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Talanta 69 (2006) 259–266
Talanta
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Amperometric determination of chloroguaiacol at submicromolar levels after on-line preconcentration with molecularly imprinted polymers

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Received 22 August 2005; received in revised form 4 October 2005; accepted 4 October 2005
Available online 2 November 2005

Abstract

In this study, a sorbent flow preconcentration system coupled to amperometric detector for the chloroguaiacol (4-chloro-2-methoxyphenol) determination at submicromolar levels is described. The satisfactory selectivity of the proposed method was attained by means of the use of a chloroguaiacol-imprinted polymer, whose the synthesis was carried out by bulk polymerization. Flow and chemical parameters associated to the preconcentration system, such as sample pH, preconcentration and elution flow rates, concentration of the carrier solution (KCl) and eluent volume were investigated through multivariate analysis. The flow preconcentration of chloroguaiacol was not affect by equimolar presence of structurally similar phenolic compounds including catechol, 4-chloro-3-methylphenol, 4-aminophenol and 2-cresol, thus showing the good performance of the imprinted polymer. Under the best experimental conditions, it was obtained a preconcentration factor of 110-fold and low detection and quantification limits of 27 and 78 nmol L^{-1}, respectively. The analytical curve covered a wide linear range from 0.05 up to 5.0 μmol L^{-1} ($r > 0.999$) and satisfactory precision ($n = 8$) evaluated by relative standard deviation (R.S.D.) were respectively, 5.5 and 4.2%, for solutions of 1.0 and 5.0 μmol L^{-1} chloroguaiacol. Other parameters related to the performance of the flow system were also evaluated including concentration efficiency of 27.5 min^{-1} and consumptive index of 0.09 mL. Recoveries varying from 93 up to 112% for water samples (tap water and river water) spiked with chloroguaiacol concentration were achieved, thus assuring the accuracy of the proposed flow preconcentration system.

Keywords: Sorbent flow preconcentration; Molecularly imprinted polymers; Chloroguaiacol; Amperometric determination

1. Introduction

Biomimetic materials based on molecularly imprinted polymers (MIP) have been widely used as an outstanding and powerful tool in different field of analytical sciences [1]. Basically, the synthesis of the MIP comprises the self-assembly in a porogenic solvent of a monomer around a template molecule followed by polymerization in the presence of a cross-linking reagent and an initiator. With the subsequent removal of the template molecule the polymer provides cavities complementary to the template capable to selectively recognize the molecule by means size, functional groups or shape. Due to its selective characteristics, MIP commonly has been used as chiral stationary phase for high-performance liquid chromatography (HPLC) [2], sorbent for solid-phase extraction (SPE) [3] as well as on the development of selective electrochemical [4] and optical sensors [5]. By now, the literature has reported a fast growing on the development of different approaches aiming the preparation and application of MIP for several matrices. However, as far as it is known, the majority of analytes investigated include amino acids, antibiotics and herbicides, where few attentions have been devoted for analytes of environmental interest, such as phenolic compounds [6–8]. These compounds are present in natural waters and effluents as consequence of many industrial processes and, even at low concentration, they have toxic effect on life organisms beyond to give undesirable taste and unpleasant smell in drinking water and fish [9].

According to literature data, nearly all publications concerning the application of MIP focusing the analyses of phenolic compounds are related to analytical protocols based on

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doi:10.1016/j.talanta.2005.10.001

solid-phase extraction in an off-line mode as clean-up procedure for liquid chromatography and as stationary phase for liquid chromatography [10,11]. The main studies have been directed for the following phenolic compounds 2,4,6-trichlorophenol, 4-nitrophenol, 4-chlorophenol and bisphenol [10,12,13]. Indeed, these methods already developed offer good selectivity and sensitivity; however they present some disadvantages, once in the solid-phase extraction procedures in off-line mode for example, high consumption of organic solvents are noted while the instrumentation cost of HPLC is relative high. Therefore, methods based on coupling of the flow preconcentration system with amperometric detection using MIP as selective sorbent can be used as an excellent alternative for the development of analytical procedures for phenolic compounds determination. This configuration joins the advantages of flow injection technique as well as the relative low cost of the electrochemical instrumentation. A flow molecularly imprinted solid-phase preconcentration system coupled to amperometric detection for the phenolic compounds determination has not been reported in literature at least in our knowledge. In this sense, a reliable flow preconcentration procedure with amperometric determination of chloroguaiacol using MIP as selective sorbent is proposed. The influence of flow and chemical parameters related to the performance of the flow preconcentration system was investigated by using a 2^{5-1} fractional factorial design and Doehlert design [14]. The usefulness of the method was evaluated after chloroguaiacol determination in spiked tap and river water.

