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Automated Solid Phase Extraction of Cd(II), Co(II), Cu(II) and Pb(II) Coupled with Flame Atomic Absorption Spectrometry Utilizing a New Sol-Gel Functionalized Silica Sorbent

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Abstract: In this study a simple and sensitive on-line sorbent extraction platform coupled with flame atomic absorption spectrometry for trace metals determination was developed. The system utilized for the first time a novel sol-gel thiocyanatopropyl functionalized silica as adsorbent for metal's separation and preconcentration. The main factors affecting the performance of the on-line system were investigated and optimized. The effect of potential interfering species that occur naturally in environmental and biological samples, as well as some toxic elements, was evaluated. Under optimum conditions the enhancement factors ranged between 73 and 152 for the target analytes. The LODs of the proposed methods were 0.15 μg L^{-1} for Cd(II), 0.5 μg L^{-1} for Co(II), 0.5 μg L^{-1} for Cu(II) and 1.9 μg L^{-1} for Pb(II) for 120 s preconcentration time. The relative standard deviation values for all elements were less than 3.8%, indicating good method precision. Moreover, the sol-gel thiocyanatopropyl functionalized silica-packed microcolumns exhibited limited flow resistance and excellent packing reproducibility. Finally, the proposed method was utilized for the analysis of environmental and biological samples.

Keywords: sol-gel; FAAS; preconcentration; lead; copper; cadmium; cobalt; flow injection; automation; on-line

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1. Introduction

The rapid development of industrialization is associated with ever-increasing environmental pollution. Undoubtedly, toxic metals are among the most dangerous pollutants, due to their wide distribution and their high toxicity. Thus, the presence of toxic metals in the natural environment is a significant threat for public health and therefore their monitoring is necessary [1]. Atomic spectrometric techniques serve as excellent analytical systems for the determination of metals. Typical examples of these techniques include flame atomic absorption spectrometry (FAAS), flame atomic emission spectroscopy (FAES), cold vapor atomic absorption spectrometry (CV-AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) [2]. Furthermore, the combination of flow systems, such as flow injection (FI), that serve as simple, handy and versatile tools, with robust analytical techniques has proven to be a powerful tool for metal determination [3].

Due to the low concentration of toxic metals and the simultaneous presence of other matrix constituents in environmental, biological and food samples, a separation/preconcentration step is typically required [4]. Among the available sample preparation techniques that are commonly utilized for the preconcentration of metal ions, solid-phase extraction (SPE) is the most frequently used. SPE is characterized by many advantages such as straightforward operation, versatility, reliability and high overall efficiency. SPE can be implemented either in "off-line" or in "on-line" mode. The later approach minimizes the operator labor and time required for sample preparation, thus eliminating the losses during this step [5]. Until now, various sorbents have been employed for the development of automated FI-FAAS methods for the separation/preconcentration of metal ions [6–9].

The continuous exploration of novel materials as adsorbents is currently in the forefront of research in analytical chemistry. In compliance with the guidelines of Green Chemistry, the utilization of materials with enhanced selectivity in micro-extraction techniques could make possible the replacement of hazardous solvents, the reduction of sample and organic solvent amount and the reusability of the sorbent. Towards the exploration of novel materials in sample preparation, a plethora of novel sorbents has been synthesized and evaluated [10]. Typical examples of new materials include graphene and its derivatives [11], molecularly imprinted polymers [12], metal-organic frameworks [13] and sol-gel materials [14]. Among them, materials produced based on the well-known sol-gel technology have attracted the interest of analytical chemists working in the field of sample preparation. These materials are usually employed for the development of sorbents for solid phase extraction (SPE), solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE) and fabric phase sorptive extraction (FPSE) [10,15,16].

The main benefit of the sol-gel process is the possibility of using mild reaction conditions for the preparation of inorganic and organic-inorganic hybrid polymers with good thermal and chemical stability. Moreover, through the modification of the synthesis conditions, the morphology, the surface properties and pore structures of the sorbents can be controlled. Among the typically used inorganic polymers prepared by the sol-gel process, silica-based materials are by far the most used. Due to the presence of abundant silanol groups in silica-based sorbents, post-synthetic modification of silica-based sorbents can be carried out to tune the selectivity of the material [10,14]. Until now, sol-gel materials have been proven to be appropriate sorbents for the development of on-line separation/preconcentration methods for the determination of metals in complex samples. Castro et al. [17] synthesized a silica sorbent through sol-gel approach that was functionalized with 2-aminothiazole. The novel material was packed in a column and employed for the preconcentration of Cd(II), Co(II) and Ni(II). Sol-gel sorbents coated on fabric disks [18], on polyester fibers [19] and on glass fiber substrates [20] have also been explored for the extraction of metals.

Thiocyanate functional group has long been known to form complexes with d block metals [21]. To exploit the affinity of thiocyanatopropyl functional group towards metals, Sui et al. described a procedure to synthesize thiocyanatopropyl functionalized silica particles [22]. However, the process was cumbersome, time consuming and of low yield. The current study proposes a novel synthesis protocol to produce sol-gel thiocyanatopropyl functionalized silica adsorbent which is simple, fast and economically more viable. The sol-gel thiocyanatopropyl functionalized silica particles were subsequently evaluated for the on-line column preconcentration of Cd(II), Co(II), Cu(II) and Pb(II) followed by FAAS determination. The main factors that could potentially affect the performance of the automated method were optimized. The novel sorbent was characterized and employed for the analysis of environmental and biological samples.

