



Adsorption behaviour of cadmium on *L*-methionine immobilized on controlled pore glass

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ABSTRACT

The adsorption behaviour of Cd onto the relative non-polar *L*-methionine was studied. To this end, *L*-met was immobilized on controlled pore glass (CPG), incorporated in a microcolumn and inserted in a flow injection system for Cd preconcentration from aqueous solutions. Binding constant of the system was calculated and it turned to be of 1.99, with sites capacity of $n=3.12$. The ratio of Cd moles bound to *L*-met moles was calculated and it was 0.03:1 at pH 9.0. On-line breakthrough curves were used to study the effect of pH, analyte concentration and influent flow rate on Cd retention. A complementary pH study was added with a titration curve. Transient peak areas revealed that Cd stripping from the column occurred instantaneously. The system achieves an enrichment factor of 130, reaching a detection limit of 0.63 ng L^{-1} when 10 mL of the solution were passed through the column. The method was successfully applied to Cd determination in the standard reference material (SRM), QC METAL LL2 metals in natural water, as a validation study.

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1. Introduction

Amino acids and peptides have showed growing interest as new substrates for metal preconcentration and/or speciation analysis due to their metal binding capacity. They were successfully applied in different fields such as nanotechnology [1], metal remediation [2–6] and metal preconcentration and separation [7–10].

Amino acids possess different functional groups with different metal binding capacities. Selectivity, strong binding capacity and environmental innocuity [11–14] are some properties that amino acids present turning them into ideal molecules for trace element preconcentration. The immobilization possibility into solid supports like controlled pore glass (CPG) provides the opportunity of column packing. Under these conditions, amino acids acquires a specific binding orientation in the presence of a complexing metal. The high specific surface of CPG enhances the number of immobilized molecules, providing a higher metal retention with easy release and reusability [2]. These columns inserted into on-line systems can be used for analytes retention and their subsequent determination by atomic spectrometric techniques such as inductively coupled plasma optical emission spectrometry (ICP OES).

L-methionine has a bulky hydrophobic and non polar aliphatic side chain. These are not the ideal properties for metal retention, even though many studies have been reported in the literature about the non-covalent interactions of *L*-met with metals [1,15–17]. Barth et al. obtained nano-gratings from the non-covalent interactions of *L*-met chains with an Ag surface [1]. Rusu et al. ascribed square-planar local symmetry for *L*-met complexes with Cu ion. Even more, complexes of *L*-met with Cd have also been described [16,17].

Amino acids act as bidentate ligands with coordination involving the carboxyloxygen and the nitrogen atom of amino group [15]. Amino acids are immobilized through its amino group to CPG, providing a free carboxylate group to interact with metals. The terminal carboxylic acid and SH-binding site in metallothioneins is more stable than binding by SH groups alone [18]. In the particular case of *L*-met, the thioether group of the molecule is not the primary binding site in any case, but depending on the number and location of this group, they have some contribution to the binding [19,20]. *L*-met specifically retained ions such as seleniate [21,22], antimoniate [23], vanadate [24], and aluminium [25].

Previous work with immobilized biopolymers used for cation exchange has demonstrated that a quantitative release can be achieved by simply lowering the pH of the solution [7,10,26–28]. It was first suggested [27] and later shown [28] that acids can cause a reversible change in the tertiary structure of amino acids providing efficient and rapid release of metals from the binding cavity. It is of particular importance for analytical applications because the target metal can be easily released enhancing the preconcentration ratio.

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Previous studies demonstrated that metals retained by *L*-met were released with a quantitative recovery [21–25] and that elevated enrichments factors can be reached [25].

Although some studies have been carried out about elemental retention capacity of *L*-met [21–25], no one of them attempt to reveal the elemental adsorption behaviour onto *L*-met-CPG. The aim of the present study is to assess the sorption behaviour of Cd onto the *L*-met-CPG system. Influent concentration, pH, influent flow rate, elution profile and binding constants were used as parameters for characterization. Analytical features were also studied. For validation, the system was applied to Cd determination in QC-LL2 standard reference material. To the best of our knowledge this is the first time that Cd retention onto *L*-met-CPG is studied for analytical purposes.