2. Experimental

2.1. Reagents and standards

For synthesis of chloroguaiacol-imprinted polymers by bulk polymerization it was used the following reagents: chloroguaiacol as template and 4-vinylpyridine, ethylene glycol dimethacrylate (EGDMA) and 2,2′-azobis-isobutyronitrile (AIBN) as monomer, cross-linking reagent and initiator, respectively, purchased from Sigma-Aldrich (Steinheim, Germany). Methanol and acetic acid were purchased from Tedia (Rio de Janeiro, Brazil). All solutions of chloroguaiacol were prepared by using water from a Millipore Milli-Q purification system. Britton-Robison buffer solution at a concentration of 0.05 mol L^{-1} and KCl solution used as supporting electrolyte/carrier solution were purchased from Merck (Darmstadt, Germany) used without further purification. In the selectivity studies it was employed the following structurally analogue phenolic compounds: 4-chloro-3-methylphenol, 4-aminophenol, 2-cresol and catechol purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Instrumentation

Amperometric measurements were carried out with a potentiostat/galvanostat Autolab® PGSTAT-12 (Eco Chemie B.V., The Netherlands) equipped with a wall-jet electrochemical cell using glassy electrode carbon as working electrode, an Ag/AgCl electrode as reference electrode and a platinum wire as auxiliary electrode. The solutions were propelled by using an Ismatec Model IPC peristaltic pump with silicone tubes. A home-made injector commutator made of Teflon® (PTFE, polytetrafluoro ethylene) was used to select the preconcentration and elution steps. A mini-column (3 cm of length) used for packing the MIP was made of polyethylene and glass wool was inserted in the both sides of mini-column in order to prevent loss of the polymer during preconcentration and elution steps. The actual pH values were determined with a Corning pH/Ion Analyser model 350.

In order to evaluate the morphological characteristics of MIP a JEOL JMT-300 scanning electron microscope was used. For this task, the polymer particles surface was covered with a thin layer of carbon and alloy of gold–palladium. The polymers morphology was acquired using an electron acceleration voltage of 20 kV. The surface area and the volume of pores were obtained by using the BET technique using ASAP 2010 equipment (Micromeritics).

2.3. Preparation of chloroguaiacol-imprinted polymers

The procedure adopted for the synthesis of the polymer based on non-covalent approach as well as the experimental details have been demonstrated in previous work [15]. The prepolymerization mixture comprised chloroguaiacol (2.0 mmol) as template dissolved with 11.0 mL of acetonitrile in a 30 mL thick-walled glass tube with 8.0 mmol of 4-vinylpyridine as monomer. Then, 40.0 mmol of cross-linking EGDMA and 1.5 mmol of AIBN as initiator were added to mixture. The prepared polymer was ground and sieved by passing the milled polymer through a steel sieve to get particle sizes between 106 and 150 μm. After ending this step, the removal of the template from the polymer was carried out by using methanol/acetic acid (4:1 v/v) solution in accordance with previous work [15]. Finally, the polymer was then dried at 60 °C and stored at room temperature for the further use. Fig. 1 shows the scanning electron micrographs (SEM) of MIP prepared by bulk polymerization. As expected, the particles have an irregular shaped; however, this characteristic does not limit their use for solid-phase preconcentration purposes. Moreover, the particles present porous surface, in which play an important role in adsorption process. The respective values obtained for the surface area and the volume of pores were 327 $m^2\,g^{-1}$ and 0.950 $cm^3\,g^{-1}$.

The imprinting effect in the MIP was evaluated by preparing corresponding blank polymers, where the same protocol described above was employed in the synthesis, but without addition of the chloroguaiacol molecule.