2. Materials and Methods

2.1. Reagents and Samples

All chemicals used in this study were of analytical grade. Ultra-pure water produced by a Milli-Q system (Merck, Darmstadt, Germany) was used throughout the study. Nitric acid 65% and ammonia solution 25% were purchased by Merck (Darmstadt, Germany). Stock standard solutions of each analyte (i.e., Cd(II), Co(II), Cu(II), and Pb(II)) were also purchased by Merck (Darmstadt, Germany). These solutions contained 1000 mg L^{-1} of each element in 0.5 mol L^{-1} HNO_3. Working standard solution was daily prepared by appropriate serial dilutions from the stock standards.

For the preparation of the functionalized sorbent, tetramethyl orthosilicate (TMOS), hydrochloric acid and isopropanol (Sigma-Aldrich (St. Louis, MO, USA)) were used. Moreover, 3-thiocyanatopropyl triethoxysilane was purchased from Gelest Inc. (Morrisville, PA, USA).

Two certified reference materials (CRMs) were analyzed for accuracy evaluation of the developed method: a NIST certified reference material (CRM) 1643e (National Institute of Standard and Technology, Gaithersburg, MD, USA) containing trace elements in water and BCR 278-R (Community Bureau of Reference, Brussels, Belgium) trace elements in mussel tissue. An amount of ca. 0.4 g of tissue was precisely weighed into Teflon crucibles, followed by addition of nitric and perchloric acid (3/2, *v*/*v*). Digestion was performed by heating the mixture at 130 °C in closed Teflon beakers placed into an autoclave. After cooling the system down to room temperature, the vessels were opened and the samples were diluted in Milli-Q water and used for the analysis.

Natural water samples were collected from Northern Greece (March 2021): Strymon River and Prespa Lake. Prior to the analysis, sample filtration took place using 0.45 μm membrane filters. The pH of samples was adjusted to ca. pH 2.0 using a dilute nitric acid solution and stored at 4 °C. The "dissolved" fraction of the metals in the above sample solutions was determined by the proposed method. A urine sample was collected from a healthy volunteer, filtered through 0.45 μm membrane filter and stored at 4 °C. The urine sample was analyzed by the proposed method after wet digestion utilizing nitric acid in an autoclave. Prior to the on-line FI-FAAS procedure the samples were adjusted to pH 5.0 with dilute HNO_3 and NH_4OH solutions.

2.2. Development of the Thiocyanatopropyl Functionalized Sol-Gel Silica Sorbent

Thiocyanate functional groups exhibit strong affinity towards d-block elements since they can interact with metal ions either in thiocyanate binding mode through the sulfur atom or in isothiocyanate binding mode through the nitrogen atom. For example, as suggested by the research group of Maveichuk, zinc and cobalt ions interact with the sorbent through the nitrogen atom, while mercury, manganese, nickel and iron interact with the sorbent through the sulfur atom [21]. Consequently, the thiocyanate moiety is a good choice for the preparation of a new sorbent for metal extraction/preconcentration.

For the preparation of the 3-thiocyanatopropyl functionalized sol-gel silica sorbent, a sol solution was initially prepared by sequential addition of TMOS, 3-thiocyanatopropyl triethoxysilane and 2-propanol in a 50 mL amber reaction bottle at a molar ratio of 1:0.3:15. The sol solution was vortexed for 2 min after the addition of each component. Afterwards, a solution of 0.1 mol L^{-1} hydrochloric acid was added to the mixture (molar ratio between TMOS and 0.1 mol L^{-1} HCl 1:5). The sol solution was thoroughly mixed and the mixture was subjected to prolonged hydrolysis at 50 °C for 8 h. Subsequently, the solution was transferred into a wide mouth vessel and a Teflon-coated bar magnet was added to it. The polycondensation was initiated by the dropwise addition of 1 mol L^{-1} NH_4OH (impregnated with 0.25 mol L^{-1} NH_4F) under constant stirring. The ratio between TMOS and 1.0 mol L^{-1} NH_4OH was maintained at 1:1.2. Within 30 min, the sol solution was turned into transparent gel and the sol-gel monolithic bed was thermally conditioned and aged for 24 h at 50 °C. Afterwards, crushing of the monolithic bed was performed, followed by drying for 24 h at 70 °C. The dried sol-gel sorbent was then crushed and pulverized into fine particles using a mortar and a pestle. Then, Soxhlet extraction with fiber-glass thimble was performed using methanol: methylene chloride 50:50 (*v*/*v*) for 4 h to clean the sol-gel sorbent from unreacted precursors, solvent and reaction byproducts. Finally, the sol-gel sorbent was dried at 70 °C for 24 h.

Major chemical reactions involving the sol-gel thiocyanatopropyl functionalized silica sorbent particles are presented in Figure 1.

