 and 160.0 μg L^{-1} Pb(II) solutions yielding values of 3.0 and 2.3 %, respectively. The preconcentration method was successfully applied to the Pb(II) ions determination in different kinds of water and *Ginkgo Biloba* samples with satisfactory recovery values (91 – 108 %). In addition, accuracy of proposed method was also checked by analysis of certified marine sediment reference material (MESS-3).

keywords: Adsorbent , carbonaceous material, silanization, on-line preconcentration, lead.

Introduction

In the last years, the search for alternative nanomaterials with large surface area, low-cost, high reusability, selectivity and chemical stability for the removal/preconcentration of pollutant metals from different sample media have been the main challenge and goal for the analytical chemists.[1] In this context, nano-carbon blacks (CB) have increasingly gained interest as alternative nanoadsorbents for the adsorption of some analytes in detriment of carbon nanotubes and activated carbon. This material is composed essentially of spherical carbon primary particles, which forms aggregates of 10 or more spheres, resulting in a large cluster of carbon layers with a pronounced ordering.[2-4] In general, according to different production processes, the CB obtained presents different diameter range, as well as differently charged surface. Furnace black produces particles with diameter range from 20 nm to 80 nm and it is considered the most important production process, giving rise to materials with surface neutral or negatively charged, a particularly important characteristic for adsorption of metal ions.[5] On the other hand, the process known as channel and thermal black produces structure of CB with particle size in the range of 9 – 30 nm and 120 – 150 nm and acidic and inactive surfaces, respectively, and their applications are limited due to their surface properties.

Approximately 90% of the world demand for CB has been used as rubber automotive products and non-automotive rubber products including industrial molded and extruded products. The remaining 10% is divided among other applications such as, pigment, UV absorbing and/or conducting agent in inks, coatings and plastics.[4,6-9] As previously mentioned, the fine particles of CB tend to form large agglomerates due to Van der Waals forces, which in turn decreases their dispersion in polar solvent, promotes instability of dispersion and, as consequence, may decrease their applicability as adsorbent of metallic ions and synthesis of nanocomposites materials for different applications. Additionally, the

virgin CB depending upon the production process may contain low amount of functional groups under their surface such as OH (hydroxyl), carbonyl (C=O) and COOH (carboxyl), which leads to low adsorption capacity of material towards the metallic ions. In order to overcome these undesirable effects and to improve the dispersability, chemical stability and heavy metals removal efficiency, many effective approaches such as oxidation,[10] surfactant adsorption,[11] polymer grafting[12-15] and dispersant addition[16] have been proposed. The oxidation process has been the simplest approach for CB modification, since it removes the impurities from CB structure and improves the reactivity of material due to increase of functional groups such as carboxyl, phenolic hydroxyl and quinonic structures.[4,17] The removal from aqueous medium of As(VI) using CB oxidized with H_2SO_4 and Cd(II) and Cu(II) from wastewater using CB oxidized with HNO_3 has recently been reported.[5,18] The results obtained clearly demonstrated significant improvements on the metal ion adsorption after chemical treatment of CB. In spite of the most of these materials to present good adsorption of metal ions, their selectivity is limited due to presence of oxygen atoms, which are able to uptake several metal ions (alkaline earth elements, and transition metal).

The adsorption capacity of CB towards the metal ions may also be achieved through chemical modification using organosilanes. In the surface silanization technique, organosilanes used as surface modifiers may contain different functional groups such as thiol, amino and pyridine, which are self-assembled on the material surface and capable to interact with metal ions. These functional groups depending upon pH chosen may provide better selectivity towards the transition metal ions based on the Person´s theory.[19] Even though very interesting, this approach for improving the adsorption properties of CB towards the metal ions has not been yet demonstrated. On the other hand, considerable progresses have been achieved on the application of sinalization of carbon nanotubes with focus on metal ion preconcentration. Carbon nanotubes modified with 3-aminopropyltriethoxysilane (AAPTS), pyridine-functionalizing agent (N-(3-

(triethoxysilyl)propyl)isonicotinamide, 3-mercaptopropyltrimethoxysilane (3-MPTS) have been applied to adsorption of Cr(VI), As(V) and Se(VI)[20], Pb(II)[21], and Cd(II) and Pb(II).[19,22]

Accordingly, in this paper a CB chemically modified with 3-MPTS was prepared and evaluated as adsorbent of Pb(II) aiming at development of a highly sensitive FIA-FAAS method. The material was characterized by Fourier transform infrared spectra (FT-IR), scanning electron microscopy (SEM), energy dispersive (EDS), Raman spectroscopy, as well as by thermogravimetric analysis and textural data. The choice of 3-MPTMS was based on the presence of thiol and sulfur, which have high retention capacity either of soft metals or borderline metals, as classified lead ions. The developed method was successfully applied in different types of water samples and medicinal herb (*Ginkgo Biloba*) and the accuracy of method was checked by analysis of certified reference material.

Materials and Methods

Reagents and Instruments

All solutions were prepared from analytical grade reagents and ultrapure water (resistivity ≥ 18.2 MΩ cm^{-1}) from a Millipore Milli-Q purification system (Bedford, MA, USA). To avoid any risk of contamination, all glassware were kept overnight in a 10% (v/v) HNO_3 solution and then washed with deionized water. Pb(II) working solutions were prepared from a 1000 mg L^{-1} Pb(II) standard solution purchased from Merck (Darmstadt, Germany) by making appropriate dilutions with ultrapure water. Sodium citrate and acetate buffer solutions were prepared from the respective salts without any purification (all from Merck) whereas Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) was obtained from Sigma-Aldrich (Steinhein, Germany). In addition, the pH of these solutions were adjusted to the desired value with NaOH (Merck) and/or HNO_3 (Vetec, Rio de Janeiro) solutions. Solutions of Fe(III), Cu(II), Ni(II), Ca(II), Mg(II), Ba(II), As(III), Cd(II) and Zn(II), used in the foreign cations studies, were prepared from their stock solutions (1000 mg L^{-1}) or from

their salts (all of analytical grade and acquired from Merck). 30% (v/v) H_2O_2 and 65% (v/v) HNO_3, acquired from Sigma-Aldrich, were used in the microwave-assisted acid digestion procedure. A commercial nanoscale Carbon Black (CB) with particle size of 20-70 nm was kindly supplied by Cabot VULCAN® (Brasil Indústria e Comércio LTDA), whereas the modifier reagent 3-mercaptopropyltrimethoxysilane (3-MPTMS) and ethanol, used in the chemical modification were acquired from Sigma-Aldrich.

