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Article

On-line Preconcentration Employing a Tannin Resin for Copper Determination in Plant Material and Food Stuff by Atomic Absorption Spectrometry

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Um sistema de análise em fluxo foi desenvolvido para pré-concentração de cobre em linha, empregando uma resina de tanino, preparada a partir de *Eucalyptus saligna sm*, com determinação por espectrometria de absorção atômica com chama. No módulo de análise, foram incluídos um injetor-comutador automático e um conjunto de válvulas solenóides controlados por um microcomputador, equipado com uma placa PCL 711, utilizando um programa escrito em QuickBASIC 4.5. Na etapa de pré-concentração, a solução de cobre era bombeada através da coluna de resina durante 60 s e a eluição era efetuada com uma solução de ácido clorídrico 1,0 mol L^{-1}. A exatidão foi avaliada mediante a determinação de cobre em digeridos de amostras certificadas e não foi observada diferença significativa em nível de confiança de 95%. Outras características importantes, tais como frequência analítica de 48 determinações por hora, consumo de reagente de 67 μL por determinação, desvio padrão relativo de 3 % (n=10) para uma amostra típica apresentado concentração de 21,75 ng mL^{-1} Cu, limite de detecção de 1 ng mL^{-1} e fator de enriquecimento de 10 vezes foram também obtidas.

A flow system for copper on-line preconcentration, employing a tannin resin prepared from *Eucalyptus Saligna Sm*, with determination by flame atomic absorption spectrometry is proposed. The manifold was designed by assembling an automatic injector-commutator and a set of three-way solenoid valves which were controlled by a microcomputer with a PCL-711 interface card running a software written in QuickBASIC 4.5. The analyte was concentrated by pumping the sample solution through the resin column during 60 s, followed by elution with a 1.0 mol L^{-1} hydrochloric acid solution. Accuracy was assessed by analyzing acidic digests of certificate reference materials and no significant difference at 95% confidence level was observed. Other profitable features such as throughput of 48 determinations per hour, reagent consumption of 67 μL per determination, relative standard deviation of 3% (n=10) for a typical sample presenting concentration of 21.75 ng mL^{-1} Cu, detection limit of 1 ng mL^{-1} and enrichment factor of 10 times were also achieved.

Keywords: preconcentration, flow system, copper determination, atomic absorption spectrometry

Introduction

Determination of metal ions at the ng mL^{-1} level generally requires prior separation and/or preconcentration steps in order to improve sensitivity. When preconcentration is carried out, separation of potential interferents, as well as minimization of the matrix effects can also be achieved[1], besides concentration of the analyte.

Since Bergamin et al.[2] proposed an on-line preconcentration procedure for the spectrophotometric determination of ammonium, a remarkable increase in the employment of preconcentration techniques, specially for spectrometric methods, have been observed[3-5].

For preconcentration of copper and other metals there are many types of resins available in the market. Despite this, in the last years many efforts have been made to prepare natural sorption materials such as natural polymers[6] and bioadsorbents[7], considering their high natural sorption power and/or high complexing ability and low cost.

Tannins extracted from different forest species have been investigated in order to verify their ability to adsorb metals[8]. Higher sorption capacity was achieved using sorbent

material prepared from *Eucalyptus saligna sm*, which contains more than 60% of oligomeric hydrolysable tannins. Plant tannins are high molecular weight natural polyphenols and their effective complexing power is due to the presence of at least two hydroxyl groups in the ortho position. Both hydrolysable and condensed tannins can link themselves to metallic cations forming highly stable tannates. Nevertheless, tannins are soluble in water, thus their immobilization on a suitable support is necessary in order to use them as sorption material[8]. Sorbent based on the immobilization of *Eucaliptus saligna sm* onto cellulose tannins was used for mercury sorption from an aqueous system[7] and for uranium preconcentration[9]. Nevertheless, there is a lack of reports in the literature on the applications of this sorbent in flow systems.

Metals are partitioned in the biota both spatially and temporally by distinct acid-base affinities and by their kinetics. One aspect of metal toxicity is the chemical combination of metal and ligands in organisms[10]. Copper is hardly absorbed by plants, but in some cases, when used as a fungicide, specially in potato and grape production, a considerable effect on copper levels occurs. This element is essential as a micronutrient in plants, however it can be absorbed from contamined soils[11]. Since copper is an useful element for plants at low levels, it is necessary to monitor its concentration to avoid contamination.