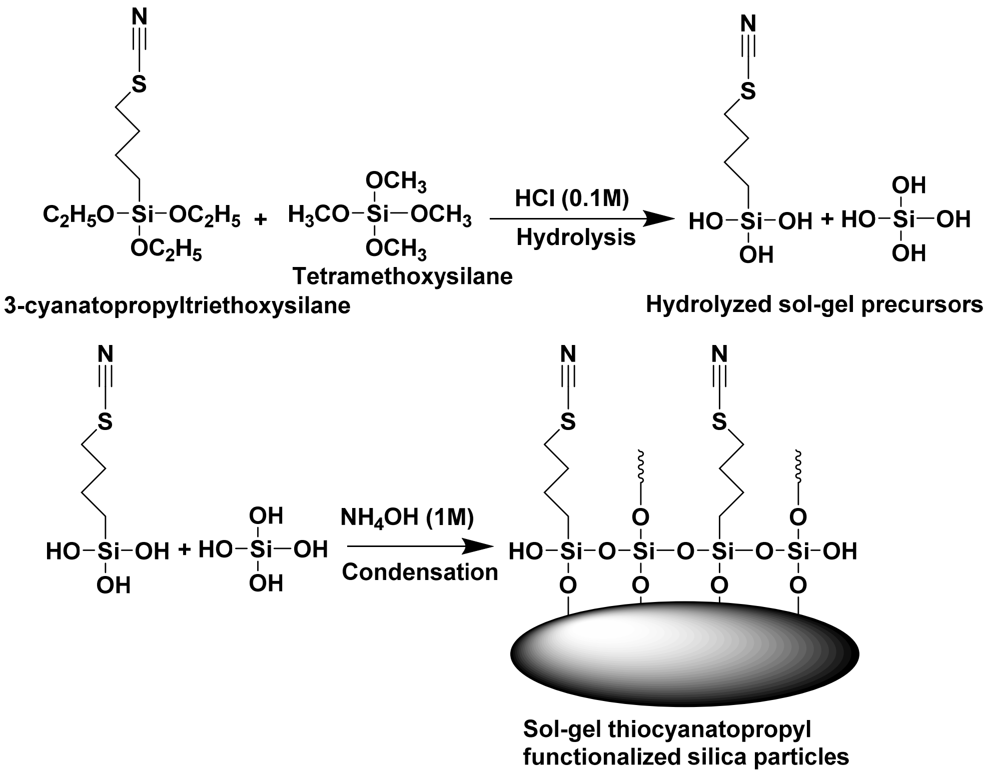

Figure 1. Major reactions involved in the synthesis of sol-gel thiocyanatopropyl silica particles.

2.3. Instrumentation

A Perkin-Elmer model 5100 PC flame atomic absorption spectrometer (Norwalk, CT, USA, https://www.perkinelmer.com/category/atomic-spectroscopy-instruments, accessed date: 7 July 2021) was used for detecting and quantifying the target analytes. The FAAS instrument was equipped with deuterium lamp background corrector, while hollow cathode lamps (HCL) were employed depending on the analyte. For cadmium the HCL operated at 4 mA, for cobalt and copper at 30 mA and for lead at 10 mA. The resonance lines were fixed at: 228.8 nm for cadmium, 240.7 nm for cobalt, 324.7 nm for copper and 283.3 nm for lead, while the spectral bandpass (slit) of the monochromator was set at 0.7 nm for cadmium, copper and lead, and at 0.2 nm for cobalt. In order to obtain oxidizing flames for all elements, the flow rate of air and acetylene was set at 10.0 L min^{-1} and 2.0 L min^{-1}, respectively. Following the recommendations of the instrument's manufacturer, the spray chamber was equipped with an internal PTFE flow spoiler to achieve good nebulization conditions.

For the on-line column preconcentration procedure, a Perkin-Elmer (Norwalk, CT, USA) Model FIAS-400 flow injection system was used and is presented schematically in Figure 2. The system was operated in preconcentration mode and it was equipped with two peristaltic pumps (P1 and P2). P1 was employed for eluent's propulsion, while P2 was employed for sample/standard solution's propulsion. An injection valve (IV) with 5 ports and 2 positions that integrated the microcolumn, fixed at ports 2 and 4, was also included in the FIAS-400 flow injection system.

Table 1. Operation sequence of the on-line FI-FAAS sorbent extraction system.

Step	IV	P1	P2	Transported Medium	Flow Rate (mL min^{-1})	Time (s)	Operation
1	Load	off	on	Sample/standard solution	10.0	120	Preconcentration
2	Elute	on	off	1.0 mol L^{-1} HNO_3	4.0	30	Elution/Measurement

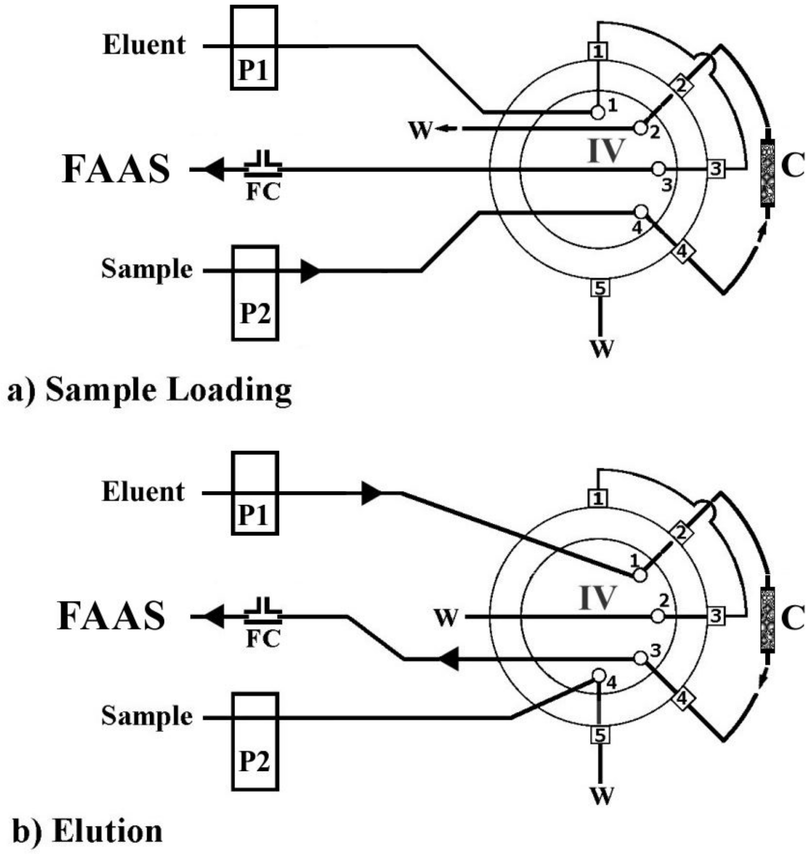

Figure 2. Schematic representation and operation sequences of FI-FAAS (**a**) step 1, sample loading and (**b**) step 2, elution (Table 1), IV; injection valve, P1 and P2; peristaltic pumps, W; waste, C; microcolumn, FC; flow compensation unit.