Measurements of Pb(II) were carried out in an AA-7000 flame atomic absorption spectrophotometer (FAAS) (Shimazdu, Kyoto, Japan) equipped with a hollow cathode lamp as radiation source (Hananatsu Photonics, K.K) and deuterium lamp as background correction. The hollow cathode lamp was operated at 8.0 mA and the wavelength was set at 217 nm, while the flame composition was maintained at acetylene at a flow rate of 1.5 L min^{-1} and air at flow rate of 10.0 L min^{-1}. A flow injection system was constructed from an Ismatec IPC-08 peristaltic pump (Glattzbrugg, Switzerland) and Tygon® tubing used for propelling samples and reagents. A homemade mini-column, made of polyethylene (3.0 cm in length and 0.70 cm at the top internal diameter) packed with 100 mg of CB modified with 3-mercaptopropyltrimethoxysilane (3-MPTMS/CB) was coupled in a homemade injector commutator for preconcentration and elution procedure. A small piece of cotton tissue was fixed at the mini-column extremities by inserting the upper end of a conical tip into the mini-column cylindrical edge, to avoid loss of material. The sample pH was measured with a Metrohn 826 mobile digital pH meter (Herisau, Switzerland). For characterization of the following materials: virgin nano-carbon black and modified nano-carbon black, a Shimadzu FT-IR-8300 Fourier transform spectrometer in transmission mode (4000 – 400 cm^{-1}) was used, whereas the surface morphology of the adsorbent material was evaluated by a scanning electron microscopy (SEM) using a Philips FEI Quanta 200 (Amsterdam, The Netherlands) equipped with probe of energy dispersive spectroscopy (EDS). The thermal stability of the studied materials was analyzed on a Perkin Elmer TGA 4000 thermogravimetric instrument in the temperature range of 30 –

800 °C (scanning rate 10 °C min^{-1}, nitrogen flow rate of 20 mL min^{-1}). Textural properties, such as specific areas, average pore sizes and volumes of the virgin and modified nano-carbon blacks were determined by a Quantachrome Nova 1200e automatic instrument. Average pore sizes and volumes of these materials were estimated by the Barrett-Joyner-Halenda (BJH) method based on nitrogen sorption experiments, whereas the specific surface areas were determined from sorption isotherms according to the Brunauer-Emmett-Teller (BET) method. Raman spectra were measured using a DeltaNu Advantage laser Raman spectroscopy system in the range 200 – 3400 cm^{-1}. For this analysis, the wavelength of the excitation beam was fixed at 532 nm and the exposure time of 15 seconds was adopted to prevent damage caused by laser irradiation. Certified reference material (MESS-3) was decomposed in a microwave oven Milestone Ethos One (São Paulo, Brazil).

Functionalization of nano-carbon black by using 3-MPTMS

Initially, 2.0 g of virgin nano-carbon black were oxidized with 50.0 mL of 65% HNO_3 under reflux at 120 °C during two hours to remove impurities from the material and increase the amount of functional groups in the surface able to react with organosilane modifier. The obtained material – (CB-oxi, oxidized nano-carbon black) was washed with ultrapure water until reaching pH ≈ 7.0 and further dried at 100 °C. After that, 1.5 g of CB-oxi were dispersed in 200.0 mL of ethanol and kept in an ultrasonic bath for 30 min. In sequence, 3.0 mL of 3-MPTMS were added in this mixture and kept under reflux at 65 °C for 3 hours. The obtained material was dried at 50 °C for 12 hours.[19,23,24] The schematic representation of CB functionalized with 3-MPTMS and interaction with Pb(II) are shown in Figure 1.

On-line preconcentration procedure and optimization strategy

The on-line preconcentration system was performed loading aliquots of 20.0 mL of sample or Pb(II) standard solution, buffered (pH 4.76) with 0.0358 mol L^{-1} acetate, through a mini-column filled with 100 mg of 3-MPTMS/CB at a flow rate of 4.0 mL min^{-1}. After the preconcentration step, the Pb(II) ions retained in the active sites of adsorbent were eluted with 2.0 HNO_3 mol L^{-1} through the mini-column toward the FAAS detector. All absorbance signals were taken as peak height.

In order to find the best performance of the on-line preconcentration of Pb(II) ions, a 2^{5-1} fractional factorial design was employed. Thus, the factors such as pH, buffer concentration (BC), eluent concentration (EC), preconcentration flow rate (PFR) and adsorbent mass (AM) which plays an important role in the preconcentration system, were evaluated and the maximum (+) and minimum (-) levels studied are shown in Table 1. After these studies, the factors sample pH and buffer concentration were studied using a Doehlert matrix for two levels. All experiments were randomly chosen, in duplicate, to avoid possible systematic errors. The significance of each factor was evaluated from the analysis of variance (ANOVA) performed at 95% confidence interval and graphically represented by a Pareto chart.

Effect of foreign ions on the Pb(II) preconcentration method

The selectivity of the proposed method for preconcentration of Pb(II) ions was evaluated through the analysis of binary solutions of the following cations: Fe(II), Cu(II), Ni(II), Ca(II), Mg(II), Ba(II), As(III), Cd(II) and Zn(II), at different proportions of the mixture analyte:interferent (w/w). The concentrations of foreign ions were chosen taking into account the maximum permissible levels in freshwater established by the Brazilian government regulating agencies.[25,26] All experiments were performed under optimized

conditions and a relative error of less than 10% was considered within the experimental error.

Breakthrough curves for the adsorption of Pb(II) onto 3-MPTMS/CB at different flow rates

Three breakthrough curves, at different flow rates, were constructed under dynamic and optimized conditions by percolating aliquots of 1.0 and 10.0 mL of 3.0 mg L^{-1} Pb(II) solution through mini-column packed with 100 mg of 3-MPTMS/CB, until saturation was reached. Each aliquot of the effluent from the mini-column was collected and analyzed by FAAS. The amount of Pb(II), adsorbed on the 3-MPTMS/CB in the flow rate of 2.0, 4.0 and 6.0 mL min^{-1}, was determined using the equation 1 depicted below and a graph of C_f/C_i vs. effluent volume (mL) plot was built, where C_f and C_i are the final and initial Pb(II) concentration (mg L^{-1}), V is the solution volume (mL) and m (g) is the mass of 3-MPTMS/CB.

$$Q(mg/g) = \frac{(C_i - C_f).V}{m} \quad \text{(Equation 1)}$$

Sample preparation

Different kinds of water samples, as well as, medicinal herb sample (Ginkgo Biloba) were used to check the applicability of the proposed method. Tap water was collected in the laboratory of the State University of Londrina (Londrina, Brazil) whereas the mineral water and serum physiologic were acquired at a local supermarket and drugstore. Lake water was collected from Igapó Lake in the city of Londrina, acidified until pH 2.0 and then was filtered through a 0.45 µm cellulose acetate membrane before use. All samples were spiked at 8.0 µg L^{-1} Pb(II) concentration and its determination was performed under optimum conditions using an external calibration curve. All samples studied had the pH

adjusted to 4.76 with 0.0358 mol L^{-1} of acetate buffer solution. To check the accuracy of the preconcentration method described herein, a mass of 237 mg of MESS-3 (certified marine sediment reference material) was weighed into Teflon flasks and decomposed with 10.0 mL of the mixture concentrate HNO_3:HCl (3:1, v/v) and 1.0 mL of concentrate HF. The mixture was kept overnight and subsequently submitted to microwave-assisted acid digestion procedure under temperature program as previously reported by research group.[27] After the sample decomposition, the solutions were heated on a hot plate almost to dryness. The residues previously cooled at room temperature were dissolved in ultrapure water and transferred to volumetric flasks of 100 mL. In addition, analytical blanks were used to confirm the absence of interfering in the analytical system. Similarly, the proposed method was applied in Ginkgo Biloba samples, weighing 500 mg and then subjected to digestion process, using 10.0 mL of 65% HNO_3 and 4.0 mL of 30% H_2O_2 for acid decomposition.