2.4. Flow preconcentration system

Fig. 2 displays the schematic diagram of the flow preconcentration system for amperometric determination of chloroguaiacol. The preconcentration step was carried out by percolating the chloroguaiacol solution at pH 4.69 buffered with 0.05 mol L^{-1} Britton-Robinson solution through 35 mg of MIP packed into a mini-column during 4.0 min at 2.5 mL min^{-1}. At

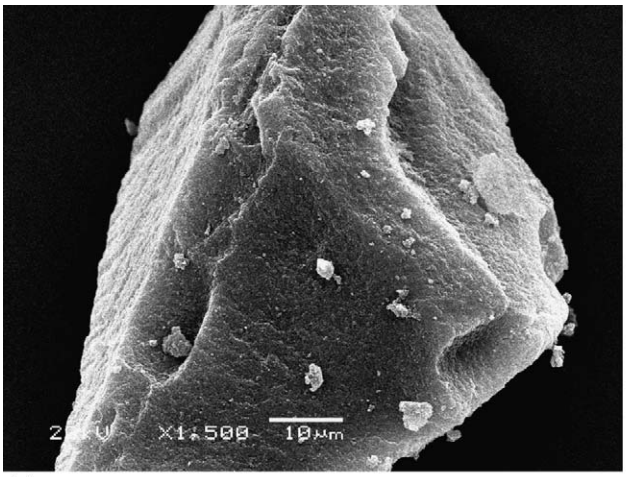

Fig. 1. Scanning electron micrographs of chloroguaiacol-imprinted polymer with particle size between 106 and 150 μm: (a) 1500× and (b) 20,000× of magnification.

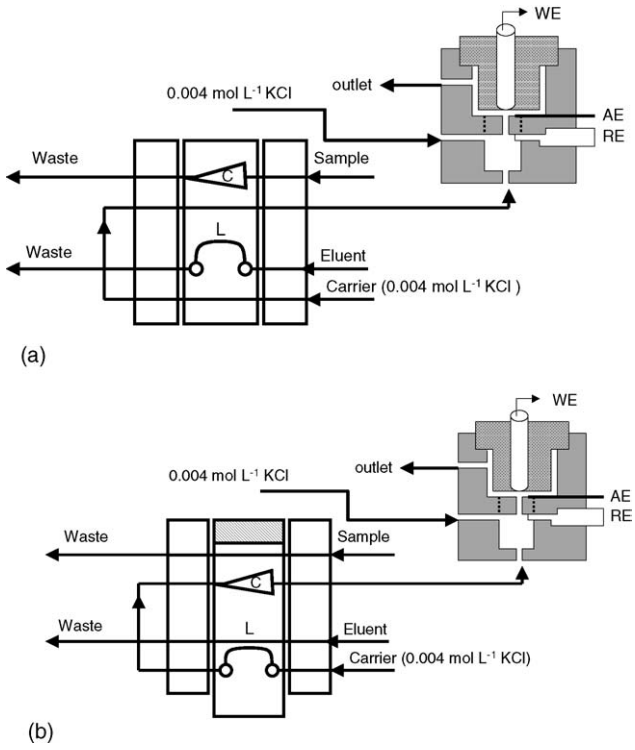

Fig. 2. Schematic diagram of sorbent flow preconcentration system for amperometric determination of chloroguaiacol: (a) preconcentration position and (b) elution position. Eluent = methanol/acetic acid (4:1, v/v); L: eluent loop (400 μL); C: mini-column packed with 35 mg of MIP; WE: working electrode (glassy carbon); AE: auxiliary electrode (platinum) and RE: reference electrode (Ag/AgCl).

this position, 0.004 mol L^{-1} KCl solution used as carrier solution flowed towards the wall-jet electrochemical cell. After this step, the injector commutator was switched to the elution position (Fig. 2b), in which 400 μL of methanol/acetic acid (4:1 v/v) displaced from the eluent loop by carrier solution at 1.0 mL min^{-1} flow rate was capable to desorb the chloroguaiacol from MIP. The methanol/acetic acid solution was chosen as eluent, since it has been successfully employed to desorb phenolic compounds from this kind of MIP [12,15]. All the amperometric measurements were obtained by fixing the oxidation potential at 1.0 V (versus Ag/AgCl). In addition, the measurements were recorded as peak height, which were proportional to the analyte concentration in the sample.

