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 biohomopolymers 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-aminopropiltriethoxysilane 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}