Instrument operation was conducted using the software program AA Lab Benchtop version 7.2. Short connecting PTFE tubing (20 cm, 0.5 mm i.d.), between the IV and the FAAS' nebulizer was utilized to eliminate the dispersion of the analyte into the zone of the eluent. It should be noted that between the FIAS-400 system and the FAAS, a VICI® (Valco Instruments Co. Inc.) (Houston, TX, USA) three-section "T" type confluence connector made of polyether ether ketone (PEEK) (0.5 mm i.d. bore size) was adapted as flow compensator.

The herein used microcolumn was fabricated from a polypropylene 1.0 mL disposable syringe (length 10 cm; i.d. 5.0 mm) with Luer slip-centered tip. The syringe body was cut properly at a length ca. 20.0 mm, so that the resulting microcolumn possessed an effective length of 10.0 mm and an internal diameter of 5.0 mm. An amount of 120 mg of sol-gel functionalized silica sorbent with a granularity between 0.180–0.500 µm was firmly packed into the microcolumn. Commercial frits were used at both ends of the microcolumn for the sorbent immobilization. The fabricated microcolumn was installed at ports 1 and 4 of the injection valve of FIAS-400 manifold, utilizing push-fit connections. This configuration of the easily prepared column exhibited certain benefits, including limited backpressure, stable flow, no channel formation, in combination with rapid replacement and repacking ability. Prior to use of a freshly prepared microcolumn, flushing with dilute nitric acid (1.0 mol L^{-1}) and deionized water took place. After at least 700 sorption/elution cycles, the performance characteristics of the herein used microcolumn were not affected and no loss of functionality was observed.

2.4. Automatic Operational Procedure

The on-line FI-FAAS analytical method is operated in two main steps, namely: sample loading and elution. The schematic representation of the on-line system and the operation steps is shown in Figure 2 and summarized in Table 1. For sample loading, the IV is in the “Load” position and P2 is employed to deliver the sample through the microcolumn (C) at a fixed loading flow rate of 10.0 mL min^{-1} for a loading time of 120 s, which also presents the preconcentration time. In this step, the metal cations are adsorbed on the sol-gel sorbent’s surface. For sample elution, the IV is actuated in the “Elute” position and P1 is activated for eluent’s propulsion. Thus, a nitric acid solution of 1.0 mol L^{-1} is aspirated and delivered through the microcolumn at a flow rate of 4.0 mL min^{-1} for 30 s to elute the analytes from the column and to deliver them in the nebulizer of the FAAS for atomization and quantification. It should be noted that the analytes desorption is done in a reverse flow direction compared to the direction of the loading step, to minimize the dispersion into the eluent’s zone. Each measurement was performed in five repetitions. Sharp recorded signals were obtained for all metals, while the peak height that was used for analyte’s quantification was proportional to the concentration of the respective analyte.

3. Results and Discussion

3.1. Optimization of Extraction/Preconcentration Conditions

The main parameters affecting the performance of the proposed on-line method, considering the sensitivity, the analysis time, as well as the reagent and sample consumption, were investigated and optimized. All experiments were performed utilizing aqueous standard solutions of 10.0 μg L^{-1} of cadmium, 60.0 μg L^{-1} of cobalt, 40.0 μg L^{-1} of copper and 100.0 μg L^{-1} of lead. Loading flow rate: 10.0 mL min^{-1}, elution flow rate: 4.0 mL min^{-1}, eluent: 1.0 moL^{-1} HNO_3 and loading time: 60 s. The well-established one-variable-at-a-time approach was adopted as optimization methodology.

Nitric acid has already proved to be a powerful eluent solvent for the desorption of the adsorbed analytes compared to other acids (e.g., HCl and H_2SO_4) [23]. The effect of concentration of nitric acid was examined between 0.1–3.0 mol L^{-1}. An increase in the absorbance was observed by increasing the concentration up to 1.0 mol L^{-1}. The absorbance remained constant for higher concentrations. Thus, a solution of 1.0 mol L^{-1} HNO_3 was adopted as eluent for further experiments.

Taking into consideration that the herein studied sorbent retains metal species in their ionic form either through its nitrogen or sulfur atom, the acidity of the sample solution should be examined to ensure that the metals exist as cations (predominant species) [21]. In this case, the pH value of sample solution is directly associated with the retention efficiency of the target analytes and affects the sensitivity of the method. The influence of pH value on the absorbance was investigated in the pH range 2.0–7.0. The experimental results are presented in Figure 3. As can be observed, the highest absorbance was obtained in a pH window from 4.0 to 6.0, for all analytes. At higher pH values, the absorbance was gradually decreased due to the formation of various potential hydrolysis species (e.g., $Co(OH)_2$, $Co(OH)_3^-$, $Cd(OH)^+$, $Cu(OH)^+$, $Pb(OH)^+$, etc.) [24–26]. Thus, pH value 5.0 was chosen for all metals. In this pH, the predominant species are the metal cations [24–26].