2.5. Methodology used for multivariate optimization

Initially, the influence of flow and chemical variables involved in the sorbent flow preconcentration system was investigated by using a 2^{5-1} fractional factorial design carried out without replicates. In this way, in order to analyse which effects from factors and their interactions that possibly to be confounded, the generator $I = abcde$ of the fractional factorial design was employed. These experiments were performed in a random order using 10 mL of chloroguaiacol solution at a concentration of 5.0 μmol L^{-1}. The variables investigated were: sample pH, preconcentration and elution flow rates, concentration of the carrier solution (KCl) and eluent volume, whose minimum and maximum levels are summarized in Table 1. The final optimization of those significant variables was further carried out using Doehlert design. All data were processed using the STATISTICAL package program (version 6.0).

Table 1
Experimental factors and their levels employed in the 2^{5-1} fractional factorial design for amperometric determination of chloroguaiacol after its flow preconcentration

Factors	Levels	
	(−) Low	(+) High
pH	1.3	11
Preconcentration flow rate (PFR) (mL min^{-1})	2.5	5.0
KCl concentration (mol L^{-1})	0.01	0.3
Elution flow rate (EFR) (mL min^{-1})	0.5	1.0
Eluent[a] volume (EV) (μL)	200	300

[a] Methanol/acetic acid (4:1 v/v).

3. Results and discussion

3.1. Fractional factorial design for assessing the effect of experimental variables

The fractional factorial design based on a fraction (1/2, 1/4, 1/8, . . ., $1/2^p$) of a full factorial is commonly indicated for those situations where several variables need to be investigated. The numbers of experiments comprises 2^{K-p} experiments where p is the size of the fraction. Thus, the total number of experiments in a fractional factorial design is much less as compared to a full factorial design method. For instance, the screening of five variables by using a fractional factorial design is accomplished with only 16 experiments, if $p = 1$, while a full factorial design requires 32 experiments. Thus, this multivariate optimization strategy was adopted in this work. As indicated in Table 1, five variables were investigated, however other variables, such as mass of MIP and type and concentration of buffer solution (Britton-Robison) were previously fixed. The mass of MIP was fixed at 35 mg in order to avoid overpressure in the mini-column due to the swelling effect after contact with the eluent [methanol/acetic acid (4:1 v/v)]. Britton-Robison buffer solution at a concentration of 0.05 mol L^{-1} was adopted because it provides very good buffering capacity in the wide studied pH range (1.3–11).

The multivariate assays were performed only after optimizing the oxidation potential of chloroguaiacol. For this task, the same schematic flow diagram indicated in Fig. 2 was used without using mini-column packed with MIP. The oxidation potential was studied within the range from 0.7 to 1.2 V, where the following conditions were established: 0.1 mol L^{-1} KCl carrier solution at 1.0 mL min^{-1} flow rate and eluent loop of 50 μL. The sample was introduced into the flow system from the eluent loop. According to Fig. 3, the peak current profile for chloroguaiacol when potential value is changed from 1.0 to 1.2 V suffers a slight variation, thus the oxidation potential of 1.0 V was established for the further experiments.

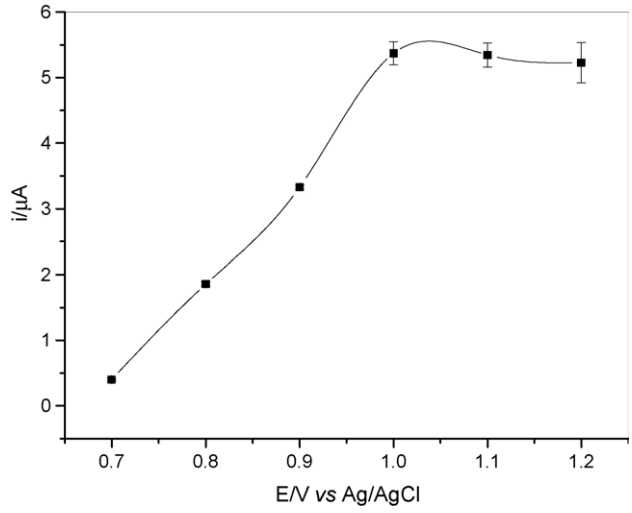

Fig. 3. Effect of oxidation potentials in the hydrodynamic responses for flow amperometric determination of chloroguaiacol.