In healthy plants, copper is normally found at low levels, requiring a preconcentration step for its determination. Thus, a flow system employing a tannin resin immobilized onto cellulose for on-line copper preconcentration with determination by flame atomic absorption spectrometry was investigated.

Experimental

Apparatus

A Perkin-Elmer 503 atomic absorption spectrometer equipped with a copper hollow-cathode lamp using an air-acetylene flame and connected to an ECB 201 strip chart recorder (Equipamentos Científicos do Brasil, São Paulo) was used. In the flow manifold a mp-13R Ismatec peristaltic pump with Tygon pumping tubes, an automatic injector-commutator[12], a set of three-way solenoid valves (NResearch 161T031 - Stow, MA) controlled by a microcomputer with a PCL-711 interface card (American Advantech, San Jose, California)[13], polyethylene tubes, three-way connecting pieces made of Perspex and a resin column were included. This column was 10 mm long and 4 mm internal diameter machined in a Perspex block and sandwiched between two other blocks fixed by two screws[14]. The column was filled with tannin resin and polyesther sieve discs were plugged in each column end to avoid resin losses. The column was connected to the injector sliding bar by mean of polyethylene tubing.

Reagents and solutions

All chemicals were of analytical reagent grade, and freshly distilled and deionized water was used throughout.

A 1,000 g L^{-1} copper stock solution was prepared by dissolving 1.000 g of metallic copper in 10 mL of a 1:1 (v/v) nitric acid solution and making the volume up to 1000 mL with water. Working solutions were prepared by suitable dilutions from stock solution.

A 1.0 mol L^{-1} potassium dihydrogen phosphate buffer solution was prepared by dissolving 13.60 g of the salt in 100 mL of water and adjusting the pH to 7.0 with a 1.0 mol L^{-1} sodium hydroxide solution and making the volume up to 200 mL with water. A 1.0 mol L^{-1} hydrochloric acid solution used as eluent was prepared by appropriate dilution from concentrated acid.

Resin preparation

The sorbent was synthesized by using a modification of the procedure developed earlier for tannic acid immobilization on epoxiactivated agarose[8]. Spherical cellulose is a white powder with high porosity and good mechanical properties which was sieved to select particles with 0.075-0.160 mm to be used as immobilization matrix. This cellulose was obtained from the Treatment Laboratories of Cellulose Materials (Cuba 9, Havana, Cuba). Tannins from *Eucaliptus saligna sm* were obtained from the Institute of Nuclear Science and Technologies and extracted from the tree bark by maceration in water. Immobilization was carried out onto spherical cellulose by chemical attachment with epichloridrine. The reagent 1,2-epoxi-3-chloropropane was employed for matrix activation. An interposition employing 1,6-hexanodiamine as spatial arranger was also included in the activation mechanism to avoid sterical effects, due to the bulky tannin molecules during immobilization process. An amount of 20 g epoxiactivated cellulose was kept in contact with a 500 mL tannin solution during 24 hours in a nitrogen atmosphere at 40 ^{o}C. The brown colour sorbent obtained was filtered and washed with 0.1 mol L^{-1} hydrochloric acid and rinsed with water subsequently until neutralization and then air dried[9].

Sample preparation

The following certified reference materials were used: unpolished rice flour (NIES 10-a), unpolished rice flour

unpolished (NIES 10-c) from the National Institute for Environmental Studies (Japan), rice flour (NIST 1568) from the National Institute of Standards and Technology (USA) and rye grass (CBR 281) from the Community Bureau of Reference (Belgium). Samples were mineralized by using a nitric-perchloric digestion as described elsewhere[15]. A 1.000 g sample amount was weighted (0.500 g for CBR 281 sample) and transferred to a digestion tube. A 5 mL concentrated nitric acid was added and the mixture was kept at laboratory temperature overnight. The tube was placed in a digestor block and warmed slowly up to 165 °C, this temperature was maintained until brown fumes releasing stopped. After cooling, a 1.3 mL perchloric acid was added and temperature was raised to 210 °C, which was maintained until white fumes appeared. After cooling, 25 mL of water were added to the digestion tube. The pH was adjusted to 5.0 with the buffer solution described above and the volume was made up to 100 mL with water.