2. Experimental

2.1. Instrumentation

Measurements were performed with a sequential ICP spectrometer Baird ICP 2070 (Bedford, MA, USA.). The 1 m Czerny-Turner monochromator had a holographic grating with 1800 grooves mm^{-1} . An ultrasonic nebulizer, U 5000 AT [CETAC Technologies (Omaha, NE, USA)], with desolvation system was used. A Minipulse 3 peristaltic pump Gilson (Villiers-Le-Bell, France) was also used. Sample injection was achieved using a Rheodyne (Cotati, CA, USA) Model 50, four-way and of 6 ports, 2 positions, rotary valves. Instrumental details and experimental conditions adopted are depicted in Table 1. The conical minicolumn was prepared by placing 50 mg of *L*-methionine-CPG into an empty conical tip using the dry packing method. To avoid filling losses when the sample solution passed through the conical minicolumn, a small amount of quartz wool was placed at both ends of the conical minicolumn. The column was then connected to an 8-roller peristaltic pump with PTFE tubing to form the preconcentration system. Tygon type pump tubing (Ismatec, Cole Parmer, Vernon Hills, IL, USA) was employed to propel the sample, reagents and eluent.

2.2. Reagents

Unless otherwise stated, the chemicals used were of analytical grade, and therefore no further purification was required. Working standard solutions were prepared immediately before use by stepwise dilution from 1000 mg L^{-1} Cd stock standard solution.

L-methionine was obtained from Fluka A. G., (Switzerland). Controlled pore glass (CPG, pore diameter 240, mesh size 240–400), 8-aminopropyltriethoxysilane and glutaraldehyde were supplied by Sigma (St. Louis, USA).

2.3. Immobilization procedure

A 0.2 g portion of *L*-methionine was suspended in 15 mL 0.1 mol L^{-1} phosphate buffer at pH 7.0. Silanization of the CPG using 8-

aminopropyltriethoxysilane and the use of the bifunctional property of glutaraldehyde to prepare the glutaraldehyde-treated CPG was reported previously [29]. Glutaraldehyde is employed due to its bifunctional property, with two functional groups on both sides: one links with silane, and the other one links with the amino group of *L*-met. The glutaraldehyde-treated CPG was filtered and washed. To the baker containing the methionine solution, 1.0 g of the treated glass was added and N_2 was flushed for 15 min. The mixture was kept at 4 °C for 24 h under a N_2 atmosphere and then air-dried filtered.

2.4. Metal binding studies

2.4.1. Procedure

The uptake and release of Cd by *L*-methionine immobilized on CPG (*L*-met-CPG) were studied with respect to pH, influent flow rate, and influent concentration using a batch procedure. Before starting the experiments, a cleaning step of 5 min with 10% HCl was performed. After that, a 0.05 mol L^{-1} ammonium acetate solution (pH 7.0) was pumped through the column for 2 min at 1 mL min^{-1} to recondition the column to the neutral pH. The Cd solutions were prepared by dilution from the metal standard into 0.05 mol L^{-1} ammonium acetate. Different volumes of 11.43 mg L^{-1} ammonium acetate-cadmium solution were then introduced onto the column at a flow rate of 1 mL min^{-1} .

The effluent solutions were collected in 25 mL glass flasks. Once the effluent concentration reached the influent concentration, the sample flow was stopped. A 10% HCl solution at a flow rate of 1 mL min^{-1} was used to strip Cd from the column for 5 min. Other eluents such as HNO_3 were tested and no significant difference was observed in comparison to HCl during the elution process. A volume of 5 mL of eluent ensures a complete removal of the metal from the column. Measurements of Cd concentration in the different solutions were made directly by ICP OES.

2.4.2. Titration studies

An acid-base titration was performed to determine the pKa of the system. Two aliquots of 0.05 g of *L*-met-CPG were dispersed in 50 mL of deionized water. One aliquot was added with 0.1 mol L^{-1} NaOH from an automatic burette while the solution pH was measured using a pH meter, under continuous shaking. The other one was titrated with 0.1 mol L^{-1} HCl in order to complete the titration curve. Finally, the titration curve was recorded and the pKa determined.

2.4.3. Elution profile

Transient peak areas were used to construct the system elution profile when on-line acid stripping was employed. This was achieved by loading the column with different Cd concentrations made up in a solution of 0.05 mol L^{-1} ammonium acetate buffer, adjusted to pH 9, where the maximum capacity was observed and stripped with 10% HCl into the USN-ICP OES system. Both, loading and elution flow rates were of 1 mL min^{-1} . The calibration graphs were set up as concentration of analyte versus peak area of the transient signal.

