Spectrochimica Acta Part B 103–104 (2015) 49–56

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

journal homepage: www.elsevier.com/locate/sab

Activated carbon-modified knotted reactor coupled to electrothermal atomic absorption spectrometry for sensitive determination of arsenic species in medicinal herbs and tea infusions

Alexander Castro Grijalba [a,b], Estefanía M. Martinis [a,b], Gustavo E. Lascalea [b], Rodolfo G. Wuilloud [a,b,*]

[a] Laboratory of Analytical Chemistry for Research and Development (QUIANID), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Padre J. Contreras 1300, (5500) Mendoza, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 10 September 2014
Accepted 24 November 2014
Available online 3 December 2014

Keywords:
Knotted reactor
Arsenic speciation
Medicinal herb
Tea
ETAAS

ABSTRACT

A flow injection system based on a modified polytetrafluoroethylene (PTFE) knotted reactor (KR) was developed for arsenite [As(III)] and arsenate [As(V)] species preconcentration and determination by electrothermal atomic absorption spectrometry (ETAAS). Activated carbon (AC) was immobilized on the inner walls of a PTFE KR by a thermal treatment. A significant increase in analyte retention was obtained with the AC-modified KR (100%) as compared to the regular PTFE KR (25%). The preconcentration method involved the on-line formation of As(III)-ammonium pyrrolidinedithiocarbamate (As-APDC) complex, followed by its adsorption onto the inner walls of the AC-modified KR. After analyte retention, the complex was eluted with acetone directly into the graphite furnace of ETAAS. The parameters affecting the flow injection system were evaluated with a full central composite face centered design with three center points. Under optimum conditions, a preconcentration factor of 200 was obtained with 10 ml of sample. The detection limit was 4 ng L^{-1} and the relative standard deviation (RSD) for six replicate measurements at 0.2 μg L^{-1} of As were 4.3% and 4.7% for As(III) and As(V), respectively. The developed methodology was highly selective towards As(III), while As(V), monomethylarsonic acid [MMA(V)] and dimethylarsinic [DMA(V)] were not retained in the AC-modified KR. The proposed method was successfully applied for As speciation analysis in infusions originated from medicinal herbs and tea.

1. Introduction

The demand for rapid, selective, and sensitive methodologies for determination of arsenic (As) species is increasing as a consequence of its deleterious impact on human health [1]. Widely known as a toxic element, As may naturally occur in environmental compartments under different species, depending on the nature of the sample [2]. Both the toxicological behavior and biochemical activity of As depend on its chemical forms [3]. Therefore, determination of total As in a sample does not fulfill the understanding about the toxicological risks derived from this element in environmental and biological studies. Consequently, speciation analysis of As becomes highly necessary.

Tea (*Camellia sinensis*) is a popular non-alcoholic and healthy beverage widely consumed in the world [4]. Even when a regular consumption of infusions of medicinal herbs and tea may contribute to the daily dietary requirements of several essential elements [5,6], some toxic metals and organic pollutants are ingested as well [7]. Specifically for As, its inorganic species, arsenite [As(III)] and arsenate [As(V)], which are much more toxic than the organic ones, generally predominate in infusion samples [8]. Therefore, considering the massive consumption of infusions in some countries [4], determination of As in infusions of tea and medicinal herbs is highly needed to evaluate As daily dietary intake [5]. Different instrumental techniques including atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES) [9], inductively coupled plasma mass spectrometry (ICP-MS) [10], and capillary electrophoresis (CE) [11] have been used to determine As levels in tea leaves and infusions. However, since the reported concentrations of total As in tea leaves are below 0.08 mg kg^{-1}, preconcentration methodologies are required prior its determination to achieve accurate, sensitive and reliable results [5].

Analytical methods for preconcentration and speciation of metals and metalloids are numerous and several devices have been used for analyte retention [12,13]. Among them, knotted reactors (KR) have been successfully employed in flow injection methodologies based on the sorption of metal complexes on the inner walls of these reactors [14]. Knotted reactors are open tubes made of fully knotted polytetrafluoroethylene (PTFE) tubing. Both the effect of centrifugal

* Corresponding author at: Laboratory of Analytical Chemistry for Research and Development (QUIANID), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Padre J. Contreras 1300, (5500) Mendoza, Argentina. Tel.: +54 261 4259738.
E-mail addresses: rwuilloud@mendoza-conicet.gob.ar, rodolfowuilloud@gmail.com (R.G. Wuilloud).

http://dx.doi.org/10.1016/j.sab.2014.11.007
0584-8547/

force generated by the steady change in flow direction due to the knots and the hydrophobic nature of PTFE surface allow the retention of analytes as precipitates [15]. Since lower back-pressures are observed in KRs compared to those observed in packed columns at similar flow rates, higher sample loading flow rates and elution efficiency can be obtained. Furthermore, a KR is easily manufactured without needing packing materials, offering an almost unlimited lifetime due to chemical inertness of PTFE. Additional advantages of KRs are: high stability under different sorption conditions, rapid mixing of sample and reagent solutions, low hydrodynamic impedance and minimum analyte dispersion, allowing high load sample rates and increased sample throughput [16]. Nevertheless, retention capacity of KRs is limited (usually lower than 30%), thus conditioning the enrichment factors obtained with on-line preconcentration methods using these reactors [17,18]. In fact, the use of regular PTFE KRs has been reported previously for preconcentration and determination of As(III) by ICP-MS [19], HG-AFS [20] and ETAAS [21]. However, the sensitivity enhancement factors obtained with these types of KRs has been low and ranged between 11 and 44.

The aim of the present work was the modification of the inner walls of a classical PTFE KR to solve one of its main drawbacks for analytes preconcentration: the low retention capacity. The modification of the inner walls of the KR was performed using activated carbon (AC), which is widely known for its excellent sorption capacity. Therefore, a novel on-line analytical methodology based on an AC-modified KR was developed for simple and highly selective preconcentration of As species. As(III) reacted with ammonium pyrrolidinedithiocarbamate (APDC) forming a precipitated complex which was adsorbed on the walls of the KR. An experimental design (CCD) was used for the optimization of the factors that influence the preconcentration of the formed complex. The complex was eluted with a few microliters of acetone directly into the graphite furnace of ETAAS for As determination. The performance of the AC-modified KR was evaluated under flow conditions with an on-line system coupled to ETAAS. The proposed methodology proved to be particularly useful to obtain quantitative information on inorganic As(III) and As(V) species concentrations in infusions of medicinal herbs and tea samples.