Loading flow rate for a fixed sample volume determines whether the adsorption procedure is fast as well as strong enough for effective sorption of the analytes. The impact of the loading flow rate on the absorbance of the analytes was studied in a range between 3.5 mL min^{-1} and 10.0 mL min^{-1}, for a fixed sample volume of 10.0 mL. As can be seen in Figure 4, the absorbance was almost constant over the studied range without significant variation in the sensitivity of the method, presenting very fast mass transfer of the analytes onto the sorbent material. As a compromise between high sensitivity and reasonable sample consumption, a loading flow rate of 10.0 mL min^{-1} was selected as optimum.

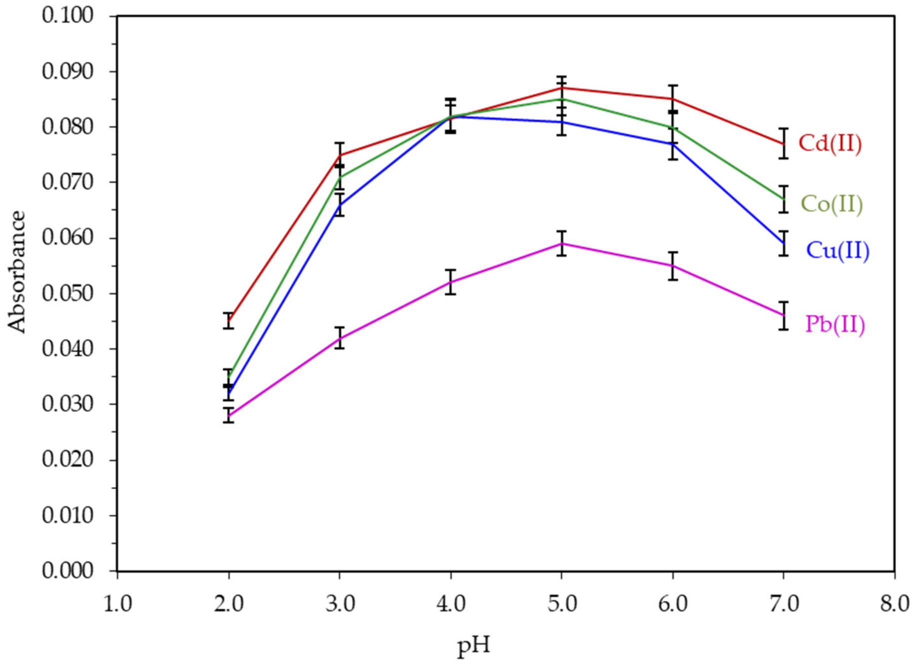

Figure 3. Effect of pH value on the absorbance of 10.0 μg L^{-1} of Cd(II), 60.0 μg L^{-1} of Co(II), 40.0 μg L^{-1} of Cu(II) and 100.0 μg L^{-1} of Pb(II). Loading flow rate: 10.0 mL min^{-1}, elution flow rate: 4.0 mL min^{-1}, eluent: 1.0 moL^{-1} HNO_3 and loading time: 60 s. The error bars were calculated based on standard deviation (±1 s).

In flow injection systems coupled with FAAS, the feeding flow rate must be compatible with the nebulizers' free uptake. On the other hand, it should facilitate the fast and quantitative desorption of the analytes. Herein, for elution flow rate optimization, diverse flow rates between 1.6 and 4.8 mL min^{-1} were studied. The highest absorbance (Figure 5) was obtained from 3.0 to 4.0 mL min^{-1}. For lower flow rates, the slightly lower absorbance was attributed to possible dispersion of the analytes into the atomizer of FAAS because of the difference between the free uptake nebulizer flow rate and the elution flow rate [27]. For higher flow rates, possible inefficient desorption results in decreased absorbance. Thus, an elution flow rate of 4.0 mL min^{-1} was chosen.

The preconcentration time is a key parameter for on-line preconcentration systems since it affects the sample consumption, the preconcentration ratio/enhancement factor, as well as the analysis time. In this study, different preconcentration time spans between 30 and 180 s were evaluated, as shown in Figure 6. As can be observed, an almost linear absorbance increase was obtained up to 180 s for all the examined analytes. Therefore, a preconcentration time of 120 s was selected as a compromise between method sensitivity, sample consumption and sample throughput.

3.2. *Study of Interference Ions*

For the complete evaluation of the sorbent, the competition of common matrix components of environmental and biological samples and the target analytes for the active sites on the sorbent was studied. The individual effect of potentially interfering ions on the recovery of 5.0 μg L^{-1} cadmium, 10.0 μg L^{-1} cobalt, 10.0 μg L^{-1} copper and 20.0 μg L^{-1} lead was investigated. In order to consider the presence of a coexisting ion as interference, a variation on the recovery above ±5% was set as criterion. As is observed from the experimental results, the tolerance limit for Fe(III), Cr(III), Cr(IV), Mn(II), Al(III) and Zn(II) is at least 2.0 mg L^{-1}. For Hg(II), the tolerance limit is up to 1.0 mg L^{-1}. Furthermore, the tolerance limit for common cations that exist in natural and biological samples, such as sodium, potassium, calcium and magnesium, is up to 500 mg L^{-1}.