Flow diagram and experimental procedure

The manifold is depicted in Figure 1 with the injector in the preconcentration position. The solenoid valves switching on/off time and its sequence is presented in the time diagram of Figure 2. In the configuration of Figure 1, valves V_2, V_3 and V_4 are off, reagent solutions are recycled and carrier stream flows towards the detector (FAAS).

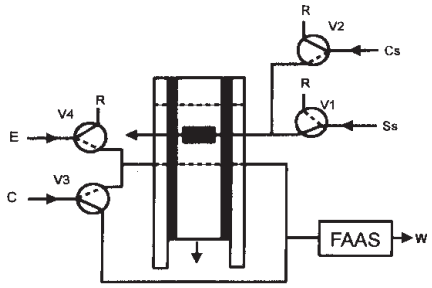

Figure 1. Diagram of the flow system The three rectangular areas are an overview of the injector-commutator, the shaded box in the center represents the resin column, the dashed lines indicate inner holes, the arrow indicates the sliding bar displacement for the eluting position; V1, V2, V3 and V4 = solenoid valves; FAAS = spectrometer; E = eluent, 0.5 mol L^{-1} hydrochloric acid solution, flow rate at 2.0 mL min^{-1}; C = carrier stream, water, flow rate at 3.4 mL min^{-1}; Ss = sample solution; flow rate at 4 mL min^{-1}; Cs = conditioning solution, flow rate at 2 mL min^{-1}; R = solution recuperation. The arrow in the flow lines indicates direction of pumping.

As indicated in the valve timing diagram (Figure 2), the analytical cycle begins by switching valve V_2 on for 2 s for resin conditioning with buffer solution. Afterwards, valve V_1 is switched on (Figure 1) directing the sample solution through the column where ion exchange takes place and the effluent flows toward waste (w). Once the column loading step is

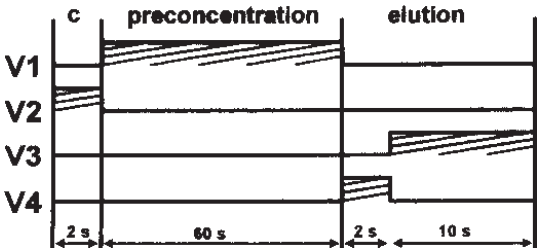

Figure 2. Time diagram of the analytical cycle. V1, V2, V3 and V4 = three way solenoid valves; c = resin conditioning step; the hatched surfaces indicate that the valve is switched on.

completed, the injector is commuted to the elution position and valve V_4 is switched on for 2 s to allow introducion of an aliquot of the eluent solution into the analytical path. Afterwards, valve V_3 is switched on for 10 s in order to direct the eluent aliquot to the column by the carrier solution (C). Thus the eluate is displaced from the column towards the spectrometer nebulizer. The next analytical cycle is started when valve V_3 is switched off and the injector is commuted back.

Parameters such as pH of the buffer solution, volume of the conditioning solution, eluent solution concentration, eluent solution volume, sample flow rate and column loading time were investigated. This was done to achieve best efficiency concerning sorption and elution, which should result in increased sensibility, as well as high analytical throughput. These studies were performed by using a 100 ng mL^{-1} copper standard solution.

Tannin resin is pH dependent, thus the effect of the conditioning solution pH was examined by using 1.0 mol L^{-1} potassium dihydrogen phosphate solution with pH adjusted to 6, 7 and 8. Subsequently, the conditioning solution volume was investigated by programming the microcomputer to switch valve V_2 on for 1, 2, 5 and 10 s, releasing solution volumes of 33, 67, 160 and 340 µL, respectively.

The effect of eluent solution concentration was studied using 0.25, 0.50, 1.00, 1.50 and 2.00 mol L^{-1} hydrochloric acid solutions. Eluent solution volume was then investigated, by programming the microcomputer to switch valve V_4 on for 1, 2, 5 and 10 s, delivering volumes of 33, 67, 160 and 340 µL, respectively.

Sample solution flow rate was studied by employing flow rates ranging from 4 to 16 mL min^{-1}, maintaining the column loading time at 60 s. Conditioning step was done with pH 7.0 buffer solution and switching on valve V_2 for 2 s. Elution step was performed using a 1.0 mol L^{-1} hydrochloric acid solution and switching valve V_4 on for 2 s, with carrier solution flow rate fixed at 3.4 mL min^{-1}.