2. Experimental

2.1. Instrumentation

Measurements were performed with a PerkinElmer (Überlingen, Germany) Model 5100 ZL atomic absorption spectrometer equipped with a transversely heated graphite atomizer with a Zeeman-effect background correction system. An As electrodeless discharge lamp (EDL) operated at a current of 300 mA (modulated operation) and a wavelength of 193.7 nm with a spectral band pass of 0.7 nm was used. All measurements were made based on absorbance signals with an integration time of 5 s. Instrumental parameters are listed in Table 1. Gilson Minipuls 3 tygon-type pump tubing was used to carry the sample and APDC solution. The eluent (acetone) was carried using a silicone tube. The sample injection and the elution were performed using six-way rotary valve from Upchurch Scientific (Oak Harbor, WA, USA). The KR flow injection system is shown in Fig. 1. The modification of inner walls of KR and its morphological changes were observed by scanning electron microscopy (SEM) (LEO 1450 VP, Zeiss, Germany).

2.2. Reagents

All the reagents were of analytical grade and the presence of As was not detected within the working range. Stock standard solutions of inorganic As(V) and As(III) species (1000 mg L^{-1}) as sodium arsenate dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$) (99.998%) (Sigma-Aldrich, Milwaukee, WI, USA) and sodium metaarsenite ($AsNaO_2$) (99%) (Fluka, Buchs, Switzerland), respectively, were prepared in 0.1 mol L^{-1} HCl.

Table 1
Instrumental and experimental conditions for As determination.

Instrumental conditions				
Wavelength (nm)				193.7
Spectral band width (nm)				0.7
EDL lamp current (mA)				300
Injection volume (μl)				50
Matrix modifiers				5 μg Pd [$Pd(NO_3)_2$] 3 μg Mg [$Mg(NO_3)_2$]
Graphite furnace temperature program				
Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Argon flow rate (ml min^{-1})
Drying	110	15	30	250
Pyrolysis	600	10	10	250
Atomization	2300	0	3	0
Cleaning	2400	1	2	250
Extraction conditions				
Sample volume (ml)				10
APDC concentration (% w/v)				0.1
HCl concentration (mol L^{-1})				0.01
Eluent				Acetone
Eluent volume (μl)				50
Sample loading flow rate (ml min^{-1})				5.0
APDC loading flow rate (ml min^{-1})				3.0
Elution flow rate (ml min^{-1})				0.2

Disodium methylarsonate ($CH_3AsNa_2O_3 \cdot 6H_2O$) (MMA, 98%) (Fluka) and dimethylarsinic ($C_2H_7AsO_2$) (DMA, 98.6%) (Fluka) stock standard solutions (1000 mg L^{-1}) were prepared with ultrapure water and stored at 4 °C in amber-colored HDPE bottles. Working solutions were prepared by diluting these stock solutions.

Hydrochloric acid (37%), purchased from Merck (Darmstadt, Germany), was used to guarantee acidic media in the samples and in As(III) standard solutions for optimization of the methodology. The chelating agent solution was prepared by dissolving APDC (Sigma-Aldrich, Milwaukee, WI, USA) in ultrapure water (18 MΩ cm) (Milli-Q water purification system, Millipore, Paris, France). Potassium iodide >99% (Fluka, Buchs, Switzerland) was used as reductant. Activated carbon (Merck) of 35–50 mesh ASTM was used for modification of KR inner surface. A 1000 mg L^{-1} palladium nitrate solution [$Pd(NO_3)_2 \cdot 2H_2O$ (Fluka)] and 150 mg L^{-1} magnesium nitrate solution [$Mg(NO_3)_2$ (Merck)], both in 0.1% (v/v) HNO_3 (Mallinckrodt Baker, Phillipsburg, NJ, USA), were prepared and used as chemical modifiers. Methanol (Merck) and acetone (Merck) were used as eluents. A 10% (v/v) nitric acid solution was prepared in ultrapure water to be assayed as elution agent. All bottles used for storing samples and standard solutions and the glassware were washed in 10% (v/v) HNO_3 for 24 h and later rinsed with ultrapure water.

2.3. Fabrication of the AC-modified KR

The immobilization of AC was performed by filling a PTFE tube (0.51 mm i.d. and 120 cm length) with dry AC. The tube was extended vertically by attaching its endings with two clamps to a universal support stand, and a small micropipette tip was installed on the top of the PTFE tube to serve as a funnel using a desk tape. The dry AC material was poured into the PTFE tube while beating the outside in order to obtain a compact filling. Subsequently, the PTFE tube was heated in a muffle furnace for 5 h at 327 °C. The PTFE tube used for this work was purchased from GRACE (Columbia, USA) and a melting point of 330 °C is specified by the manufacturer. The muffle furnace had a temperature tolerance of ±2 °C. After heating time, the tube was cooled until room temperature was reached. Then, the tube was washed with a 0.1 mol L^{-1} HNO_3 solution at 1 ml min^{-1} in order to easily remove free AC particles not immobilized on PTFE surface. A SEM micrograph showing the modification of the inner section of the AC-modified KR is shown in Fig. 2.

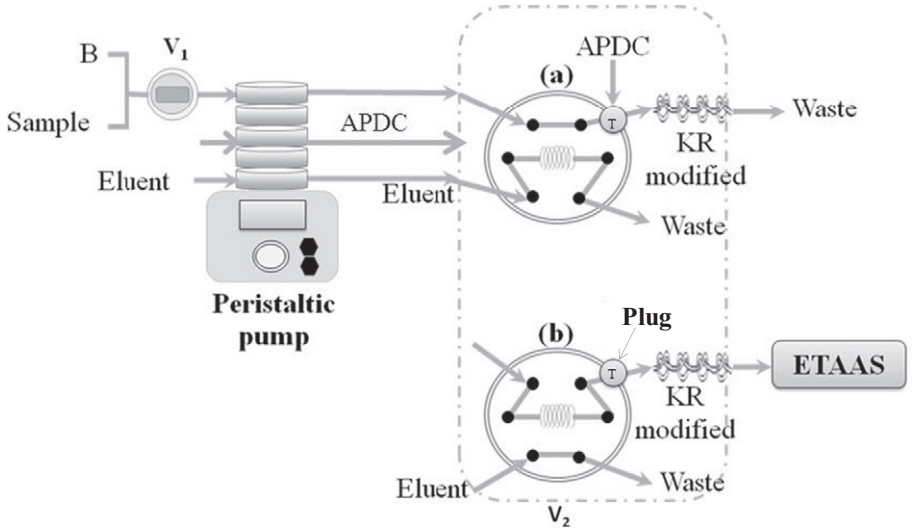

Fig. 1. Schematic diagram of the instrumental setup: V1 and V2: six-way valves; B: buffer solution; T: 0.8 mm three-way connector; KR: knotted reactor. (a) Load position. (b) Injection position.