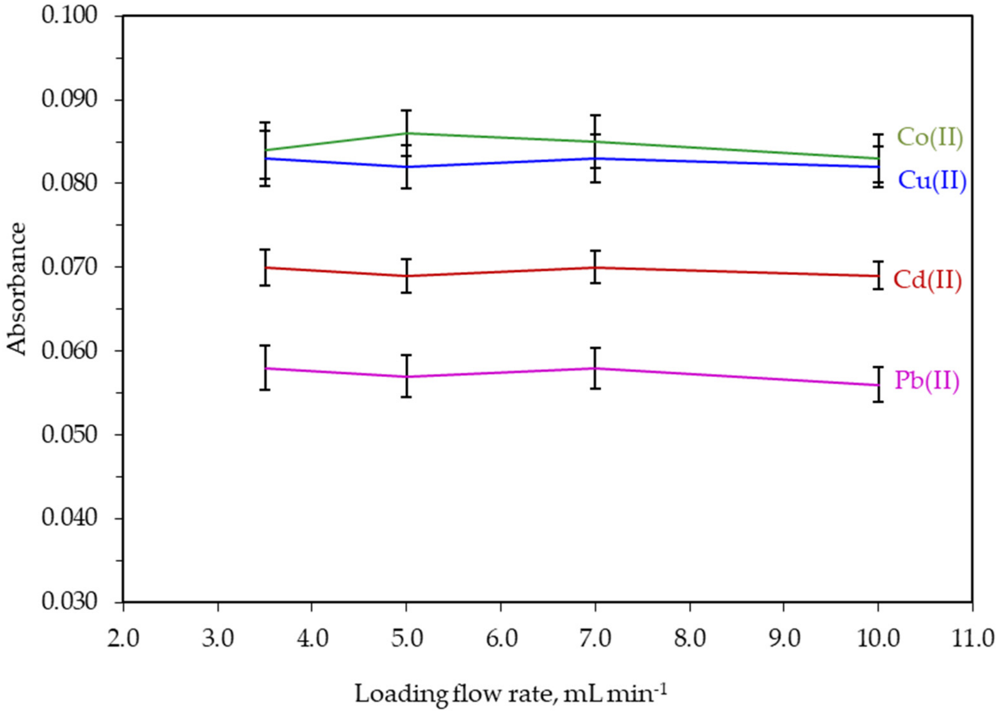

Figure 4. Effect of loading flow rate on the absorbance of 10.0 μg L^{-1} of Cd(II), 60.0 μg L^{-1} of Co(II), 40.0 μg L^{-1} of Cu(II) and 100.0 μg L^{-1} of Pb(II). Elution flow rate: 4.0 mL min^{-1}, sample pH: 5, eluent: 1.0 moL^{-1} HNO_3 and loading sample volume: 10 mL. Preconcentration time varied regarding the loading flow rate. The error bars were calculated based on standard deviation (±1 s).

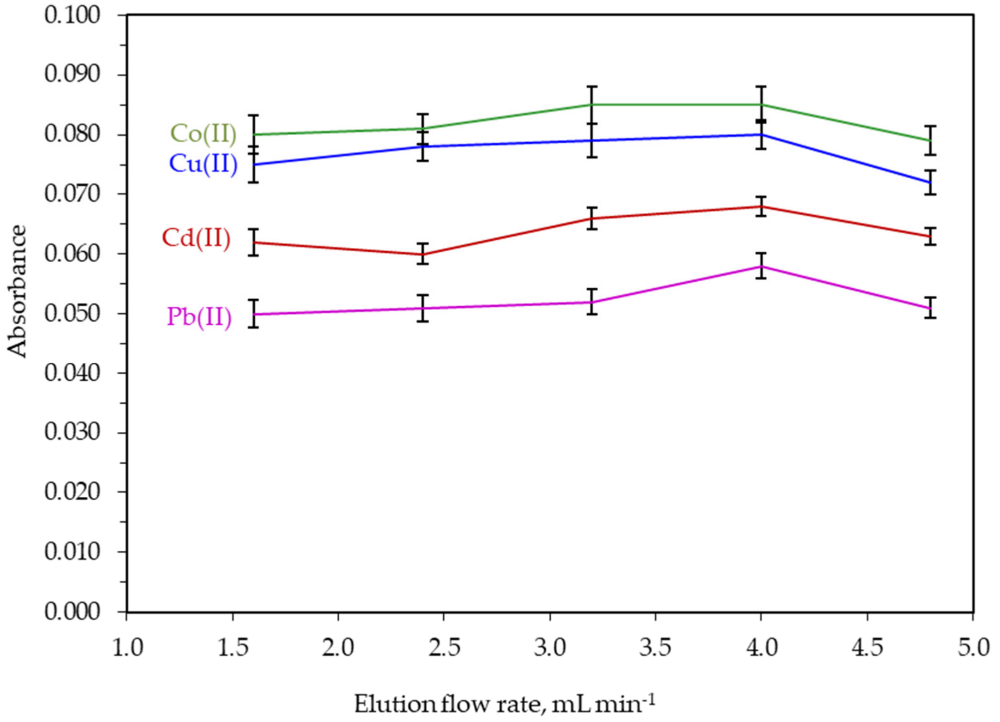

Figure 5. Effect of elution flow rate on the absorbance of 10.0 μg L^{-1} of Cd(II), 60.0 μg L^{-1} of Co(II), 40.0 μg L^{-1} of Cu(II) and 100.0 μg L^{-1} of Pb(II). Loading flow rate: 10.0 mL min^{-1}, sample pH: 5, eluent: 1.0 moL^{-1} HNO_3 and loading time: 60 s. The error bars were calculated based on standard deviation (±1 s).