Table 2
2^{5-1} fractional factorial design and the results obtained for amperometric determination of choloroguaiacol after its flow preconcentration onto MIP

Runs	pH (*a*)	PFR (*b*)	[KCl] (*c*)	EFR (*d*)	EV (*e*)	Response (peak current/μA)
1	–	–	–	–	+	6.101
2	+	–	–	–	–	2.260
3	–	+	–	–	–	3.700
4	+	+	–	–	+	1.615
5	–	–	+	–	–	3.279
6	+	–	+	–	+	1.784
7	–	+	+	–	+	3.948
8	+	+	+	–	–	1.172
9	–	–	–	+	–	5.384
10	+	–	–	+	+	3.233
11	–	+	–	+	+	5.497
12	+	+	–	+	–	1.476
13	–	–	+	+	+	5.455
14	+	–	+	+	–	1.687
15	–	+	+	+	–	2.966
16	+	+	+	+	+	1.389

The generator of fractional factorial design is $I = abcde$. PFR: preconcentration flow rate; EFR: elution flow rate; EV: eluent volume.

The experimental results for the five variables obtained from the 2^{5-1} fractional factorial design are shown in Table 2, whose estimates of the contrast were higher for pH (−2.79) and KCl concentration (−1.024) followed by preconcentration flow rate (−0.852), eluent volume (0.811) and elution flow rate (0.480) (Table 3).

In order to determine the significant effects of the variables, it was calculated the standard error $(S.E.)_e$ of the estimate of the contrast for the variables employing Eq. (1):

$$(S.E.)_e = \sqrt{\frac{\sum E_{x_i y_j}^2}{n_{x_i y_j}}} \quad (1)$$

where $E_{x_i y_j}$ is attributed to the estimate of the contrast only for the two-factor interactions, in which is not confounded with the

Table 3
Estimate of the contrast obtained from 2^{5-1} fractional factorial design

Confounding pattern	Contrast	Estimate
$a = bcde$	$l_a \rightarrow a + bcde$	$l_a = -2.79$
$b = acde$	$l_b \rightarrow b + acde$	$l_b = -0.852$
$c = abde$	$l_c \rightarrow c + abde$	$l_c = -1.024$
$d = abce$	$l_d \rightarrow d + abce$	$l_d = 0.480$
$e = abcd$	$l_e \rightarrow e + abcd$	$l_e = 0.8115$
$ab = cde$	$l_{ab} \rightarrow ab + cde$	$l_{ab} = 0.175$
$ac = bde$	$l_{ac} \rightarrow ac + bde$	$l_{ac} = 0.234$
$ad = bce$	$l_{ad} \rightarrow ad + bce$	$l_{ad} = 0.090$
$ae = bcd$	$l_{ae} \rightarrow ae + bcd$	$l_{ae} = -0.605$
$bc = ade$	$l_{bc} \rightarrow bc + ade$	$l_{bc} = 0.320$
$bd = ace$	$l_{bd} \rightarrow bd + ace$	$l_{bd} = 0.256$
$be = acd$	$l_{be} \rightarrow be + acd$	$l_{be} = 0.027$
$cd = abe$	$l_{cd} \rightarrow cd + abe$	$l_{cd} = 0.0007$
$ce = abd$	$l_{ce} \rightarrow ce + abd$	$l_{ce} = -0.095$
$de = abc$	$l_{de} \rightarrow de + abc$	$l_{de} = 0.204$
$I = abcde$	$l_I \rightarrow$ medium + ½($abcde$)	$l_I = 3.184$

a = pH; b = sampling flow rate (SFR); c = [KCl]; d = elution flow rate (EFR) and e = eluent volume (EV).