2.4.4. Evaluation of stability constants

The batch procedure followed for binding sites evaluation was accomplished by equilibrating a known mass of *L*-met-CPG with known concentrations of Cd solutions. Different Cd solutions in 0.05 mol L^{-1} ammonium acetate at pH 7.0 in a 25 mL polyethylene container were re-circulated at 1.0 mL min^{-1} through the minicolumn containing 0.05 g of *L*-met-CPG material accordingly to previous described methods [30]. To improve mass transport, the 25 mL bottles were mounted on a motor-driven wheel turning and solutions were tumbled for 18 h, to reach equilibrium conditions. Then, 10 mL of the supernatant were removed for Cd determination.

After this, Cd bound to the column was eluted with 10 mL 10% HCl at 1.0 mL min^{-1} . This solution was also analyzed by ICP OES for Cd determination. These experiments were performed by duplicate.

Table 1
USN-ICP OES instrumental parameters

ICP conditions	
RF generator power (kW).	1.0
Frequency of RF generator (MHz).	40.68
Plasma gas flow rate (L min^{-1}).	8.5
Auxiliary gas flow rate (L min^{-1}).	1
Carrier gas flow rate (mL min^{-1}).	90
Observation height above load coil. (mm).	15
Analytical line: Cd (nm).	228.802
USN conditions	
Heater temperature	140 °C
Condenser temperature	4 °C
Sample flow rate	1 mL min^{-1}

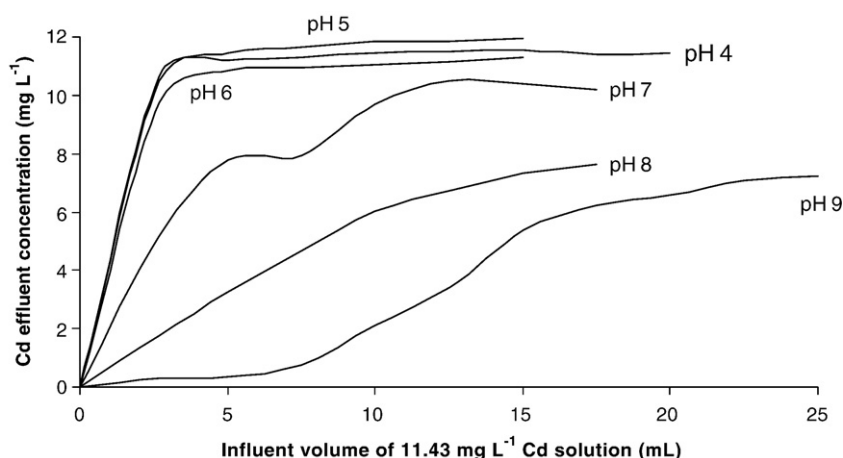


Fig. 1. Breakthrough curves on *L*-met-CPG for 11.43 mg of Cd L⁻¹ of solution (pH 4.0–9.0) loaded at 1.00 mL min⁻¹.

2.5. Analytical performance studies

2.5.1. Preconcentration procedure

Analytical performance studies were performed employing an on-line system. The flow injection system used for preconcentration, separation and subsequent determination of Cd is the same described previously [25]. Before loading, the column was conditioned at the desired pH with 0.05 mol L⁻¹ ammonium acetate buffer at pH 7.0 (valve V₁ in position B). A volume of sample was then loaded on the conical-minicolumn (M) at flow rate of 1 mL min⁻¹ with valve V₁ in position S and valve V₂ in load position (a). Finally, valve V₂ was switched to the injection position (b) and Cd retained was eluted with 10% HCl solution at 1 mL min⁻¹. After that, the eluate was introduced into the USN unit and subsequently pumped to the ICP torch. The operation conditions were established and the determination was carried out.

3. Results and discussion

3.1. Determination of column capacity

The *L*-met-CPG column capacity was determined for pH values ranging from 4.0 to 9.0, with an 11.43 mg L⁻¹ Cd influent solution pumped through the column at a flow rate of 1.00 mL min⁻¹.