Results and discussion

Characterization of materials

Figure 2 shows the FT-IR spectra of virgin CB, CB-oxi and 3-MPTMS/CB. As can be seen, intense bands observed at 3400 cm^{-1} corresponds to the vibrations of OH group from adsorbed water molecule, whereas the two bands observed at 2922 and 2850 cm^{-1}, present in the 3-MPTMS/CB can be attributed to the symmetric and asymmetric stretching vibrations of CH_2 and CH_3 from 3-MPTMS modifier.[19] The intense band observed at 1723 and 1732 cm^{-1} can be assigned to the stretching of C=O from carbonyl and/or carboxyl groups present in both CB-oxi and 3-MPTMS/CB,[4,24] while the lower band intensity at 1237 cm^{-1}observed at 3-MPTMS/CB, 1234 cm^{-1} at CB-oxi and 1113 cm^{-1} at virgin CB, can be attributed to C-O stretching vibrations.[28] A slight band observed at 691 cm^{-1} can be attributed Si-O vibrations, whereas the broad band at 1064 cm^{-1} can be assigned to the Si-O-C stretching vibrations from the 3-MPTMS, thus indicating the immobilization of

organosilane on the CB.[19,29]. The band observed at 1385 cm^{-1} for the spectrum of virgin CB, can be assigned to the O-H deformations from COOH groups, since the modification by HNO_3 favors the presence of COOH as functional groups on the surface CB.[18] In addition, the presence of peaks at 1573 cm^{-1} and 1580 cm^{-1}, observed on the spectra of 3-MPTMS/CB and CB-oxi, can be related to the physically adsorbed water in the materials.[30]

As seen in Figure 3, the morphology of materials (virgin CB, CB-oxi and 3-MPTMS/CB), as expected, showed spherical particles in shape with size, in general, lower than 200 nm, roughness surface and are considered mesoporous (average pore diameter between 2 and 50 nm).[31] It was noticed an increase on the particles aggregation of CB after the oxidation and silanization procedures. This behavior can be attributed to the incorporation of the functional groups, such as hydroxyl, carbonyl and carboxyl (CB-oxi) and organosilane both on the surface and in the inner pores of the CB matrix, leading to a significant decrease of the specific surface areas and, as consequence, decreases the volume pore, as can be seen in Table 2.[32] One should note that the average pore diameter of the CB-oxi has been lower than one achieved for the virgin CB, most likely due to the presence of hydrophilic groups on the surface of the CB-oxi, resulting in strong interaction between particles through hydrogen bonds. On the other hand, the grafting process of 3-MPTMS, a larger molecule, in the narrow pores of CB-oxi promoted a significant increase on the average pore diameter (18.65 nm).

The porosity of materials has also been evaluated by means of nitrogen adsorption isotherms (Figures 4a, 4b and 4c). As can be seen in Figure 4a, the amount of nitrogen adsorbed is small even at high relative pressure (P/P_0 = 0.7), thus suggesting the presence of pores of short volume and large diameter on the 3-MPTMS/CB. This type of isotherm belongs to type IV, which indicates the presence of mesopores, generating a hysteresis of the type H3 and the presence of different pores sizes presenting parallel shapes of wedge, cones and/or plates.[33] The volume of N_2 adsorbed by CB-oxi (Figure 4b) was considerably larger than one observed for 3-MPTMS/CB, even at low relative

pressures. Furthermore, it has been observed an almost linear behavior for the adsorption and desorption isotherms for the CB-oxi material, with isotherm characteristic of type IV and H2 hysteresis, with cylindrical pores and strangulations. The hysteresis observed even at low relative pressure shows that CB-oxi presents a higher heterogeneity of pores size among the materials studied. Finally, as shown in Figure 4c, the hysteresis phenomenon for virgin CB was not observed, since this behavior is much more pronounced in adsorbents with low pore volume.[17,33] Nevertheless, it worth emphasize that the absence of hysteresis does not mean the absence of porosity of the virgin CB, once some pore shapes may give rise to equal adsorption and desorption process.[33]

The efficiency of the CB silanization has also been assessed by the energy dispersive spectrum (Figure 5) and elemental mapping (Figure 6). As the electron beam penetrates some nanometers in the material, the technique gives an estimative of elemental composition on the surface of material and not in bulk, which is considered of paramount importance in adsorption process. From achieved results, a uniform distribution of S and Si were observed in the surface of the 3-MPTMS/CB, revealing a good interaction of the modifier reagent (3-MPTMS) with the functional groups generated at the CB surface after acid treatment, without any phase of segregation. The percentage values (wt%) for C, O, Si and S on the 3-MPTMS/CB material were found to be 55.9, 29.6, 7.3, 7.2 respectively, confirming the presence of the modifier agent on the adsorbent material matrix. Bearing in mind a silanization process, the amount of Si and S obtained can be considered very satisfactory by comparing with literature data[4,34], thus demonstrating the successful of functionalization herein adopted.

In this work, Raman spectroscopy was used to evaluate graphitization levels of CB after the chemical functionalization (Figure 7). The first-order Raman spectra of carbonaceous materials are characterized mainly by two strong peaks: the G (“Graphite”) band around 1600 cm^{-1}, indicative of sp^2-hybridized carbons and the D (“defect or disorder”) band around 1350 cm^{-1}, originates from sp^3-hybridized carbons.[35] In addition,

the intensity ratio $I_{(D)}/I_{(G)}$ and the full width at half maximum of these bands are spectral parameters that exhibit a strong relation with the microstructure of the carbonaceous materials. According to Figure 7, the $I_{(D)}/I_{(G)}$ratio decrease from 0.904 for virgin CB to 0.799 for 3-MPTMS/CB and CB-oxi, indicating that both the insertion of oxidizing functional groups, such as organosilane on the carbonaceous matrix, promotes a decrease in disorder of the graphitic structure of nano-carbon black.