Table 4
Structure of Doehlert design and results obtained for amperometric determination of choloroguaiacol after its flow preconcentration onto MIP

Runs	pH	[KCl]	Response (peak current/μA)	Predicted value by quadratic model
1	0 (6.15)	0 (0.25)	6.750/6.812	6.784
2	1 (11)	0 (0.25)	0.934/0.959	1.262
3	0.5 (8.57)	0.866 (0.50)	4.382/4.305	4.035
4	−1 (1.3)	0 (0.25)	6.125/6.235	5.871
5	−0.5 (3.72)	−0.866 (0.001)	6.958/6.845	7.210
6	0.5 (8.57)	−0.866 (0.001)	5.662/5.545	5.300
7	−0.5 (3.72)	0.866 (0.50)	6.422/6.416	6.726

The values between parentheses are the real values of variables while the first values represent the codified values from Doehlert design for two variables.

main variable (Table 3) and $n_{x_i y_j}$ is the number of these estimates of contrast used in the calculation of S.E.

The standard error for the experimental design listed in Table 2, obtained from Eq. (1) was 0.2605. By multiplying this value by critical t_{10} (1.812) with confidence interval of 95%, the error estimated became 0.472, in which is lower than those estimates of the contrast for the variables (Table 3). As consequence it is possible to note that all investigated variables are statistically significant.

The negative estimative of the contrast (−2.79) for variable pH indicates that adsorption of chloroguaiacol onto MIP takes place mainly at low pH values. It suggests that at high pH values the chloroguaiacol molecule charged negatively suffers electrostatic repulsion of binding sites of MIP (the basic pyridine group). The second more important variable, KCl concentration, has also shown negative estimate of the contrast (−1.024). This result probably suggests that using high KCl concentration the solubility of chloroguaiacol in the solvent methanol/acetic acid (4:1 v/v) is decreased due to the salting out effect. Consequently, the performance of the solvent for releasing chloroguaiacol from MIP is decreased. The estimates of the contrast for preconcentration (PFR) and elution (EFR) flow rates were found to be −0.852 and 0.480, respectively. Such results were expected, once at high preconcentration flow rate the time of contact between the chloroguaiacol and MIP is small, thus limiting the mass transfer rate of the analyte. In relation to the EFR variable, the slow elution flow rate at smaller levels results in broader peaks and, as consequence, a decrease of the analytical signal since it was recorded as peak height. Finally, even using eluent volume up to 300 μL it was verified memory effect after each preconcentration/elution cycle. In this sense, the eluent volume optimization was further optimized by univariate method ranging from 200 to 500 μL. From the achieved results, the necessary volume for complete release of chloroguaiacol from MIP without memory effect was 400 μL (data not shown). Thus, this value was chosen as best condition.

According to the overall obtained data, the variable levels selected for the preconcentration and elution flow rates were 2.5 and 1.0 mL min^{-1}, respectively, while the final optimization of the sample pH and KCl concentration was carried out by using Doehlert design.

3.2. Use of Doehlert design for final optimization

In this design, seven required experiments were carried out in duplicate being five levels used for the pH variable and three for the KCl concentration (Table 4). According to the real data applied to Doehlert design the following quadratic function (Eq. (2)) was obtained, in which establishes the relationship between the variables studied and the analytical response (peak current):

$$Y = 4.31 + 1.28\text{pH} - 0.136\text{pH}^2 + 1.52[\text{KCl}] - 2.60[\text{KCl}]^2 - 0.32\text{pH}[\text{KCl}] \quad (2)$$

The response surface constructed from Eq. (2) is shown in Fig. 4. The presence of a maximum point under this surface was checked by using Lagrange's criteria. It is based on calculation of Hessian determinant Eq. (3) establishing that when $H(a_0, b_0) > 0$, $\delta^2 Y/\delta A^2\ (a_0, b_0) < 0$ or and $\delta^2 Y/\delta B^2\ (a_0, b_0) < 0$, there is a maximum point, where A and B are the evaluated variables:

$$H(A, B) = \left(\frac{\partial^2 Y}{\partial A^2}\right)\left(\frac{\partial^2 Y}{\partial B^2}\right) - \left(\frac{\partial^2 Y}{\partial A\,\partial B}\right)^2 \quad (3)$$