Loading time was varied from 60 to 140 s with increments of 20 s to evaluate its influence on the analytical signal magnitude. For this study sample flow rate was fixed at 4 mL min^{-1}.

A solution containing the main concomitant ions present in the samples was prepared in order to evaluate their effect on the analytical signal. For this study, a solution containing 100 ng Cu mL^{-1} plus concomitant ions (Ca^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+} and Zn^{2+}) was prepared. Their concentration in this solution was approximately 1000 higher than that found in the sample solutions considered.

Results and Discussion

Tannins contain phenolic groups which are weakly acid, so that at low pH they are not available to form chelate complexes. As shown in Figure 3, best results were obtained with solutions at pH 7, thus, sample and resin conditioning buffer solutions at this pH value were used throughout. The observed decrease in analytical signal at pH higher than 7 was probably caused by competition with copper hydrolysis products.

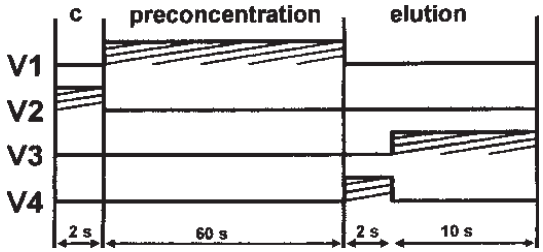

Figure 3. Effect of the acidity. Preconcentration time = 60 s; standard solution = 100 ng mL^{-1} Cu. System parameters as in Figure 1.

Prior to the resin loading step, the conditioning step was carried out by switching on valve V_2 (Figure 1) for 2 s and delivering 67 µL of conditioning solution which was enough to assure resin conditioning. With a smaller volume, a decrease in analytical signal was observed, indicating a lessening in the sorption process. No significant increase in signal was observed using a conditioning solution volume higher than 67 µL.

As can be seen in Table 1, the analytical signal presented an increase of 8 % when eluent concentration varied from 0.25 to 1.00 mol L^{-1} HCl, nevertheless, a decrease of 80 % occurred when eluent concentration was 2.00 mol L^{-1}. Presumably, eluent solution with higher concentration causes difficulties in the diffusion process, due to acid high viscosity. In this case, the elution process could be hampered, increasing eluate dispersion and consequently reducing the analytical signal[16].

Eluent solution aliquot (67µL) was inserted into the analytical path at a flow rate of 2.0 mL min^{-1}, by switching valve V_4 on for 2 s, and was transported through the column by the carrier solution at a flow rate of 3.4 mL min^{-1}. Under this condition, the eluate was released from the column into a small volume of solution, thus contributing to enhance sensitivity. This flow rate was adjusted to equal the aspiration rate of the spectrometer in order to avoid air bubbles delivering[17]. Elution step was performed in reverse mode to minimize dispersion of eluate inside the resin column and also to avoid excessive resin compaction at the column end[18].

Table 1. Effect of eluent concentration

Concentration (M)	Absorbance
0.25	0.12
0.50	0.12
1.00	0.13
1.50	0.09
2.00	0.07

The effect of sample flow rate on the analytical signal is shown Figure 4, which shows a constant value for flow rates higher than 12 mL min^{-1}. Despite the beneficial effect of a flow rate increase on the analytical signal, sorption efficiency decreases as flow rate increases. When the flow rate is too high, there is not enough time to allow analyte sorption and therefore thus part of the analyte is delivered to the efluent[16]. This effect was verified by collecting and analyzing the column effluent. Additionally, inner pressure of the system augments as flow rate increases and this could cause fluid leakage. Nevertheless, higher flow rates could be employed in order to improve sensitivity, since there was not leakage problem and there was a large sample volume available.