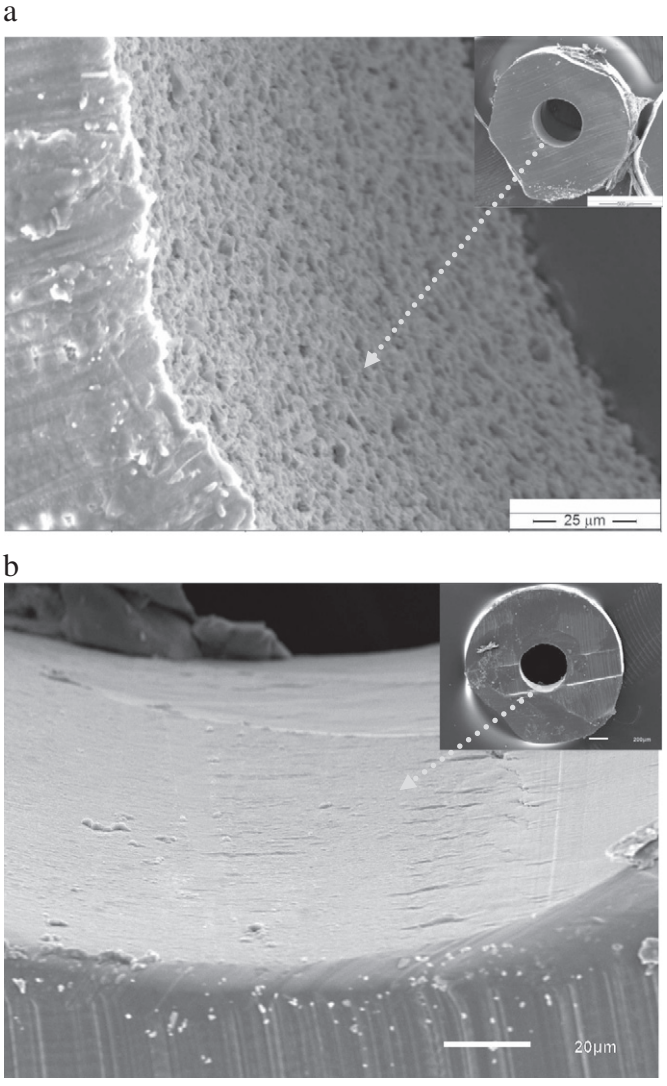

Fig. 2. SEM micrograph of the inner walls of (a) the AC-modified KR and (b) a regular PTFE KR.

2.4. Sample collection and preparation of the infusions

Medicinal herbs and tea [yerba mate (*Ilex paraguariensis*), tila (*Tilia platyphyllos*), camomile (*Matricaria chamonilla*), and tea (*C. sinensis*)] were purchased from local markets of Mendoza City (Argentina). Samples were stored in their original package until analysis. The infusions were prepared by the following extraction procedure: 10 ml of ultrapure water and 10 g of medicinal herb or tea were mixed in a glass beaker and heated for 15 min at 80 °C on a hot plate. The infusions were filtered with filter paper and diluted with ultrapure water up to 10 ml in a volumetric flask (extract).

2.5. Preconcentration and determination of As(III) species

Initially, the AC-modified KR was washed with 1.0 ml of methanol at a flow rate of 1.0 ml min^{-1} followed by 1.0 ml of water at a flow rate of 1.0 ml min^{-1}. The preconditioning was made with 1.0 ml of a 0.01 mol L^{-1} HCl solution at a flow rate of 1.0 ml min^{-1}. These washes were needed to avoid a possible contamination of the KR inner surface. Under the optimized conditions, no washes were performed except for the preconditioning step.

The operational sequence of the on-line AC-modified KR preconcentration system is described in Table 2. For As(III)–APDC complex formation and retention in the AC-modified KR, 10 ml of the extract containing As(III) with a final concentration of 0.01 mol L^{-1} of HCl at a flow rate of 5.0 ml min^{-1} were mixed on-line with a 0.1% (w/v) APDC solution at a flow rate of 3.0 ml min^{-1} by a three-way connector. The mixture was then loaded into the KR with valve V1 in sample position and valve V2 in load position (a) (Fig. 1). After sample loading, air was pumped into the system for emptying the AC-modified KR. Simultaneously, a 50 µl PTFE loop was filled with the eluent. After sample and eluent were loaded, the injection valve V2 was switched to the injection position (b) and the retained analyte was eluted with acetone at a flow rate of 0.2 ml min^{-1}. During the elution, the APDC flow was stopped and blocked in the three-way connector with a plug. The eluate was directly injected into the graphite furnace of ETAAS for As determination. Instrumental and preconcentration conditions are shown in Table 1. Pyrolysis and atomization temperatures of ETAAS were optimized to obtain the highest absorbance-to-background signal ratio. The effect of pyrolysis temperature was studied within the range of 600–1200 °C and the effect of atomization temperature was studied within the range of 2000–2350 °C. Thus, pyrolysis and atomization temperatures selected were 600 °C and 2300 °C, respectively. Furthermore, different

Table 2
Operational sequence of the on-line AC-modified KR preconcentration system for ETAAS determination of As(III), As(V) and total As (see Fig. 1).

Step	Function	Valve position	Duration (s)	Solution pumped	Flow rate (ml min^{-1})	Read
1	Preconditioning	a	60	HCl 0.01 mol L^{-1}	1.0	
2	Sample loading	a	120	Sample	5.0	
	Filling of eluent loop			Acetone	0.2	
	ADPC loading	a		0.1% (m/v) APDC	3.0	
3	Elution of the retained analyte complex	b	15	Acetone	0.2	yes

mixtures of $Mg(NO_3)_2$ and $Pd(NO_3)_2$ were tested as chemical modifiers. A signal improvement was observed when 3 μg of Mg and 5 μg of Pd were injected into the graphite furnace.

2.6. Preconcentration and determination of As(V) species

Concentrations of As(V) species were calculated by the difference between total As and As(III) concentrations. Total As was determined after reduction of As(V) to As(III) species. Selective reduction of As (V) to As(III) species was performed using a KI solution: 10 ml of the extracts were placed in a digestion flask and 0.1 ml of 20% (w/v) KI was added. The mixture was then heated for 10 min at 90 °C on a hot plate. After complete reduction, total As concentration was evaluated by following the same procedure as described earlier for As(III) species.