Thermal stability of materials has been evaluated through thermogravimetric analysis (data not shown). A weight loss of ca 2.32, 4.7 and 24.2 % for 3-MPTMS/CB, virgin CB and CB-oxi, respectively, was observed in the temperature interval of 30 – 77.5 ℃, which is ascribed to the removal of physically adsorbed water. The higher loss of water observed to CB-oxi can be attributed to the higher amount of hydrophilic groups such as, hydroxyl, carbonyl and carboxylic in the surface of this materials. Virgin CB is the most thermally stable material with no significant weight loss in the evaluated temperature range (30 – 800 ℃). For 3-MPTMS/CB two well defined peaks were observed at 360 ℃ and 514 ℃, which can be attributed, respectively, to decomposition side groups created during after oxidation process still present in the material and the organosilane 3-MPTMS bonded to carbonaceous matrix.[4,36] In addition, based on thermogravimetrics analysis, the higher amount of residue of about 64.9% for 3-MPTMS/CB in comparison to CB-oxi (52.0 %), can be explained due to the presence of silica in the modified CB, confirming the functionalization process.[4,30,37]

On-line preconcentration procedure and optimization strategy

The optimization procedure of the on-line preconcentration method was carried out by using a 2^{5-1} fractional factorial design and results were analyzed through a Pareto chart with a confidence interval of 95% (Figure 8). As can be observed from Pareto chart, all studied variables and the majority of the interactions were statistically significant. As

regards the pH, the negative effect (-13.47) demonstrated that adsorption of Pb(II) on the surface of 3-MPTMS/CB occurs preferably in acidic medium. Considering that in experimental domain (pH 4-7), the 3-MPTMS bonded on the CB is in its protonated form (for instance pKa of thiol group is 10.2), the results of pH on adsorption of Pb(II) can be explained based on the formation of hydroxyl species, such as $Pb(OH)^{+}$ at higher pH values, which leads to less effective interaction on the surface of adsorbent. Similarly, high adsorption of Pb(II) ions was observed when the buffer concentration was used in its low level (0.01 mol L^{-1}). The use of concentrated buffer solution provides a highly electrolytic medium, which in turn may difficult the mass transfer of Pb(II) towards binding sites of 3-MPTMS/CB. The adsorbent mass (AM) was the third most important factor on the preconcentration system and the use of the mini-column containing a large amount of 3-MPTMS/CB provides a positive effect, which can be attributed to the higher amount of active sites available on the adsorbent material. So, 100.0 mg was chosen for further studies under dynamic conditions. The eluent concentration (EC) was significant at its higher studied level. So, the 2.0 mol L^{-1} HNO_3 was chosen for all experiments to avoid possible memory effect during several preconcentration/elution steps. A negative effect was observed for the flow of preconcentration (FPR) (-4.83) when experiments were performed in the experimental domain (4.0 to 6.0 mL min^{-1}), thus suggesting that higher flow rates decreases the analytical signal due to slow adsorption kinetics of Pb^{2+} on the material. Thus, as a compromise between sensitivity and sample throughput, 4.0 mL min^{-1} was set as the best condition for further experiments. According to aforementioned results, it was necessary to perform the final optimization of pH and buffer concentration and, for this task, a Doehlert matrix[38] (Figure 9) was employed whose results are expressed in Table 3. To avoid systematic errors, the assays carried out in triplicate were performed randomly. The quadratic model (Equation 2) obtained from Doehlert matrix was considered to be statistically significant, since lack of fit was not significant [(experimental

$F_{1,2}(2.860)$ < tabulated $F_{1,2}$ (18.51)].[39] Then, the pH and buffer concentration values defined from the quadratic model were found to be 4.76 and 0.0358 mol L^{-1}, respectively.

$$Abs = -0.0926 + 0.1691pH - 0.0241pH^2 - 5.7251CT - 22.8741CT^2 + 1.6414pH \times CT$$

(Equation 2)

Effect of interfering ions on the preconcentration system

The effect of various interfering ions on the Pb(II) ions preconcentration was investigated. For this task, 20.0 mL of binary solution containing 200.0 µg L^{-1} Pb(II) ions at different proportions (analyte:concomitant ion) (Table 4) were submitted to the optimized preconcentration procedure and the results of . As shown in Table 4, in general, high analytical recoveries for Pb(II) were achieved even in the presence of possible interfering ions. The explanation about the absence of interference on solid phase is not an easy task, but according to Pearson's theory[40] and the high adsorption capacity, Pb(II) is prone to establish higher interaction with 3-MPTMS/CB when compared to the other ions. For the Ba^{2+} and Mg^{2+} ions, a positive interference was observed when studied in the proportion 1:100 (w/w), probably due to higher affinity these ions for oxygen atoms in the flame, favoring the formation of oxides, influencing strongly the thermodynamic equilibrium theses ions after atomization.[41] On the other hand, for Fe^{3+} ions, studied in the proportion 1:50 (w/w), exhibited a negative interference, probably due to formation of $Fe(OH)_3$.

Analytical performance of the proposed on-line preconcentration method

The analytical performance of the proposed on-line preconcentration method was estimated for the 3-MPTMS/CB material, under the optimum conditions. A linear graph was constructed from solutions of Pb(II) in the concentration range from 0.0 – 220 µg L^{-1},

providing the equation Abs = 0.0025 + 0.0011[Pb(II), μg L^{-1}] with a linear correlation coefficient of 0.9997. For this equation, the limits of detection and quantification, calculated according to the IUPAC definition[42], were found to be 1.33 μg $L^{-1}$and 4.45 μg L^{-1}, respectively. The precision evaluated as repeatability for 10 measures of Pb^{2+} at 10.0 μg L^{-1} and 160.0 μg L^{-1} concentrations, was found to be 3.0 and 2.3 % (Relative standard deviation, RSD %). Under optimized conditions, the preconcentration factor (PF), determined as the ratio between the slopes calculated for the Pb(II) preconcentration using the 3-MPTMS/CB adsorbent and the Pb(II) without preconcentration process (direct aspiration into the FAAS detector), was found to be 28. The enhanced preconcentration efficiency, promoted by functionalization of CB was confirmed by the ratio between the slopes obtained from two calibration curves using 3-MPTMS/CB and CB-oxi as adsorbents. An increase of about 40% on the sensibility of the method was obtained when 3-MPTMS/CB was used as adsorbent. This result demonstrates that even CB-oxi having higher surface area, its performance to uptake Pb(II) was lower than 3-MPTMS/CB, thus clearly justifying the modification of CB with functional organosilanes for metal ions uptake. Other figures of merit commonly evaluated, such as concentration efficiency (CE), consumptive index (CI) and sample throughput (ST) were determined for assessing the efficiency of the proposed method.[43] The concentration efficiency (CE), which is defined as the preconcentration factor obtained by absorbent during 1 min of preconcentration step was found to be 5.6 min^{-1}, whereas the consumptive index (CI), defined as the minimum volume of sample required to achieve one unit of preconcentration factor, was found to be 0.714mL. The sample throughput (ST) was 12 h^{-1}. The analytical performance of the preconcentration method was compared with previously published method using other carbonaceous materials as solid phase and FAAS as detector (Table 5). The low sample consumption (20.0 mL), as well as the wide linear range and low limit of detection can be considered the highlight of the proposed method. The chemical stability of 3-MPTMS/CB can also be considered an advantage of proposed method, because more than 300

preconcentration/elution cycles under acidic conditions were carried out without any loss of adsorption capacity. Therefore, up to now it is not possible to accurately mention the life time of mini-column, but the number of cycles already obtained is considerably very satisfactory.