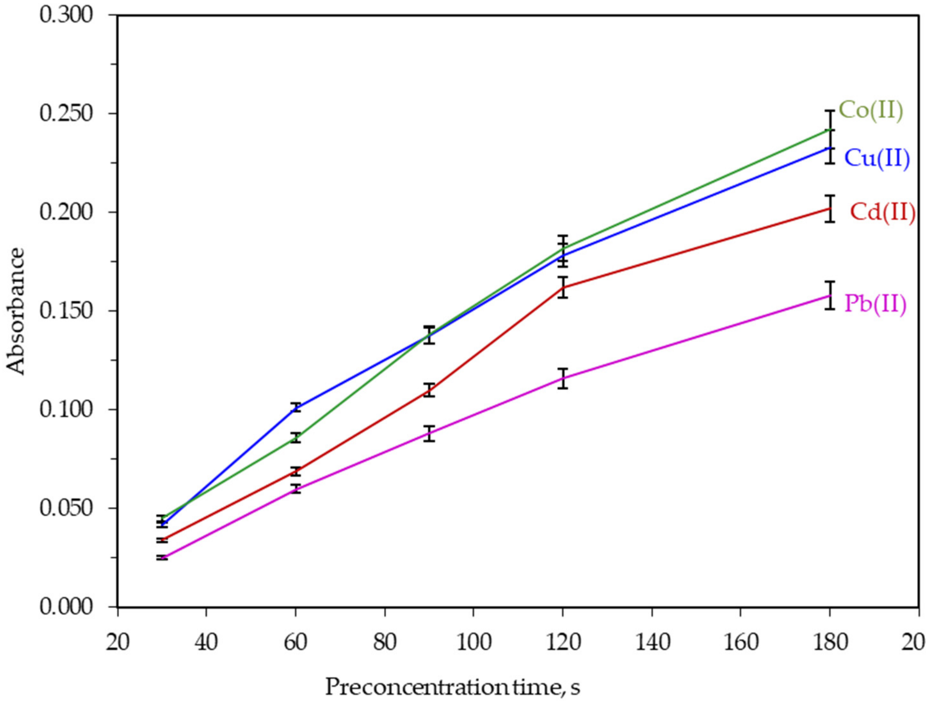

Figure 6. Effect of preconcentration time on the absorbance of 10.0 μg L^{-1} of Cd(II), 60.0 μg L^{-1} of Co(II), 40.0 μg L^{-1} of Cu(II) and 100.0 μg L^{-1} of Pb(II). Loading flow rate: 10.0 mL min^{-1}, elution flow rate: 4.0 mL min^{-1}, sample pH: 5 and eluent: 1.0 moL^{-1} HNO. The error bars were calculated based on standard deviation (±1 s).

3.3. Figures of Merit

The figures of merit of the developed FI-FAAS method are summarized in Table 2. The sample throughput for a preconcentration time of 120 s was 25 per h (h^{-1}). The detection limit (LOD) and the quantification limit (LOQ) of the proposed method were calculated by using the 3 s and 10 s criterion, respectively, based on IUPAC recommendation. As such, the LOD and LOQ values equals 3 and 10 times the standard deviation of absorbance of a blank sample solution (n = 10) divided by the slope of the regression equation for each studied analyte.

The evaluation of the precision of the FI-FAAS method was assessed in terms of relative standard deviation (RSD) of the absorbance of each analyte calculated from 10 repeated measurements of a standard solution containing 5 μg L^{-1} of cadmium, 20.0 μg L^{-1} of cobalt, 10.0 μg L^{-1} of copper and 30.0 μg L^{-1} of lead. The RSD values for all analytes were less than 3.8%, showing good method precision. Finally, the enhancement factor of the FI-FAAS for all metals was calculated by dividing the slope of the calibration curve of the FI-FAAS method with preconcentration by the slope of the calibration curve with direct aspiration without preconcentration, for the respective analyte. For all analytes, the EF values were found to be between 73 and 152, indicating the effectiveness of the sorbent.

Table 2. Analytical characteristics for Cd, Co, Cu and Pb determination by the FI-FAAS method.

Parameter	**Cd(II)**	**Co(II)**	**Cu(II)**	**Pb(II)**
Preconcentration time (s)	120	120	120	120
Sampling frequency (h^{-1})	25	25	25	25
Regression equation	A = 0.0017 + 0.0154 [Cd(II)]	A = 0.0026 + 0.0043 [Co(II)]	A = 0.0024 + 0.0045 [Cu(II)]	A = 0.0032 + 0.0012 [Pb(II)]
Enhancement factor	152	105	73	126
Detection limit (3 s, μg L^{-1})	0.15	0.5	0.5	1.9
Quantification limit (10 s, μg L^{-1})	0.5	1.7	1.7	6.4
Linear range (μg L^{-1})	0.5–25	1.7–80	1.7–80	6.4–300
Precision (RSD, n = 10) (%)	2.6	3.8	2.8	3.2
Correlation coefficient (r)	0.9994	0.9985	0.9995	0.9989

For the evaluation of method accuracy, the following certified reference materials were used: CRM 1643e and BCR 278-R. Student *t*-test was used to investigate the trueness of the method, comparing the experimental results and the certified values. The analytical results together with the t_{exp} values for all metals are presented in Table 3. All the t_{exp} values are less than the t_{crit} = 4.30, indicating that there are no statistically significant differences at the 95% probability level for each analyte and good agreement exists between the certified values and the experimental results. Subsequently, the FI-FAAS method was employed for the analysis of environmental waters from Strymon River and Prespa Lake collected from the Northern Greece area and human urine sample. The obtained results are presented in Table 4. The calculated recoveries of the analytes ranged between 92.0 and 105.0%, showing the potential application of the FI-FAAS method for the determination of these metals in similar matrices.