2.7. Optimization strategy

Different parameters could affect the preconcentration of As(III) in the AC-modified KR such as eluent, pH, APDC concentration and flows of the different reagents used during the formation of the complex. Since, the effect of pH on the formation of As(III)–APDC complex has been already studied by other authors, this factor was not covered in this work [21]. Then, a concentration of 0.01 mol L^{-1} of HCl was selected to guarantee the right pH for complex formation [21]. The loading flow rate of As(III) and APDC solutions, and the concentration of APDC solution were optimized using a multivariate optimization. Design Expert® 7.0 (Stat-Ease Inc., Minneapolis, USA) was used to process all the results obtained in this work. The eluent, the elution volume and the eluent flow rate were optimized by univariate method.

3. Results and discussion

3.1. Surface modification of the PTFE knotted reactor

Immobilization of AC on the inner walls of a PTFE tube was based on the thermal properties of the materials involved. Activated carbon is a versatile and efficient sorption material as consequence of its high surface area (500–1500 m^2 g^{-1}), microporous structure and high degree of surface reactivity. It can react with oxygen at temperatures below 400 °C resulting mainly in the chemisorption of oxygen and the formation of carbon–oxygen surface groups. These groups influence not only the superficial features of the material such as wettability, polarity, and acidity but also its physical–chemical properties such as catalytic or electrical characteristics and its chemical reactivity [22,23].

Initially, the PTFE tube filled with AC was placed in a muffle furnace at 250 °C for the immobilization of the sorption material. However, under these conditions no immobilization was observed, and the AC came out easily with the sole passage of ultrapure water when the KR was used under flow conditions. This fact could be attributed to acceptable thermal stability of PTFE for temperatures up to 260 °C [24]. In a second series of experiments, temperatures higher than 250 °C were used and optimum results were found at 327 °C, only three grades under PTFE melting point. Although at this temperature the shape of

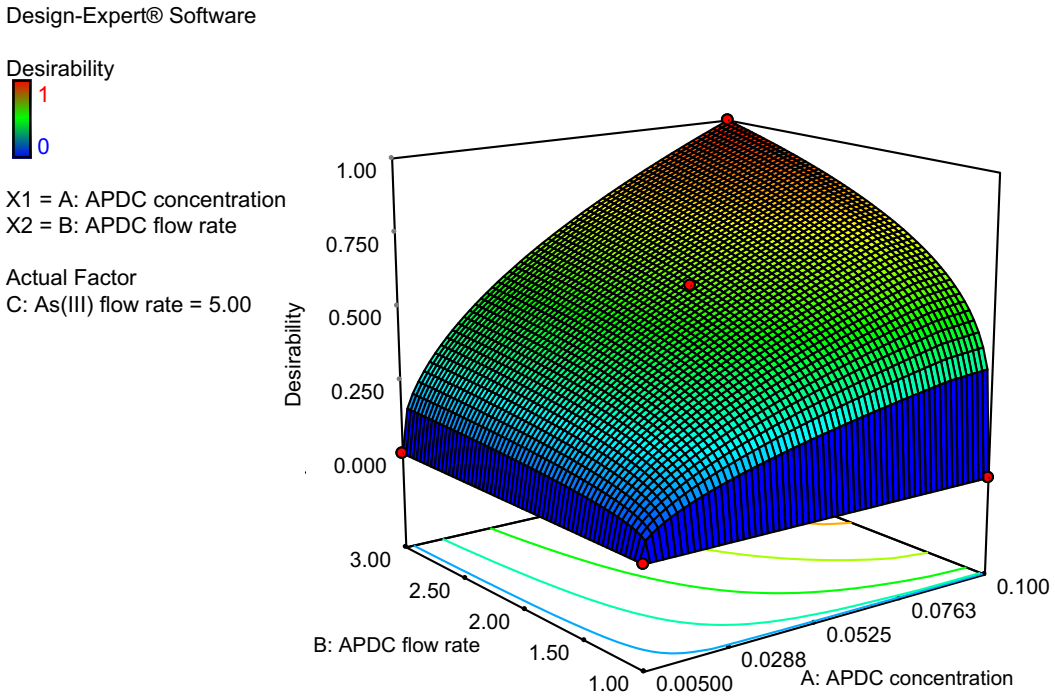

Fig. 3. Desirability function indicating the chosen conditions for optimization when As(III) sample loading flow rate is 5 ml min^{-1}.

PTFE tube remained quite unaltered, a decrease on its superficial stiffness was evident. Both the softening of the plastic material and the thermal expansion of the bulk PTFE of the tube promoted a full contact between the partially fluid PTFE inner walls and the AC particles, which, after cooling, remained well attached to the inner surface of the KR. On the other hand, at temperatures higher than 400 °C, chemical decomposition of the dense but liquid PTFE was observed as expected [24,25]. Thus, AC was successfully immobilized on PTFE surface of the KR, which remained unaltered even after hundreds of loading-elution cycles. A SEM micrograph (Fig. 2) shows a clear modification of the KR, evidenced by the increased roughness of the inner surface due to the dense covering of the wall that was fully coated with immobilized AC particles. The AC material covers the inner walls of the PTFE KR, leaving a central hole that allows the use of high flow rates for sample loading. Moreover, high flow rates can be applied with KRs as compared to other preconcentration devices, such as packed columns, due to the minimal back-pressure built up inside these reactors and upon the circulation of solutions [26,27].

3.2. Optimization of the on-line preconcentration system

The variables involved in complex formation along with its retention and elution were studied to maximize the analytical performance of the AC-modified KR. The effects of APDC concentration, its flow rate, and the sample loading flow rate used in the proposed on-line preconcentration system were carefully studied. APDC was chosen as the complexing agent since it is a common reagent used for As preconcentration with KRs [19–21]. Thus, the low solubility of the metal complexes formed with APDC and the high stability of this reagent at low pH, make it an excellent choice for preconcentration methods [14].

Initially, to optimize these variables for the highest analyte retention in KR a multivariate methodology was developed. Thus, 10 ml of 1 μg L^{-1} As(III) solution in 0.01 mol L^{-1} HCl was employed as a model loading solution. After on-line reaction between As(III) species and APDC was performed, and the complex was retained in the KR, the analyte was desorbed with 50 μl of acetone to assure total elution. The eluent solution was injected in the graphite furnace of ETAAS for As determination. A response surface methodology was used to optimize these variables and obtain a model to predict the response (analyte retention) as a function of the input variables. A full central composite face centered design with three center points for these three factors was applied to optimize the parameters for improving the retention of the complex As(III)–APDC on the inner walls of the AC-modified KR. The total number of design point (N) was calculated by means of the following equation:

$$\mathbf{N} = \mathbf{2^f} + \mathbf{2f} + \mathbf{Cp} \quad (1)$$

where f is the number of factors and Cp the number of center points [28]. Therefore, 17 experiments were needed for this central composite design. The low and high levels of these factors were as follows: As (III) solution flow rate (1.0–5.0 ml min^{-1}), APDC concentration (0.005–0.1% w/v) and APDC solution flow rate (1.0–3.0 ml min^{-1}). These experimental ranges were pre-selected according to previous works where KRs without surface modification have been used with APDC for preconcentration of As(III) [19,21]. The evaluated response was As(III) retention on the AC-modified KR.