Study of the adsorption capacity of 3-MPTMS/CB under dynamic conditions (breakthrough curve)

In order to evaluate the maximum adsorption capacity of 3-MPTMS/CB under dynamic conditions towards adsorption of Pb(II), breakthrough curves were performed at different flow rates. As can be seen in Figure 10, breakthrough volumes of 8.0, 10.0 and 22.0 mL for the flow rates of 2.0, 4.0 and 6.0 mL min^{-1} were obtained, yielding adsorption of 0.024, 0.030 and 0.066 mg g^{-1}, respectively. Yet, the respective maximum adsorption capacities of 0.96, 2.06 and 2.36 mg g^{-1} were obtained when 98.0, 240.0 and 376.0 mL of 3.0 mg L^{-1} Pb(II) solution at 2.0, 4.0 and 6.0 mL min^{-1} flow rates were percolated through mini-column. The results of breakthrough volumes reflect the differences on the flow rates, i.e., the higher the flow rates the greater the breakthrough volumes, due to low contact time of solution on the mini-column. On the other hand, in spite of low contact time, the breakthrough volume for flow rate of 6.0 mL min^{-1} has been the highest one (22.0 mL), which explain the highest adsorption of 0.066 mg g^{-1}.

It worth emphasize that the new adsorbent showed a high adsorption capacity in any flow rate when compared to carbon nanotube functionalized with 3- MPTMS (0.48 mg g^{-1}).[22]

Application of the method to real samples

The feasibility of the method in real samples was evaluated by analysis of different kinds of water and food samples, followed by adding known amounts of Pb(II) to samples.

The results of the method application are listed in Table 6. The percentage of recoveries by preconcentration system ranged from 90.81 to 107.63 %, which demonstrated that Pb(II) could be determined in water and food samples subjected to acid digestion without matrix effect. Furthermore, the accuracy of the method was checked from the analysis of the certified reference material MESS-3 (marine sediment). The obtained result by the method (19.91±1.00 mg Kg^{-1}) was statistically equal to the Pb(II) concentration in the certified material (21.1±0.70 mg kg^{-1}) at 95% confidence interval (*Student t-test*) (n=3) (Table 6).

Conclusion

In this paper we described the development of a new solid phase extraction method based on on-line preconcentration of Pb(II) using CB modified with 3-mercaptopropiltrimetoxysilane. The modification of CB was successfully confirmed through FT-IR, SEM, EDS, TG, Raman spectroscopy and surface analysis by the BET method. The use of 3-MPTMS/CB as a low-cost and efficient adsorbent material in detriment to other nanocarbonaceous material, such as carbon nanotubes, was assured by good analytical performance including low sample consumption, low limit of detection and applicability to a wide range of samples without any matrix effect. In summary, due to advantages of developed method assured by performance of 3-MPTMS/CB as adsorbent, the strategy herein adopted, which is still largely unexplored, may provide new insight into application on cheap and abundant nanocarbonaceous materials in the field of separation science.

Acknowledgements

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Figure Captions

Figure 1. Schematic representation of CB functionalized with 3-MPTMS and interaction with Pb(II)

Figure 2. FT-IR spectra for virgin CB, CB-oxi and 3-MPTMS/CB.

Figure 3. SEM images of CB with magnification of 50000 (a) virgin (b) CB-oxiand (c)3-MPTMS/CB. Conditions: accelerating voltage of 30.0 kV.

Figure 4. Nitrogen adsorption isotherms on (a) 3-MPTMS/CB, (b) CB-oxi and (c) virgin CB

Figure 5. EDS spectrum of 3-MPTMS/CB

Figure 6. Elemental mapping of Si and S of 3-MPTMS/CB.

Figure 7. Raman spectra of virgin CB, CB-oxi and 3-MPTMS/CB.

Figure 8. Pareto chart showing the principal effects and their interactions using absorbance as analytical response.

Figure 9. Surface response to the variables: pH and buffer concentration.

Figure 10. Breakthrough curves for the CB-3 MPT using 3.0 mg L^{-1} Pb(II) solution at 2.0, 4.0 and 6.0 mL min^{-1}.

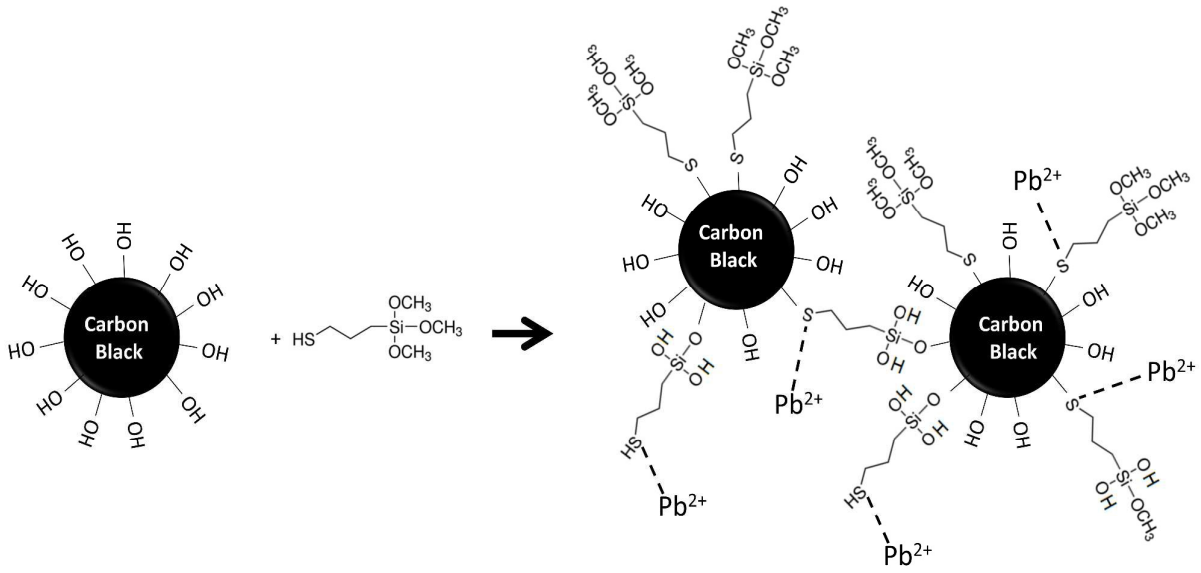


Figure 1.Nanicuacua et. al.