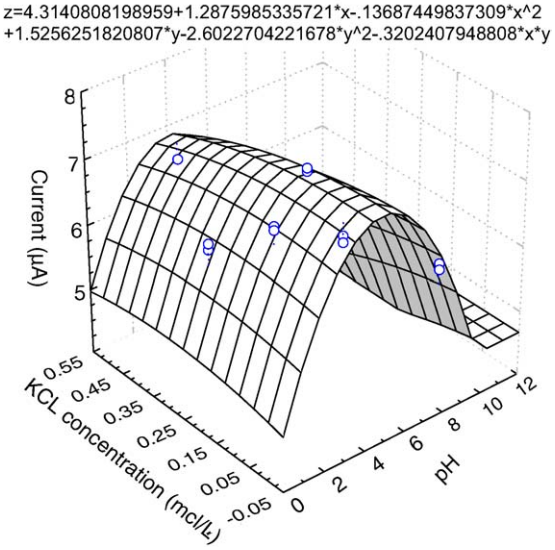

Fig. 4. Surface response obtained from Doehlert design employed for optimization of sample pH and KCl concentration.

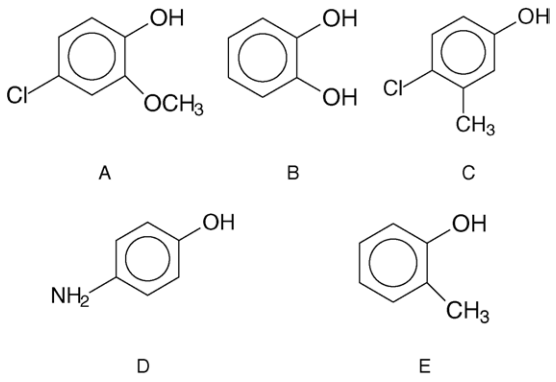

Fig. 5. Chemical structures of chloroguaiacol and similar phenolic compounds used in the interference studies: (A) chloroguaiacol, (B) catechol (C) 4-chloro-3-methylphenol, (D) 4-aminophenol and (E) 2-cresol.

The resultant values obtained from application of Lagrange's criteria were $H\ (a_0,\ b_0) = 1.312$, $\delta^2\text{current}/\delta\text{pH}^2 = -0.27$ and $\delta^2\text{current}/\delta[\text{KCl}]^2 = -5.2$, thus indicating a maximum on the surface response. These maximum values were calculated by solving the system $\delta\text{current}/\delta\text{pH} = 0$ and $\delta\text{current}/\delta[\text{KCl}] = 0$ as follows:

$$\delta\text{current}/\delta\text{pH} = 0 = 1.28 - 0.27\text{pH} - 0.32[\text{KCl}],$$

$$\delta\text{current}/\delta[\text{KCl}] = 0 = 1.52 - 5.2[\text{KCl}] - 0.32\text{pH}$$

The maximum values were 4.7 and 0.004 mol L^{-1} for pH and KCl concentration, respectively. The good agreement to the experimental values with those predicted from Eq. (2), as observed in Table 4, demonstrates the significance of the quadratic model.

3.3. Interference studies

Biomimetic polymers based on molecular imprinting have an inherent characteristic that is the selectivity. However, when working with molecules, that present similar structures to the template molecule, commonly the concomitants are adsorbed onto MIP surface by means of non-specific interactions and also in those selective sites of MIP. Hence, the specificity of the MIP for chloroguaiacol was evaluated by comparing the adsorption behaviour of some phenolic compounds with analogous structure that present nearly the same potential of the chloroguaiacol: catechol, 4-chloro-3-methylphenol, 4-aminophenol and 2-cresol (Fig. 5). As can be seen in Table 5, the higher relative analytical response for the chloroguaiacol template than those verified for the concomitants suggests a good imprinting effect in the synthesis of polymer. It was confirmed after performing the preconcentration procedure using MIP or NIP, whose peak current values were found to be 6.47 and 3.14 μA, respectively, thus confirming the imprinting effect.

Table 5
Comparison of relative response of analytical signal of phenolic compounds with analogous structure of chloroguaiacol

Compounds	Current peak/μA	Response (%)
Chloroguaiacol	6.47	100
Catechol	3.85	59.5
4-Chloro-3-methylphenol	5.27	81.4
4-Aminophenol	0.88	13.6
2-Cresol	4.96	76.6

The concentration employed in this study was 5.0 μmol L^{-1}.