According to the results obtained by this approach, a lineal model with interactions was obtained as expressed by the following equation:

$$[\mathrm{As(Ret)}]^{1/2} = 0.615 + 0.248A + 0.0632B - 0.00483C - 0.214AB + 0.804\,AC - 0.0129BC \quad (2)$$

where A = APDC concentration, B = APDC solution flow rate and C = As(III) solution flow rate. The model consisted of three main effects and three two-factor effects. R^2 was 0.975 and adjusted R^2 was 0.954.

Table 3
Criteria for the optimization of response to obtain the overall desirability (D).

Factor/Response	Goal	Lower limit	Upper limit	Optimal conditions
APDC loading flow rate	Maximize	1.0	3.0	3.0
Sample loading flow rate	Maximize	1.0	5.0	5.0
APDC concentration	In range	0.005	0.1	0.1
As(III) retention	Maximize	54.6	100	100

These values indicated a good quality of fit for the model equation. The response, in this case As(III) retention, had to be transformed in order to obtain a significant lack of fit. Therefore, the results were fit to the obtained model. These transformations are possible in the multivariate optimization [29,30]. By Eq. (2), it can be verified how the factors and their mutual interactions affected the response. The highest As retention was 100%. The graphics of predicted vs. actual values showed an adequate distribution, so optimum values were obtained using the desirability function (see Fig. 3 and Table 3). After the analysis of the results, the following conditions were chosen as optimal for next assays of the on-line preconcentration system: 0.1% (w/v) APDC at 3 ml min^{-1} and a loading flow rate of 5 ml min^{-1} for As(III) solution. The results obtained in this work are really outstanding with respect to the limited retention of regular PTFE KRs (30%), since a 100% As retention could be achieved with the AC-modified KR under the optimized conditions.

The length of the AC-modified KR was also evaluated. The length of the KR is an important factor to assure sufficient contact time between the analyte complex and the AC covering material. Thus, if the KR is too short the contact of the As(III)–APDC complex is insufficient and the retention would decrease. On the other hand, when the KR is too long, although the analyte adsorption efficiency would improve, the analyte dispersion would increase too, leading to higher dilution of the analyte during the elution step [19]. Four lengths: 90 cm, 120 cm, 160 cm and 200 cm were evaluated. With the shortest KR (90 cm) the analyte retention was less than 80%. The maximum recovery (100%) was obtained with a 120 cm AC-modified KR. Longer KRs required more than 50 μl eluent volume for efficient elution towards the graphite furnace of ETAAS instrument. Furthermore, since the elution step was performed by injecting a defined volume of solvent enclosed by air injected in the flow line, analyte dispersion inside the KR was limited. Thus, elution of the analyte was possible with only 50 μl of solvent.

In order to establish the best elution agent, three kinds of solvents were evaluated: methanol, acetone and a 10% (v/v) HNO_3 solution. In this part, a univariate methodology was used. When there are variables fixed at discrete values as 1 step, 2 steps or 3 steps for microextraction processes or different microextraction solvents such as carbon tetrachloride, chloroform among others, a mix of univariate and multivariate optimizations can be performed since the use of an experimental design

Table 4
Selectivity of the method for As(III) determination at 1.0 μg L^{-1} (95% confidence interval, n = 6).

Concentration ratio	As(III)	
As(V)/As(III)	Found (μg L^{-1})	Recovery (%)
2	0.98 ± 0.042	98
5	0.99 ± 0.049	99
10	1.02 ± 0.051	102
MMA[a]/As(III)		
2	1.03 ± 0.051	103
5	0.99 ± 0.042	99
10	0.96 ± 0.046	96
DMA[b]/As(III)		
2	0.98 ± 0.042	98
5	1.04 ± 0.052	104
10	0.97 ± 0.042	97

[a] Monomethylarsonic acid.
[b] Dimethylarsinic acid.

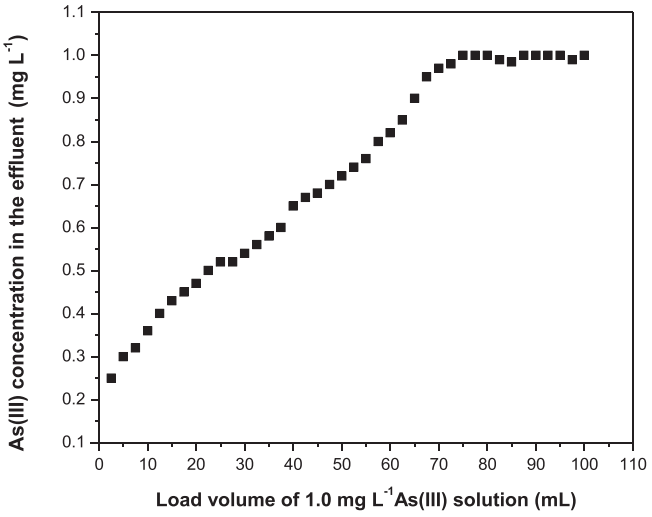

Fig. 4. Breakthrough curve of As(III) retention in the AC-modified KR. Concentration of As (III) in the sample loading solution was 1.0 mg L^{-1} and flow rate was 5 ml min^{-1}.

to study all variables could have limitations. The behaviors in the response can be completely different and cannot be adjusted to a model [31,32].

The flow rates obtained in multivariate optimization were selected for the next evaluations. The elution volume used at this step was 50 μl and 10 ml of a 1.0 μg L^{-1} As(III) solution was prepared for the preconcentration step. The selected eluent was acetone because it allowed full elution of the analyte from the KR. The rest of the solvents assayed as eluents showed poorer efficiency, meaning 95% and 76% for methanol and 10% (v/v) HNO_3, respectively. These results are related with the low polarity of acetone that makes feasible the total solubilization of As(III)–APDC hydrophobic complex. Moreover, acetone was easily volatilized during the drying step of ETAAS measurement, thus avoiding any damage to inner surfaces of the graphite furnace, as that caused when strong oxidants are injected [33].