The total amount of Cd bound, expressed in μmol g⁻¹ of *L*-met-CPG, was determined by integration of the breakthrough curves in Fig. 1 and it was verified by comparison with the amount of Cd recovered in the 25 mL 10% chloride effluent solution (Table 1). A study was performed to determine the minimal volume of effluent needed for Cd stripping from the column. It turned to be of 1 mL and beyond this volume the amount of stripped metal from the column decreased significantly. The total Cd recovered in the effluent was in good agreement with calculations from the breakthrough curves. Cd effluent concentration reached the influent concentration of 11.43 mg L⁻¹ only for pH 4, 5 and 6. This indicates that for these pH values, the binding sites of *L*-met-CPG are saturated. From pH 7 and on, a different behaviour starts and the initial concentration of the influent solution cannot be reached (Table 2).

Table 2
Effective Cd retention capacities for *L*-met-CPG

pH	Cd bound (μmol g ⁻¹ of CPG)	Cd recovered (%)
4	0.42 ± 0.09	105.87
5	0.31 ± 0.12	100.70
6	0.96 ± 0.29	102.84
7	3.34 ± 0.57	116.94
8	7.05 ± 0.26	87.07
9	15.11 ± 2.58	112.43

3.2. Evaluation of conditional stability constants

Considering previous experimental data such as Cd bound to the column and its easy stripping, it was plausible to think that Cd is not strongly bound to *L*-met-CPG. Scatchard analysis has been previously employed to estimate conditional stability constants for metal-binding by *L*-met-CPG [3,10]. The Scatchard function expresses that:

$$\frac{[\text{CdX}_i]}{[\text{Cd}]} = K_i(n_i - [\text{CdX}_i]) \quad (1)$$

where [CdX_i] (expressed as μmol g⁻¹ resin) is the number of complexed sites of type *i*, [Cd] is the concentration of free Cd (μmol L⁻¹), *n_i* is the total concentration (μmol g⁻¹ resin) of type *i* sites, and *K_i* is the stability constant for the *i*th site. This equation was re-formulated as follows in order to obtain an adequate equation for binding studies:

$$\frac{[\text{CdX}_i]}{[\text{Cd}]} = K_i n_i - K_i [\text{CdX}_i] \quad (2)$$

This expression has been described previously. The model assumes that the binding is a simple reversible bimolecular reaction that obeys mass action laws and non specific binding does not occur [30]. This equation describes a straight line which slope corresponds to $-K_i$ value, and the intersection of the straight line with ordinate axis corresponds to the product of $K_i n_i$. Several concentrations were tested in order to build the straight line. The ordinate axis represents the quotient between [CdX_i] and [Cd]; and abscises axis represents [CdX_i]. Cd influent concentrations were lower than 8 mg L⁻¹ to avoid the baseline region or “shoulder” between 5 and 7.5 mL of influent volume in the breakthrough curve for pH 7.0. The values obtained for *K* were of 1.99, *n*=3.12. The obtained value is one order of magnitude higher than that obtained for Pb binding on algae, where the same method was employed [30].

3.3. Effect of pH on Cd binding

The amino acid immobilization into the solid support elapses through its amino group with the carboxyl and the functional group available to interact with metals. The deprotonation of the carboxyl group in *L*-met occurs at pH 2.28. Based on this, Cd should be retained at any pH over this value. As it can be seen in Fig. 1, retention is more effective from pH 7.0 and on. Then, the retention capacity is not completely related to a deprotonation process of functional groups. Spatial distribution, binding orientation and tertiary structure are also important parameters to consider [31].

A certain amount of weaker binding sites appears and Cd effluent concentrations cannot reach the concentration of the initial solution, 11.43 mg L⁻¹. The breakthrough curve for pH 7.0 shows a particularity,