Table 3. Analytical results of Cd, Co, Cu and Pb in CRMs by the FI-FAAS method.

Certified Reference Material	Certified Value (μg L^{-1})	Found *	$t_{exp.}$	Recovery (%)
CRM 1643e				
Cd	6.568 ± 0.073	6.3 ± 0.2	2.321	95.9
Co	27.06 ± 0.32	25.6 ± 0.7	3.613	94.6
Cu	22.76 ± 0.31	22.5 ± 0.5	0.901	98.9
Pb	19.63 ± 0.21	19.1 ± 0.5	1.836	97.3
BCR 278-R				
Cd	0.348 ± 0.007	0.33 ± 0.02	3.118	94.8
Co	-	-	-	-
Cu	9.45 ± 0.13	9.2 ± 0.4	1.083	97.4
Pb	2.00 ± 0.04	1.96 ± 0.06	1.155	98.0

* Mean value ± standard deviation based on three replicates (*n* = 3). $t_{crit.}$ = 4.30 at 95% probability level.

Table 4. Real samples analysis by the FI-FAAS method.

Sample	Analyte (μg L^{-1})	Added *	Found *	Recovery (%)
Strymon River	Cd(II)	-	ND	-
		5.0	4.7 ± 0.2	94.0
	Co(II)	-	2.2 ± 0.1	-
		10.0	11.9 ± 0.4	97.0
	Cu(II)	-	5.2 ± 0.2	-
		10.0	15.0 ± 0.4	98.0
	Pb(II)	-	ND	-
		30.0	29.1 ± 0.9	97.0
Prespa Lake	Cd(II)	-	ND	-
		5.0	5.1 ± 0.2	102.0
	Co(II)	-	ND	-
		10.0	9.6 ± 0.3	96.0
	Cu(II)	-	4.2 ± 0.1	-
		10.0	13.6 ± 0.4	94.0
	Pb(II)	-	ND	-
		30.0	28.5 ± 1.1	95.0
Urine	Cd(II)	-	ND	-
		5.0	4.6 ± 0.15	92.0
	Co(II)	-	ND	-
		10.0	10.5 ± 0.4	105.0
	Cu(II)	-	6.5 ± 0.2	-
		10.0	16.23 ± 0.4	98.0
	Pb(II)	-	ND	-
		30.0	29.5 ± 0.9	98.3

* ND: not detected.

3.4. Comparison of FI-FAAS Method with Other On-Line Sorbent Extraction Studies

A comparison of the proposed FI-FAAS method with other automated SPE-FAAS methods is presented in Table 5. As can be observed, the herein presented protocol exhibits satisfactory precision and sensitivity with lower or similar LODs to other on-line methods utilizing diverse sorbents.

Table 5. Comparison of the FI-FAAS method with other studies for the determination of Cd(II), Co(II), Cu(II), Pb(II).

Analyte	Sorbent/Support	Ligand	SC (mL)	PT (s)	LOD (μg L^{-1})	RSD%	EF	Ref.
Cd(II), Co(II), Cu(II), Mn(II), Pb(II)	Amberlite XAD-4 2,6-pyridinedicarboxaldehyde functionalized	-	10	660	0.13–2.19	-	23.6–28.9	[6]
Cd(II), Pb(II)	Oasis HLB©	DDTP	12	90	0.09–0.9	<2.9	155–180	[7]
Cd(II), Co(II), Cu(II), Pb(II)	HypersepSCX	-	15	150	0.14–2.1	<3.3	77–99	[8]
Cd(II), Cu(II), Pb(II)	Nobias chelate PA-1	-	20	120	0.1–1.0	<3.3	98–106	[9]
Cd(II), Pb(II)	polyester fabric fibers coated sol-gel PDMS	DDTC	18	90	0.3–1.6	<2.9	40–167	[19]
Pb(II)	Glass fiber coated sol-gel PDPS	APDC	16	120	1.1	3.0	215	[20]
Cd(II), Cu(II), Pb(II)	Polyester fabric disks coated sol-gel PCL-DMS-CL	APDC	18	90	0.15–1.62	<3.5	36–250	[28]
Cd(II), Cu(II)	MWCNTs	-	4.3	60	0.11–0.30	<2.4	24–25	[29]
Pb(II)	MWCNTs	-	5	240	2.6	<7.7	44	[30]
Cd(II), Co(II), Cu(II), Pb(II)	Sol-gel Silica thiocyanatopropyl functionalized		20	120	0.15–1.9	<3.8	73–152	This work

4. Conclusions

A novel sol-gel thiocyanatopropyl functionalized silica sorbent for direct metal extraction was constructed and characterized for the first time. The presented sorbent was based on sol-gel technology, which is a well-established technique for effective sorbents on various substrates. The efficiency of the sorbent as packing material in on-line microcolumn systems was investigated. The developed method was employed for the separation/preconcentration of Cd(II), Co(II), Cu(II) and Pb(II) as model analytes. The microcolumn exhibited excellent reproducibility, high extraction efficiency, as well as satisfactory sensitivity. The proposed FI-FAAS method was used for the analysis of environmental and biological samples enabling the utilization of high-throughput on-line sample preparation. It can be concluded that the novel on-line platform is a simple and rapid method for metal determination.

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