The elution volume was also evaluated for the following values: 20, 50, 100 and 500 μl. Injection of 20 and 50 μl of eluent were performed directly into the graphite furnace of ETAAS, while a 60 μl-fraction of the total eluent volume was injected when 100 and 500 μl were assayed. Calculations were made considering these types of injections. The efficiency of the elution process was 100% for volumes in the range of 50 to 500 μl. On the other hand, the analyte was not fully eluted from the KR when 20 μl of acetone was used. In order to obtain the highest preconcentration factor possible, 50 μl of acetone was selected. Likewise, the elution flow rate is an important variable to be optimized in on-line preconcentration systems as it influences analyte removal from the KR and injection in ETAAS [34]. The effect of flow rate on analyte elution from the KR was evaluated at 0.1, 0.2, 0.5 and 1.0 ml min^{-1}. The highest elution efficiency (100%) was obtained at 0.2 ml min^{-1}. Above this value (0.2 ml min^{-1}) analytical recoveries were lower than 50%.

3.3. Interference study

The effects of representative and potential interfering species (at concentration levels commonly present in the samples studied) were also tested. It is well known that transition metals form complexes with APDC. Therefore, Mo(II), Ni^{+}, Zn^{2+}, Fe^{3+}, Se(IV), Cu^{2+}, Mn^{2+}, Co^{2+}, Cr^{3+}, Cd^{2+}, and Pb^{2+} were evaluated as potential interferences [35]. The study was performed by loading onto the AC-modified KR, 10 ml of solutions containing 1 μg L^{-1} of As(III) and the concomitants ions at the concentration levels at which they might occur in the samples or even higher. The retention efficiency was not altered by the presence of the above-mentioned ions and could be tolerated up to, at least, 2500 μg L^{-1}. Furthermore, analytical response was not influenced by these ions in this concentration range. Ions were considered to interfere if it resulted in an analytical signal variation of ± 10%.

3.4. Selectivity of As species determination

Since the occurrence of methyl groups in organoarsenic molecules reduces the polarity with respect to inorganic As species, it was required to determine if that decrease in polarity could be enough to cause the retention of organoarsenic species on AC material covering the inner surface of the KR. Therefore, a volume of 10 ml of MMA or DMA solutions at 1 μg L^{-1} were loaded into the AC-modified KR. The results demonstrated that no retention of organoarsenic species on AC occurred under the optimized conditions. The proposed method is then capable of retaining As(III) species, while As(V), MMA and DMA can only be determined if appropriate sample treatment (oxidation/reduction) is applied before the preconcentration step.

Moreover, selectivity of As(III) preconcentration in the presence of As(V) and organoarsenic species (MMA and DMA) was assayed for the proposed method by developing a recovery study. In order to test the selectivity of the methodology for As(III) retention in the presence of other As species, standard solutions at different concentration ratios of As(V)/As(III), MMA/As(III) and DMA/As(III) were loaded into the KR. The results showed that As(III) species was completely separated and quantitatively recovered even in the presence of other As species (Table 4). Therefore, the method had an acceptable recovery under the evaluated conditions with percentages for As(III) between 96% and 104%.

3.5. Analytical performance

The time required for preconcentration of 10 ml of sample was about 3.25 min. This time includes the following steps: 1 min at 1 ml min^{-1} for KR preconditioning with 0.01 mol L^{-1} HCl solution, 2 min at 5 ml min^{-1} for sample loading, and 15 s at 0.2 ml min^{-1} for elution of As(III)–APDC with acetone. Based on the total elapsed time, the sample throughput was about 18 samples per hour. Likewise, the sorption capacity of the AC-modified KR was calculated using the following equation:

$$q = V(C_o\text{-}C_e)/m \quad (3)$$

where q is the sorption capacity (mg g^{-1}), V is the volume of

Table 5
Characteristic performance data obtained by using the proposed methodology and others reported based on KR for As species determination.

Method	Species determined	LOD (μg/L)	RSD (%)	EF	Sample	Sample volume (ml)	Calibration range (μg/L)	Ref.
KR-ETAAS	As(III); As(V)	0.008	As(III): 4.5	44	Sea water	10.0	n.r.[a]	[21]
KR-ICP-MS	As(III); As(V)	0.021	As(III): 2.8; As(V): 3.9	22	Water	5.0	n.r.[a]	[19]
KR-HG-AFS	As(III); As(V)	0.023	1.3	11	Water	6.0	0.1–10	[20]
AC-Modified-KR-ETAAS	As(III); As(V)	0.004	As(III): 4.3; As(V): 4.7	200	Medicinal herbs and tea infusions	10.0	0.01–1.5	This work

[a]n.r.: not reported.

Table 6
Results of As speciation analysis in medicinal herbs and tea infusions (95% confidence interval, n = 6).

Sample		As(III)			As(V)		
		Added (μg L^{-1})	Found (μg L^{-1})	Recovery (%)[a]	Added (μg L^{-1})	Found (μg L^{-1})	Recovery (%)[a]
Yerba mate *(Ilex paraguariensis)*	1	0	<LOD	–	0	<LOD	–
	2	0.50	0.47 ± 0.02	94	0.5	0.48 ± 0.02	96
	3	1.00	0.99 ± 0.04	99	1.00	0.98 ± 0.05	98
Tila *(Tilia platyphyllos)*	1	0	<LOD	–	0	0.16 ± 0.01	–
	2	0.50	0.48 ± 0.02	96	0.5	0.65 ± 0.03	98
	3	1.00	0.99 ± 0.05	99	1.00	1.16 ± 0.05	100
Camomile *(Matricaria chamonilla)*	1	0	<LOD	–	0	<LOD	–
	2	0.50	0.49 ± 0.02	98	0.50	0.49 ± 0.03	98
	3	1.00	0.97 ± 0.04	97	1.00	0.99 ± 0.05	99
Tea *(Camellia sinensis)*	1	0	0.11 ± 0.01	–	0	0.19 ± 0.01	–
	2	0.50	0.60 ± 0.03	98	0.50	0.47 ± 0.02	94
	3	1.00	1.09 ± 0.05	98	1.00	1.00 ± 0.05	100

[a] 100 × [(found − initial)/added].

solution, C_o is the initial As concentration, C_e is the As concentration (mg L^{-1}) at equilibrium and m is the mass of adsorbent (g), in this case, AC [36]. The sorption capacity was evaluated by loading a 1.0 mg L^{-1} As(III) solution into the AC-modified KR under the optimized conditions given in Table 2. Aliquots of 100 μl-volume each were sampled every 30 s and As was determined by ETAAS after a 10-fold dilution. The saturation of the retention material was reached when 75 ml of a 1.0 mg L^{-1} As(III) solution was loaded into the AC-modified KR (Fig. 4). Thus, the dynamic sorption capacity was determined to be 660 μg As g^{-1} AC. This result is in agreement with those reported in other works where AC used as sorbent for As remediation in waters showed values in the ranges of 370–1250 μg g^{-1} [36] and 149–1320 μg g^{-1} [37].