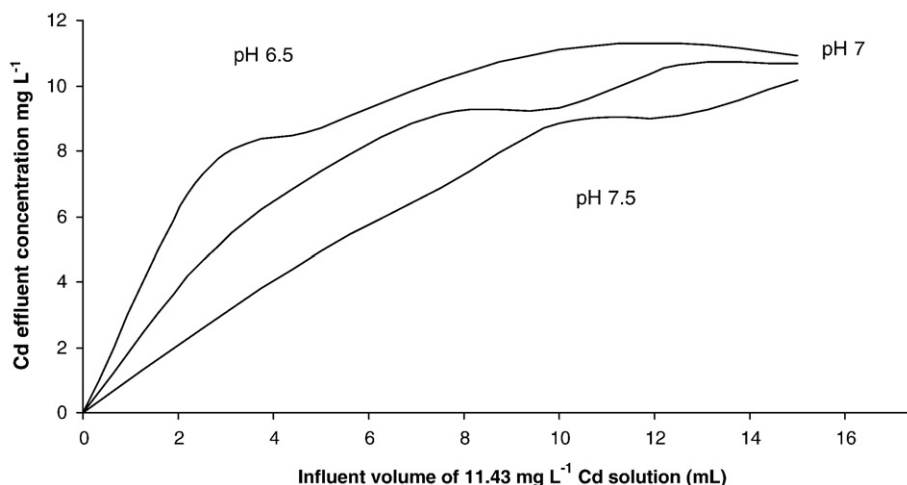


Fig. 2. Breakthrough curves on *L*-met-CPG for 11.43 mg of Cd L⁻¹ of solution (pH 6.5–7.5) loaded at 1.00 mL min⁻¹.

as it can be seen in Fig. 1. It exhibits a baseline region or “shoulder” between 5 and 7.5 mL of influent solution, reaching an effluent concentration of ~8 mg L⁻¹. After this, the sloped region continues until 11.43 mg L⁻¹. To reject the possibility of an experimental error, this experiment was repeated but this particular behaviour remained. This motivated another experiment: the analysis of the breakthrough curves from pH 6.5 to 7.5, close to pH 7. These curves can be observed in Fig. 2. The baseline region appears at higher influent volumes with increased pHs, from 6.5 to 7.5. A possible explanation to this behaviour is the generation of new binding sites as long as Cd uptake by *L*-met-CPG progresses. The saturation process enables new binding sites that were encrypted before Cd uptake by *L*-met-CPG. This process has been described by Miller et al. [28] attributing this to the different spatial distribution that amino acids acquire in the metal binding process and as a secondary effect; it could enable new binding sites that were inaccessible when the amino acid was not complexed.

The increase of pH is also a factor involved in changes of the tertiary structure, affecting Cd uptake [7]. We observed that the higher uptake of Cd by *L*-met-CPG was at pHs 8 and 9. As it can be seen in Table 1, the highest value was 15.11 μg of Cd g⁻¹ of *L*-met-CPG. Also at these pHs values a number of weaker sites appear since the effluents concentrations do not reach the concentration of the initial solution loaded on the column, which corresponds to 11.43 mg L⁻¹.

The calculation of the mol ratio (moles of Cd bound at pH 9 to moles of *L*-met) turned to be 0.03:1. This value seems low compared to

those obtained for poly-*L*-cysteine (6.2:1) [7]. If we consider that poly-*L*-cys has an average of 50 residues per chain, *L*-met ratio appears to be quite acceptable.

A study of Cd binding to siloxyl groups onto CPG surface has been described previously, even for different stages of immobilization [26]. The binding capacity of CPG to Cd corresponds to 0.41, <0.1 and <0.1 μmol g⁻¹ for acid-activated CPG, silanized-CPG and glutaraldehyde-CPG respectively at pH 7.0. Comparing these results with those obtained for Cd binding onto *L*-met-CPG at the same pH, we conclude that CPG by itself does not contribute significantly for Cd binding.

In Fig. 3 a titration curve of *L*-met-CPG system can be observed. This study was performed to reveal the system behaviour in aqueous solution versus pH variations. *L*-met-CPG system has an initial slightly acid pH, ~5.49, corresponding to siloxyls and aldehydes of CPG, and carboxylic groups of *L*-met. The titration was performed in two ways, 1) with an acid solution and 2) with an alkali solution. The amount of acid solution needed to reach the baseline was minor compared with the alkali one. The equivalence point was reached with the alkali solution at around pH 7.25. This result agrees with the breakthrough curves obtained for Cd uptake by *L*-met-CPG where the retention is more effective from pH 7.0 and on.

3.4. Effect of influent concentration on Cd binding

Different Cd influent solutions were loaded onto the column at pH 7.0, with a flow rate of 1 mL min⁻¹. This study was performed at pH 7.0 where the column is completely saturated with a 100% recovery. This study determined the uptake capacity of *L*-met-CPG at different Cd influent concentrations. The influent concentrations tested were 22.86 and 5.71 mg L⁻¹ and the amount of metal bound was 6.42 and 1.59 μmol of Cd g⁻¹ CPG, respectively.