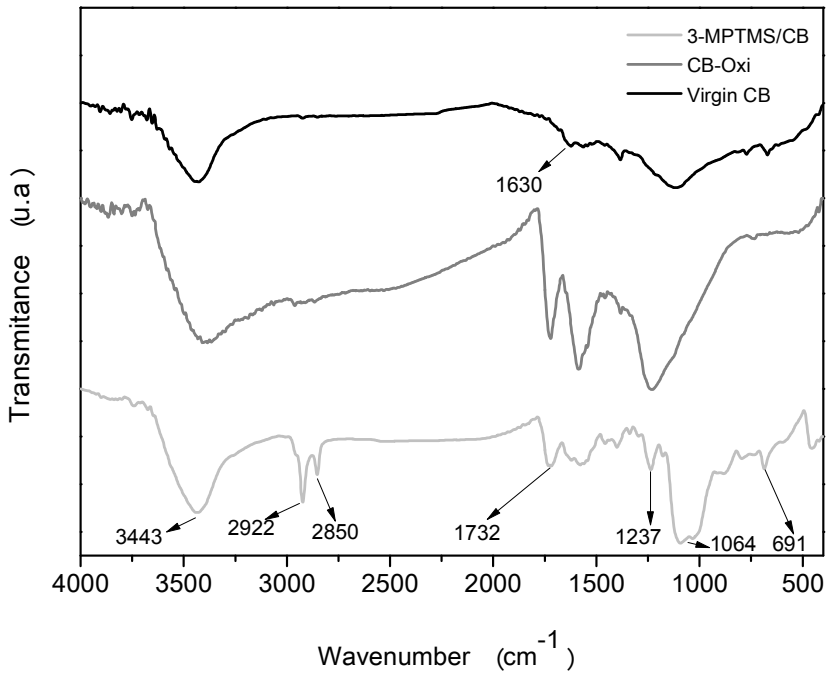


Figure 2. Nanicuacuaet. al.

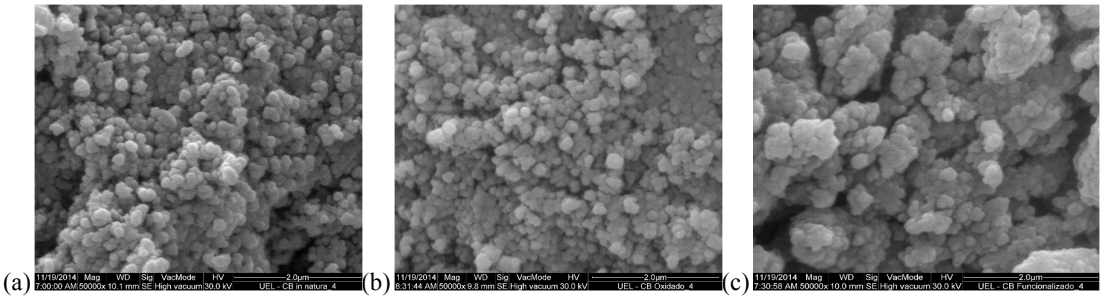
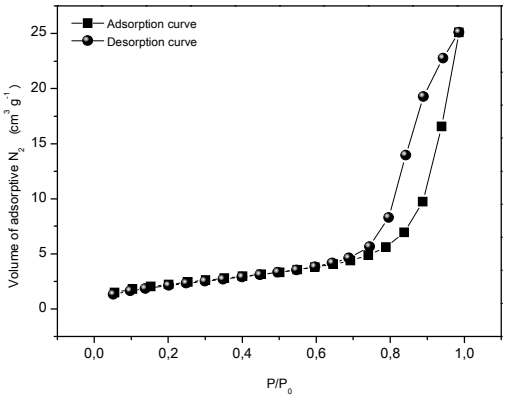
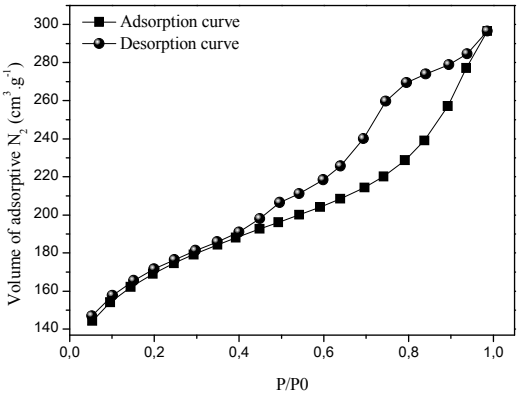


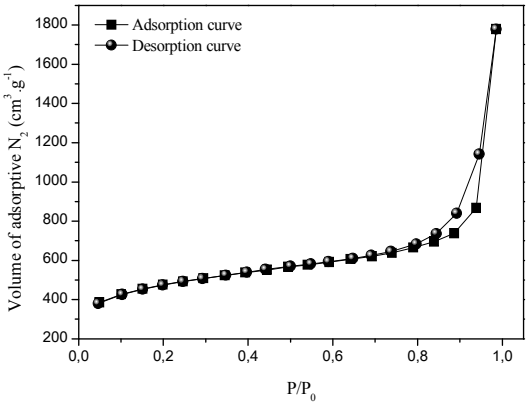
Figure 3. Nanicuacua et. al.



(a)



(b)



(c)

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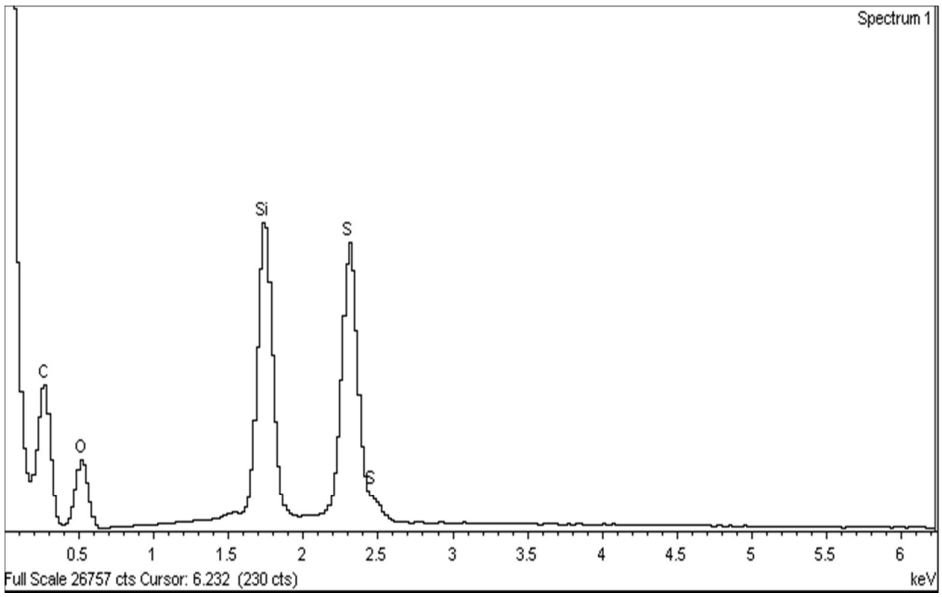


Figure 5. Nanicuacua et. al.