The analytical performance of the on-line preconcentration method for sensitive As speciation analysis is summarized in Table 5. The enrichment factor (EF) was obtained from the slope ratio of calibration graphs obtained with and without application of the preconcentration method. An EF of 200 was obtained under optimum experimental conditions.

The limit of detection (LOD), calculated based on the signal at intercept and three times the standard deviation about regression of the calibration curve, was 4 ng L^{-1} (4 pg g^{-1} wet mass). The calibration graph for ETAAS determination of As was linear at concentrations near the LOD and up to at least 1.5 μg L^{-1}, with a correlation coefficient of 0.9993. The relative standard deviation (RSD) for six replicate measurements at levels of 0.2 μg L^{-1} was 4.3% and 4.7% for As(III) and As(V), respectively. The highest analyte retention obtained with the AC-modified KR under optimum conditions was 100%.

Due to the lack of a standard reference material with a known As concentration in herbs and infusions, the accuracy of the proposed method was evaluated by analyzing the standard reference material NIST SRM 1643e "Trace Elements in Water", with a reported As content of 60.45 ± 0.72 μg L^{-1}. The total As content determined in this SRM was 60.28 ± 2.59 μg L^{-1}, indicating that acceptable accuracy is obtained with the proposed methodology.

Finally, a comparative study on analytical performance of the proposed method with others already reported in the literature is shown in Table 5. Accordingly, a significant improvement in the EF value was achieved with the proposed method, mainly due to an increase on As retention onto the AC-modified inner walls of the KR. Furthermore, KRs have been previously tested for As species determination in samples with matrices of relatively low complexity such as water samples. On the other hand, it has been demonstrated in this work, that enhancement in sorption capacity of PTFE KRs by covering their inner surface with AC material, could be an excellent approach to allow the analysis of complex matrix samples.

3.6. Determination of As species in medicinal herbs and tea infusions

In earlier studies, it has been already reported that As(III) and As(V) are the most common As species found in medicinal herbs and tea infusions [5,8,10]. Taking this into account, the proposed methodology was applied for inorganic As species determination in infusions (Table 6). Concentrations of As species found in these samples were in the range of n.d. to 0.11 μg L^{-1} for As(III) and n.d. to 0.19 μg L^{-1} for As(V). These concentrations were not significantly different to those reported by Shi et al. [38] and Desideri et al. [39] for similar samples.

Finally, a recovery study of As species determination in medicinal herbs and tea infusions was performed. Consequently, accuracy of the proposed method for As species determination was evaluated by analyzing different samples added at equimass levels of As(III) and As(V). As shown in Table 6, both As species were completely separated and quantitatively recovered. The method showed an acceptable reliability at all concentration ratios, with recovery ranges of 94–98% and 94–100% for As(III) and As(V), respectively.

4. Conclusions

In this work, a simple and inexpensive approach to modify the inner surface of a PTFE KR, and increase its retention capacity, is presented. Based on the excellent analytical performance and practical advantages observed with the AC-modified KR, the device can be considered as an efficient alternative to regular PTFE KRs to obtain much higher analyte retention, thus allowing the development of highly sensitive preconcentration methods. Likewise, the present work demonstrates the feasibility of the developed method for sorption, preconcentration, separation, and determination of trace As(III) and As(V) species in medicinal herbs and tea infusions. In comparison with conventional KRs, higher preconcentration efficiency can be achieved with the proposed modification using AC as sorption material, mainly due to high retention and sorption capacity. Moreover, the low cost, easy operation and high sensitivity of the current system make it an attractive alternative for routine speciation analysis of inorganic As species in samples with complex matrices, such as medicinal herbs and tea infusions.

Acknowledgments

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) (PICT-BID), Universidad Nacional de Cuyo (Argentina), and the Organisation for the Prohibition of Chemical Weapons (OPCW).