Comparing this result with the initial Cd concentration of 11.43 mg L⁻¹, which retained 3.34 μmol of Cd g⁻¹ of *L*-met-CPG at pH 7 we can conclude that *L*-met-CPG retains Cd proportionally to the concentration loaded.

3.5. Effect of flow rate on Cd uptake

Influent flow rate is a very important parameter to consider in this kind of studies because it is directly related to sample throughput. The influent flow rate effect on Cd binding by *L*-met-CPG was studied by loading an 11.43 mg L⁻¹ Cd solution at 0.5 and 2.0 mL min⁻¹ (all at pH 7.0). These results were compared with the breakthrough curve at 1.0 mL min⁻¹, as it can be seen in Fig. 4.

At low flow rates, Cd was strongly retained into the column while at higher flow rates the metal uptake decreased. From these results we

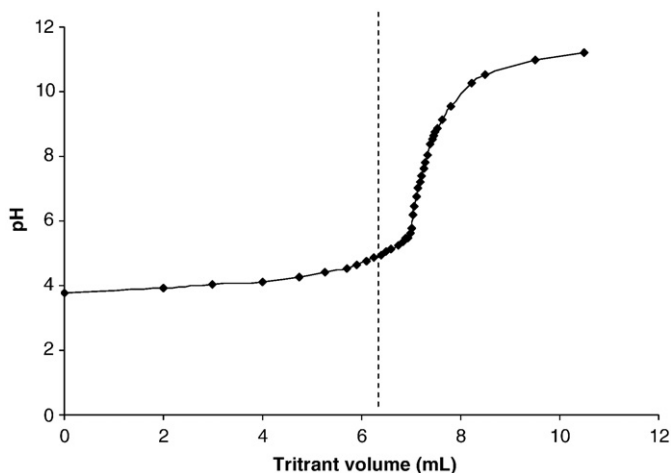


Fig. 3. Titration curve corresponding to 50 mg of *L*-met-CPG. The interrupt line indicates the division of the two titrations.

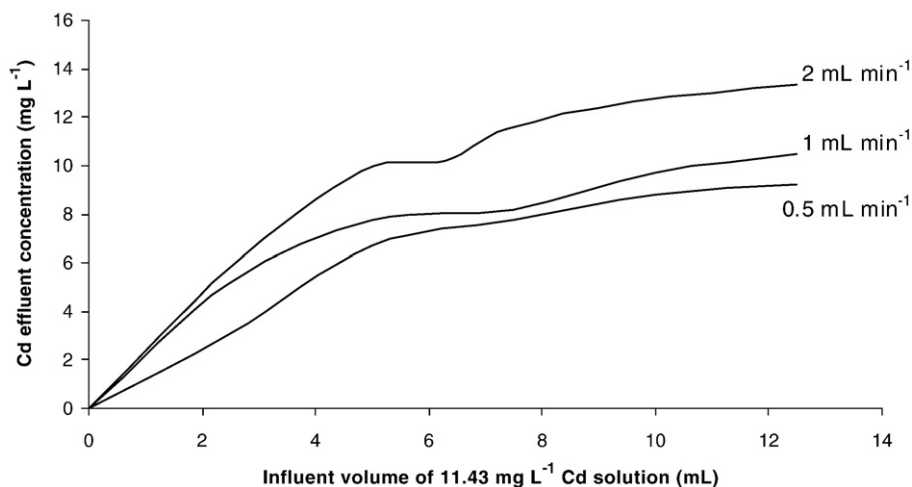


Fig. 4. Breakthrough curves on *L*-met-CPG for 11.43 mg of Cd L⁻¹ of solution (pH 7.0) loaded at flow rates of 0.5, 1.0 and 2.0 mL min⁻¹.

conclude that at flow rates higher than 1.0 mL min⁻¹, *L*-met-CPG is not binding Cd under equilibrium conditions. The baseline region shows a decrease in Cd binding with increased influent flow rate. Higher influent flow rates diminished the possibility of interaction between Cd and *L*-met-CPG.