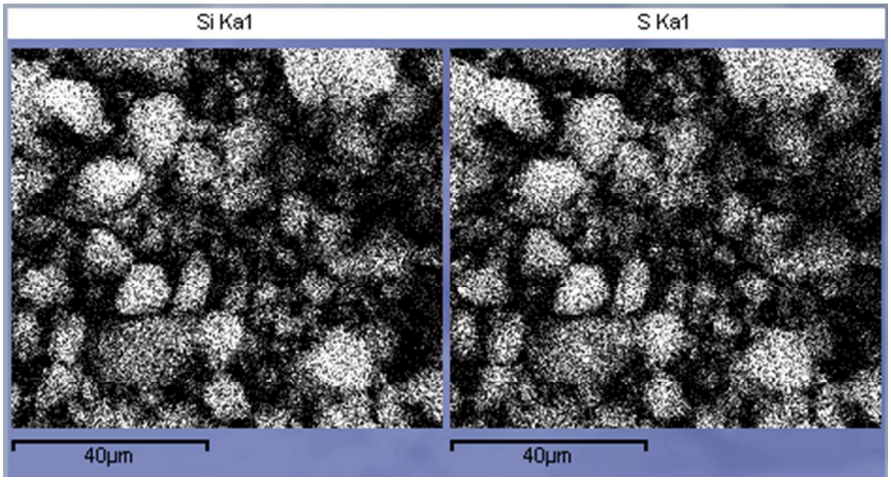


Figure 6. Nanicuacua et. al.

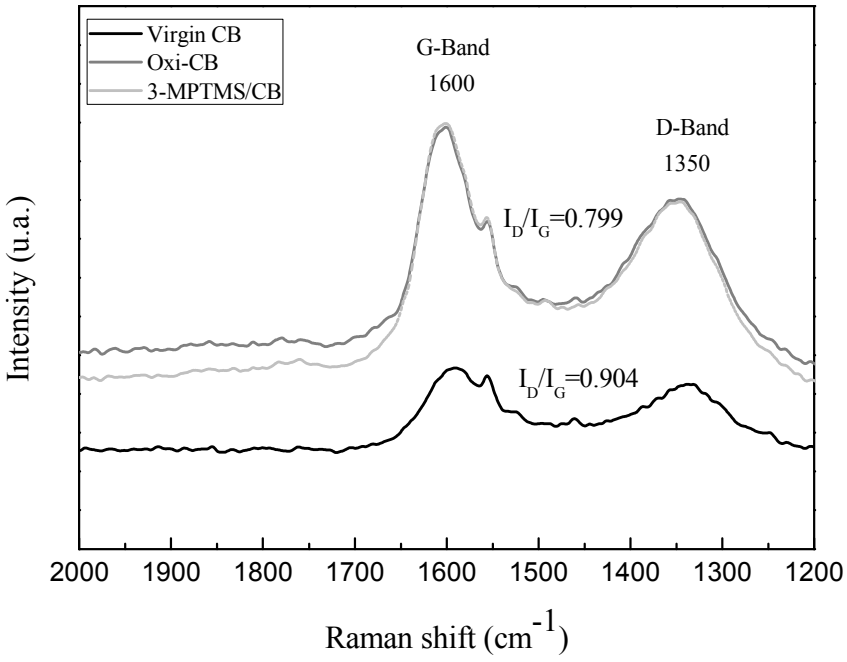
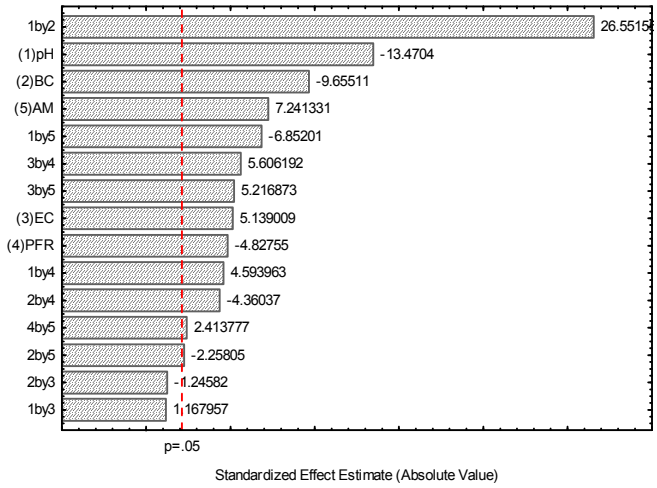


Figure 7. Nanicuacua et. al.



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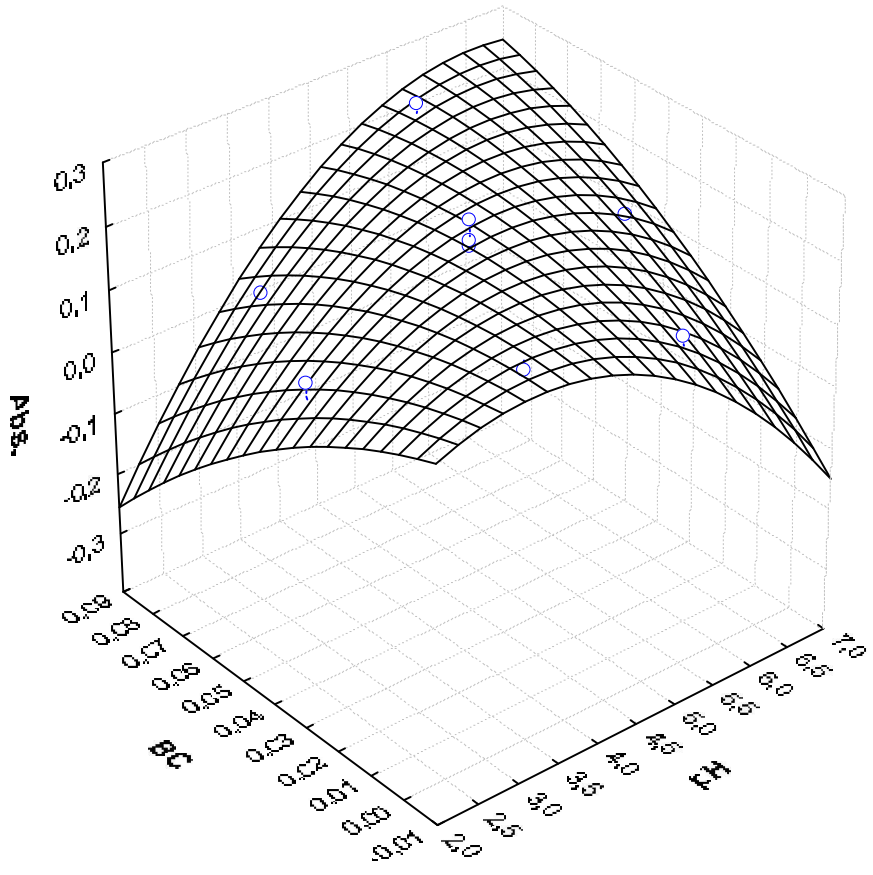

Figure 9. Nanicuacua et. al.