References

[1] F. Baroni, A. Boscagli, L.A. Di Lella, G. Protano, F. Riccobono, Arsenic in soil and vegetation of contaminated areas in southern Tuscany (Italy), J. Geochem. Explor. 81 (2004) 1–14.
[2] G.F. Nordberg, B.A. Fowler, M. Nordberg, L. Friberg, Handbook on The Toxicology of Metals, Third ed. Academic Press, Atlanta, 2007.
[3] R. Cornelis, J. Caruso, H. Crews, K. Heumann, Handbook of Elemental Speciation II: Species in the Enviroment, Food, Medicine and Occupational Health, First ed. John Wiley and sons Ltd, Chichester, 2005.
[4] K.J. Duffey, B.M. Popkin, Shifts in patterns and consumption of beverages between 1965 and 2002, Obesity 15 (2007) 2739–2747.
[5] T. Karak, R.M. Bhagat, Trace elements in tea leaves, made tea and tea infusion: a review, Food Res. Int. 43 (2010) 2234–2252.
[6] V.K. Ananingsih, A. Sharma, W. Zhou, Green tea catechins during food processing and storage: a review on stability and detection, Food Res. Int. 50 (2013) 469–479.
[7] X. Li, Z. Zhang, P. Li, Q. Zhang, W. Zhang, X. Ding, Determination for major chemical contaminants in tea (*Camellia sinensis*) matrices: a review, Food Res. Int. 53 (2013) 649–658.
[8] C. Yuan, E. Gao, B. He, G. Jiang, Arsenic species and leaching characters in tea (*Camellia sinensis*), Food Chem. Toxicol. 45 (2007) 2381–2389.
[9] S. Shekoohiyan, M. Ghoochani, A. Mohagheghian, A.H. Mahvi, M. Yunesian, S. Nazmara, Determination of lead, cadmium and arsenic in infusion tea cultivated in north of Iran, Iran J. Environ. Health 9 (2012) 37–38.
[10] F. Zhu, X. Wang, W. Fan, L. Qu, M. Qiao, S. Yao, Assessment of potential health risk for arsenic and heavy metals in some herbal flowers and their infusions consumed in China, Environ. Monit. Assess. 185 (2012) 3909–3916.
[11] Y.S. Fung, K.M. Lau, Separation and determination of cations in beverage products by capillary zone electrophoresis, J. Chromatogr. A 1118 (2006) 144–150.
[12] M.-L. Chen, L.-Y. Ma, X.-W. Chen, New procedures for arsenic speciation: a review, Talanta 125 (2014) 78–86.
[13] W.A. Wan Ibrahim, L.I. Abd Ali, A. Sulaiman, M.M. Sanagi, H.Y. Aboul-Enein, Application of solid-phase extraction for trace elements in environmental and biological samples: a review, Crit. Rev. Anal. Chem. 44 (2014) 233–254.
[14] S. Cerutti, L.D. Martinez, R.G. Wuilloud, Knotted reactors and their role in flow-injection on-line preconcentration systems coupled to atomic spectrometry-based detectors, Appl. Spectrosc. Rev. 40 (2005) 71–101.
[15] R.A. Gil, J.A. Gásquez, R. Olsina, L.D. Martinez, S. Cerutti, Cloud point extraction for cobalt preconcentration with on-line phase separation in a knotted reactor followed by ETAAS determination in drinking waters, Talanta 76 (2008) 669–673.
[16] E.H. Hansen, J. Wang, On-line sample-pre-treatment schemes for trace-level determinations of metals by coupling flow injection or sequential injection with ICPMS, Trends Anal. Chem. 22 (2003) 836–846.
[17] S.L., W. Som-Aum, E.H. Hansen, Flow injection on-line preconcentration of low levels of Cr(VI) with detection by ETAAS: comparison of using an open tubular PTFE knotted reactor and a column reactor packed with PTFE beads, Anal. Chim. Acta. 463 (2002) 99–109.
[18] H. Wu, Y. Jin, Y. Tian, S.P. Bi, Applications of flow injection knotted reactor on-line separation and preconcentration coupled with atomic spectrometry, Chin. J. Anal. Chem. 35 (2007) 905–911.
[19] X.P. Yan, R. Kerrich, M.J. Hendry, Determination of (ultra)trace amounts of arsenic(III) and arsenic(V) in water by inductively coupled plasma mass spectrometry coupled with flow injection on-line sorption preconcentration and separation in a knotted reactor, Anal. Chem. 70 (1998) 4736–4742.
[20] X.P. Yan, X.B. Yin, X.W. He, Y. Jiang, Flow injection on-line sorption preconcentration coupled with hydride generation atomic fluorescence spectrometry for determination of (ultra)trace amounts of arsenic(III) and arsenic(V) in natural water samples, Anal. Chem. 74 (2002) 2162–2166.
[21] P. Herbello-Hermelo, M.C. Barciela-Alonso, A. Bermejo-Barrera, P. Bermejo-Barrera, Flow on-line sorption preconcentration in a knotted reactor coupled with electrothermal atomic absorption spectrometry for selective As(III) determination in seawater samples, J. Anal. Atom. Spectrom. 20 (2005) 662–664.
[22] M.S. Shafeeyan, W.M.A.W. Daud, A. Houshmand, A. Shamiri, A review on surface modification of activated carbon for carbon dioxide adsorption, J. Anal. Appl. Pyrol. 89 (2010) 143–151.
[23] R.A., A. Mohammad-Khah, Activated charcoal: preparation, characterization and applications: a review article, Int. J. Chem. Technol. Res. 1 (2009) 859–864.
[24] M. Bruk, Radiation-thermal crosslinking of polytetrafluoroethylene, High Energ. Chem. 40 (2006) 357–369.
[25] K. Lunkwitz, U. Lappan, U. Scheler, Modification of perfluorinated polymers by high-energy irradiation, J. Fluor. Chem. 125 (2004) 863–873.
[26] J. Rutkowska, K. Kilian, K. Pyrzynska, Removal and enrichment of copper ions from aqueous solution by 1,8-diaminonapthalene polymer, Eur. Polym. J. 44 (2008) 2108–2114.
[27] Y.F. Huang, Y. Li, Y. Jiang, X.P. Yan, Magnetic immobilization of amine-functionalized magnetite microspheres in a knotted reactor for on-line solid-phase extraction coupled with ICP-MS for speciation analysis of trace chromium, J. Anal. Atom. Spectrom. 25 (2010) 1467–1474.
[28] S.L.C. Ferreira, W.N.L. dos Santos, C.M. Quintella, B.B. Neto, J.M. Bosque-Sendra, Doehlert matrix: a chemometric tool for analytical chemistry-review, Talanta 63 (2004) 1061–1067.
[29] L. Vera Candioti, M.M. De Zan, M.S. Cámara, H.C. Goicoechea, Experimental design and multiple response optimization. Using the desirability function in analytical methods development, Talanta 124 (2014) 123–138.
[30] A.M. De Llanos, M.M. De Zan, M.J. Culzoni, A. Espinosa-Mansilla, F. Cañada-Cañada, A.M. De La Peña, H.C. Goicoechea, Determination of marker pteridines in urine by HPLC with fluorimetric detection and second-order multivariate calibration using MCR-ALS, Anal. Bioanal. Chem. 399 (2011) 2123–2135.
[31] P. Berton, L. Vera-Candioti, H.C. Goicoechea, R.G. Wuilloud, A microextraction procedure based on an ionic liquid as an ion-pairing agent optimized using a design of experiments for chromium species separation and determination in water samples, Anal. Methods 5 (2013) 5065–5073.
[32] A. Morado Piñeiro, J. Moreda-Piñeiro, E. Alonso-Rodríguez, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, Arsenic species determination in human scalp hair by pressurized hot water extraction and high performance liquid chromatography-inductively coupled plasma-mass spectrometry, Talanta 105 (2013) 422–428.
[33] G. Schlemmer, B. Radziuk, Analytical graphite furnace atomic absorption spectrometry, A Laboratory GuideFirst ed., Birkhäuser, Berlin, 1999.
[34] Z. Fang, Flow Injection and Preconcentration, First ed. Wiley-VCH, Weinheim, 1993.
[35] K.L. Cheng, K. Ueno, T. Imamura, Handbook of Organic Analytical Reagents, First ed. CRC, Florida, 1982.
[36] A.V. Vitela-Rodriguez, J.R. Rangel-Mendez, Arsenic removal by modified activated carbons with iron hydro(oxide) nanoparticles, J. Environ. Manage. 114 (2013) 225–231.
[37] M. Asadullah, I. Jahan, M.B. Ahmed, P. Adawiyah, N.H. Malek, M.S. Rahman, Preparation of microporous activated carbon and its modification for arsenic removal from water, J. Ind. Eng. Chem. 20 (2014) 887–896.
[38] Y.Z. Shi, J.Y. Ruan, L.F Ma, W.Y. Han, F. Wang, Accumulation and distribution of arsenic and cadmium by tea plants, J. Zhejiang Uni. Sci. B 9 (2008) 265–270.
[39] D. Desideri, M.A. Meli, C. Roselli, L. Feduzi, Determination of essential and nonessential elements in herbal tea and camomile by polarised X rays fluorescence spectrometer (EDPXRF), J. Radioanal. Nucl. Chem. 290 (2011) 391–396.