3.6. Elution profile

As mentioned previously, Cd stripping from *L*-met-CPG is fast with quantitative recoveries reaching elevated enrichment factors. To provide a better analysis of this situation, the elution profile of Cd elution from *L*-met-CPG was studied. Fig. 5 shows the time-dependent, transient signal obtained from stripping the metal from the column. From the shape of the strip peaks we conclude that the elution step is fast and instantaneous. The transient signals lasted for less than 20 s, which corresponds to 0.6 mL of HCl 10% solution for metal removal. The trailing edges of the strip peaks shows a very straighten increase followed by a slightly decay of the signal with a "tale" at the end of the strip, describing a very acute peak. The straighten increase could be attributed to accessible binding sites of *L*-met-CPG to the eluent allowing a fast proton exchange, this is mostly due to the monolayer formed by *L*-met on CPG with no ramifications. On the other hand, the slightly decay could be attributed to certain regions of the CPG with decrease access to the eluent.

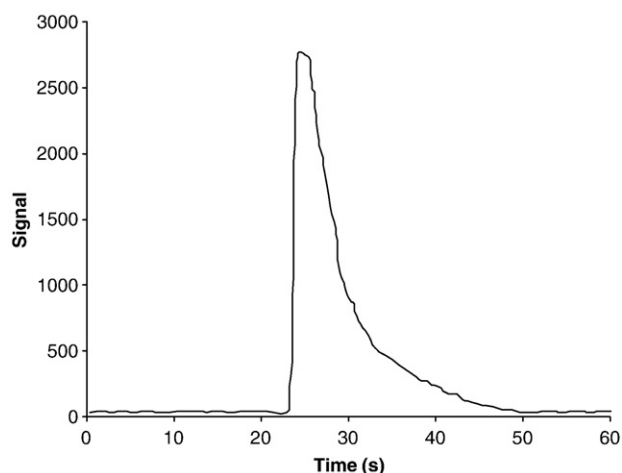


Fig. 5. Transient absorbance signal observed when stripping Cd on-line with 10% HCl.

3.7. Analytical performance

In order to establish the analytical potential of *L*-met-CPG for Cd preconcentration and separation, a study of the detection limit, enhancement factor and possible interferences was carried out.

The analytical performance was studied by loading 10 mL of a 11.43 µg L⁻¹ Cd solution on the column. The study was performed at pH 9.0, where the retention was maximal. The detection limit (LoD), calculated on the basis of the 3σ criterion, and with the preconcentration of a 10 mL sample turned out to be 0.62 ng L⁻¹ (n=7). The precision (RSD) for 5 replicate determinations of a solution containing 1.0 µg L⁻¹ of Cd were 3.4%. A total enrichment factor of 130 was obtained. Linearity was obtained from the limit of quantification (LoQ) till at least 2000 µg L⁻¹, the coefficient of correlation (r²) was 0.9995.

The effect of potential interferent species was tested. For this purpose, synthetic Cd solutions were prepared and the signal was monitored, after the preconcentration procedure, in the presence of Cu(II), Fe(III), Ni(II) and V(V) up to at least 1000 mg L⁻¹. Other elements such as alkaline and alkaline earth elements were not retained on the immobilized methionine under the working conditions. On the other hand, the effects of anions such as CO₃²⁻ and SO₄²⁻ were tolerated up to at least of 1000 mg L⁻¹.

In order to validate the proposed method, Cd was determined in the standard reference material (SRM) QC METAL LL2 metals in natural water with a Cd certified concentration of 1.97 ± 0.11 µg L⁻¹. The standard addition technique was applied to Cd determination. Three aliquots of 20 mL of the SRM at pH 9.0 were added with 1, 4 and 8 µg L⁻¹ of Cd solution and diluted with distilled water into glass flasks to 25 mL. Using the proposed method, the content of Cd found was 2.1 ± 0.09 µg L⁻¹ (95% confidence interval; n=6).

4. Conclusion

The results obtained show that *L*-methionine immobilized on CPG is an appropriate substrate for cadmium extraction and preconcentration. A 100% of Cd retention was reached in a wide pH range. A linear response of *L*-met-CPG system to different Cd influent concentrations provides versatility to the system. This is directly related to different sample analysis with different Cd concentration. The monolayer disposal of *L*-met on CPG surface allowed a fast and instantaneous strip of Cd from the column by the eluent.

The analytical features of the system were correlated with the mentioned characteristics of *L*-met-CPG. The fast kinetic adsorption/desorption process, allowed a high enrichment factor, reaching a very low detection limit when USN-ICP OES is used as detection system. The results of this study will be extended to other amino acids and

other analytes. These aspects of amino acids and the possibility of their immobilization into different solid supports are under further investigation.

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