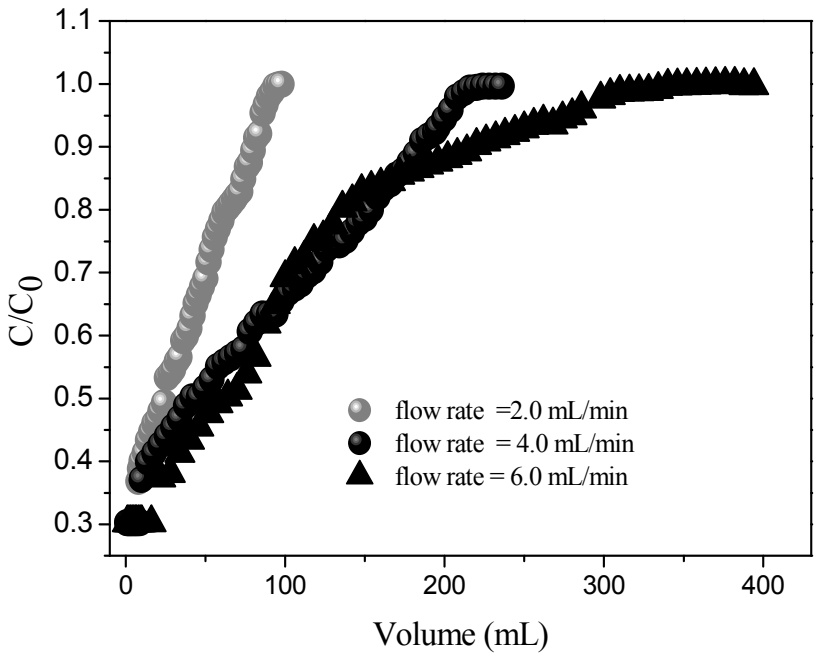

Figure 10. Nanicuacua et. al.

Table 1. Factors and their levels employed on the 2^{5-1} fractional factorial design.

Factors	Levels	
	Low (-)	High (+)
pH	4.0[a]	7.0[b]
BC (mol L^{-1})	0.01	0.1
EC HNO_3 (mol L^{-1})	1.0	2.0
PFR (mL min^{-1})	4.0	6.0
AM (mg)	50.0	100.0

BC: buffer concentration; EC: eluent concentration; PFR preconcentration flow rate; AM: adsorbent mass. [a] = acetate/acetic acid buffer solution; [b] = Tris-HCl buffer solution

Table 2. Textural parameters of virgin CB, CB-oxi and 3-MPTMS/CB

Carbon black	Surface area ($m^2 g^{-1}$)	Pore volume ($cm^3 g^{-1}$)	Average pore diameter (nm)
Virgin	1573	2.751	6.99
Oxidized	551.1	0.458	3.33
3-MPTMS/CB	8.33	0.039	18.65

Table 3. Doehlert matrix used on the optimization of Pb(II) solid phase preconcentration using 3-MPTMS/CB

Assays	Buffer concentration (mol L^{-1})	pH	Average of absorbance (peak height)
1	0 (0.0405)	0 (4.5)	0.2107
2	0 (0.0405)	1 (6.5)	0.1377
3	0.866 (0.081)	0.5 (5.5)	0.2411
4	0 (0.0405)	-1 (2.5)	0.0911
5	-0.866 (0.001)	-0.5 (3.5)	0.1897
6	-0.866 (0.001)	0.5 (5.5)	0.1273
7	0.866 (0.081)	-0.5 (3.5)	0.0416

The first number represents the Doehlert matrix coded values, whereas the values between parentheses stand for the real values. pH 3,5, 4.5, 5.5 and 6.5 buffered with acetate/acetic acid and pH 2.5 buffered with KCl/HCl

Table 4. Effect of interfering ions on the preconcentration of Pb(II) using 3-MPTMS/CB as adsorbent

Foreign ions	Ratio (Pb(II)/foreign ion) (w/w)			
	1:1	1:10	1:50	1:100
	% Recovery			
Zn(II)	99.5	101.85	108.1	109.1
Ni(II)	103.2	104.4	104.54	93.0
Cd(II)	103.7	101.7	94.5	91.2
As(III)	107.89	90.78	100.7	97.3
Cu(II)	97.9	107.7	104.2	90.6
Ca(II)	-	-	96.2	107
Ba(II)	-	-	108.8	127.5
Mg(II)	-	-	109.8	127
Fe(III)	99.4	101.4	61.8	-

Table 5. Comparison of the analytical performance of different solid phase preconcentration for Pb(II) determination by FAAS.

Adsorbents	Modifier	PF	Linear range ($\mu g\ L^{-1}$)	VP (mL)	LOD ($\mu g\ L^{-1}$)	Application	Ref.
MWCNT	3-MPTMS	31.5	5.0 – 130.0	20	1.71	Waters samples and certified reference materials (PACS-2)	22
MWCNT	HNO_3	44.2	8.6 – 775	20.0	2.6	Waters, physiological serum, synthetic seawater, garlic and Ginkgo Biloba	44
MWCNT	Poly (2-aminothiophenol)	304	3.0 – 110	500	1.00	Water, sediment and fish samples	45
MWCNT	Ammonium pyrrolidinedithiocarbamate	80	---	400	0.6	Waters samples and certified reference materials	46
MWCNT	Ethylenediamine	60	---	300	0.3	Water samples	47
MWCNT	HSPIMP	17.9	0.02 – 0.35	250	2.89	Leek, radish, and banana	48
MWCNT	o-cresolphtaleincomplexone	40	---	400	3.52	Real environmental water sample	49
Carbon black	3-MPTMS	27.5	10.0 – 220	20	1.36	Waters and Ginkgo Biloba samples and certified reference material	This work

PF = preconcentration factor; VP = volume of preconcentration; LOD = limit of detection; 3-MPTMS = 3-mercaptopropyltrimethoxysilane; HSPIMP = 3-hydroxy-4-((3-silylpropylimino)methyl) phenol.

Table 6. Application of the on-line preconcentration method of Pb(II) using CB-3 MPT as solid phase in real samples.

Samples	$[Pb^{2+}]$ (μg.L^{-1})		
	Amount added (μg L^{-1})	Amount found[a] (μg L^{-1})	Recovery (%)
Tap water	-	ND	-
	8.0	7.80±0.81	97.5
Mineral water	-	ND	-
	8.0	7.50±0.17	93.7
Physiological serum	-	ND	-
	8.0	8.74±0.05	107.6
Water Igapó lake	-	ND	-
	8.0	8.46 ± 0.03	105.7
Ginkgo Biloba[b]	-	0.80±0.07	-
	0.8[b]	1.45±0.23	90.8

ND, below limit of detection
[a]Results are expressed as mean value ± standard deviation based on three replicates (n=3)
[b] Units expressed in μg g^{-1}

Table 7. Determination of Pb(II) in certified reference material

	Certified value	Value founded by the analytical method[a]
MESS-3 (marine sediment) mg Kg^{-1}	21.10 ± 0.70	19.91 ± 1.00

[a]Results expressed as mean ± standard deviation based on three repetitions; confidence interval of 95% (Student's t test).

GRAPHICAL ABSTRACT