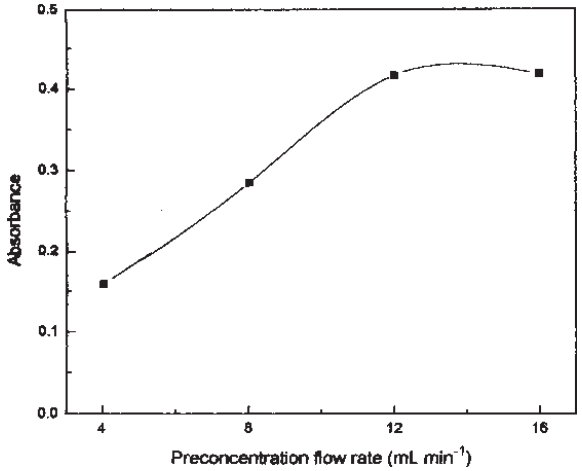

Figure 4. The effect of sample flow rate. Preconcentration time = 60 s; standard solution = 100 ng mL^{-1} Cu at pH 7; eluting solution 1.0 mol L^{-1} HCl; conditioning, phosphate buffer.

The signal magnitude is also a function of loading time, therefore if the preconcentration time is increased, a larger amount of analyte is introduced in the resin column, enhancing sensitivity. When loading time was varied from 60 to 140 s, a 2.6 times signal increase was observed, as shown

in Figure 5. However, long sample loading time causes analytical throughput decrease, thus a compromise between both parameters must be considered.

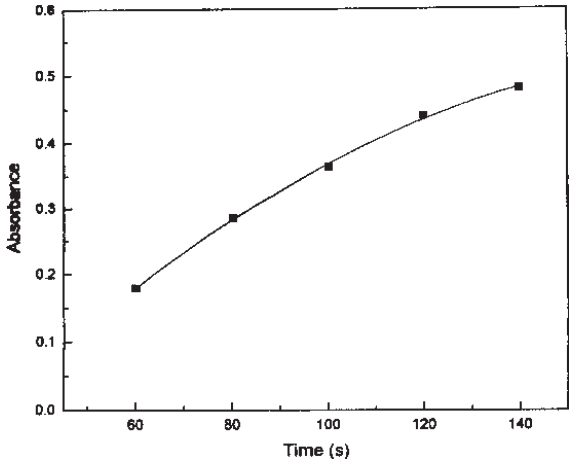

Figure 5. The effect of sample loading time. Solutions and other system parameters as indicated in Figure 4.

As sample solution enters into column, the sorption zone moves from the column inlet towards the outlet. By increasing the loading time the sites are occupied gradually and remaining sites diminish continuously their exchange capacity. Thus, a loss in sorption efficiency occurs, so that breakthrough limit is reached before all sites are occupied[16]. Although elution was performed in reverse mode, each portion of the eluate runs through different path lengths inside the column, which is presumably associated with efficiency reduction and tendency to asymptotic behaviour[19].

Tests carried out with concomitant ions showed that only calcium and sodium ions caused serious interference when concentrations were higher than 0.3 % (w/w), which is probably due to mass effect. Other concomitants have caused analytical signal decrease, nevertheless, it should be stressed that their concentration was at least 1000 fold higher than those found in real sample solutions. No interference was observed considering the real concentrations of analyte and concomitants in the real sample solutions.

The effectiveness of the tannin resin for copper preconcentration was ascertained by analyzing certified samples yielding the results of Table 2. No significant difference at the 95 % confidence level was observed by applying the *t*-test. Other profitable features, such as detection limit of 1 ng mL^{-1}, estimated as suggested by IUPAC[20], relative standard deviation of 3% (n=10), reagent consumption of 67 mL per determination and sample throughput of 48 determinations per hour were achieved. By employing a 60 s preconcentration time interval and a 4.0 mL min^{-1} loading flow rate, a 10 fold enrichment factor was obtained. Since analyte enrichment depends on loading time and/or sample flow rate, an improvement in the preconcentration factor could be reached by increasing these parameters. After six months work, no significant variation on resin efficiency was verified.

Table 2. Comparison of results

Samples	Certified Results (mg g^{-1})	Obtained Results (mg g^{-1})
NIES 10-a	3.50 ± 0.30	3.41 ± 0.05
NIES 10-c	4.10 ± 0.30	4.34 ± 0.01
NIST 1568	2.20 ± 0.3	2.34 ± 0.2
CBR 281	9.65 ± 0.38	9.24 ± 0.81

Conclusions

The proposed methodology is very simple, efficient, precise and accurate since results obtained with the analysis of the certified samples presented agreement with the certified results. Tannin resin presents as advantages low cost, no swelling problems and ability to form quelates with the analyte. Besides some previous test proved the resin is also capable to form quelates with others elements such as cadmium, lead, cobalt and manganese, therefore, it could be employed for multielement determinations.

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