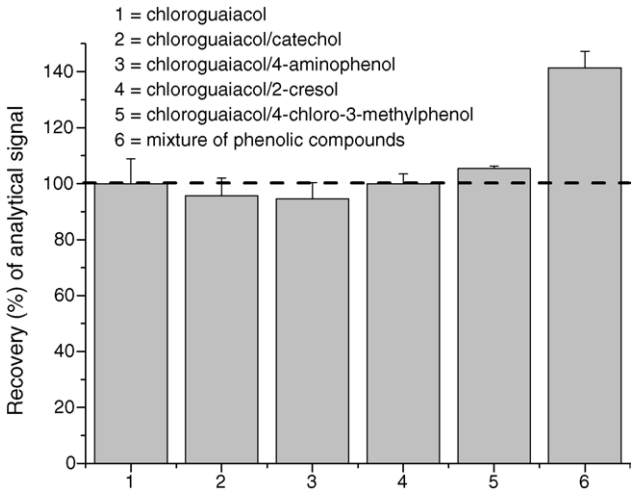

Fig. 6. Recovery (%) of analytical signal (current of peak) for chloroguaiacol preconcentration in the presence of phenolic compounds with similar structures.

In order to check the feasibility of the proposed method in relation to the selectivity of MIP, binary solutions of chloroguaiacol in the presence of each concomitant at a concentration of 5.0 μmol L^{-1} were preconcentrated onto MIP. Moreover, chloroguaiacol solution containing five phenolic compounds already defined at the same concentration was analysed in order to verify the selectivity of the method. According to Fig. 6, no interference was observed after preconcentration of binary solutions. On the other hand, an increase of ca. 40% on the relative response was observed when the co-existing phenolic compounds were preconcentrated; however, it is important to stress that this condition is not usually found in real samples, such as natural waters.

3.4. Figures of merit

Using the optimized conditions for the proposed method a calibration graph was prepared within the concentration range from 0.05 up to 5.0 μmol L^{-1} with satisfactory correlation coefficient ($r > 0.999$). Typical flow diagram curve obtained for the sorbent flow preconcentration system is displayed in Fig. 7. The detection and quantification limits calculated according to IUPAC recommendations [16] were found to be 27 and 78 nmol L^{-1}, respectively. The relative standard deviation (R.S.D.) were 5.5 and 4.2% for $n = 8$ analysing of standard solution containing 1.0 and 5.0 μmol L^{-1}, respectively. Obviously, these results indicate the repeatability of the measurements with one single MIP mini-column, but also confirm the anti-fouling properties of the eluent methanol/acetic acid solution (4:1 v/v) as already demonstrated [15].

The performance of the proposed method in relation to the flow preconcentration system was evaluated from

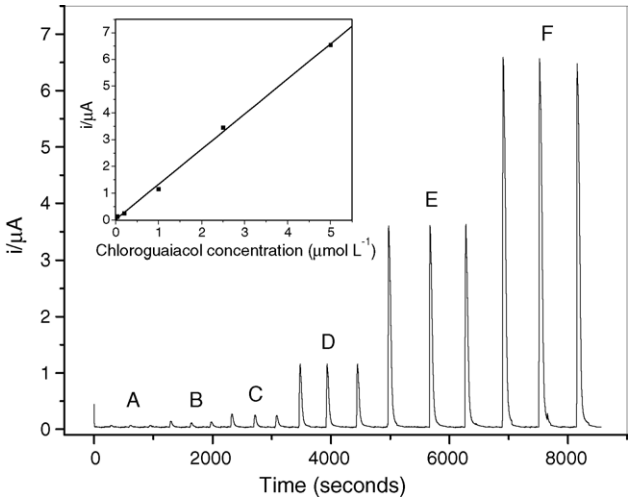

Fig. 7. Typical flow diagram curve obtained for the sorbent flow preconcentration system: A = blank; B = 0.05; C = 0.2; D = 1.0; E = 2.5 and F = 5.0 μmol L^{-1}. For experimental details, see text.

preconcentration factor (PF), concentration efficiency (CE) and consumptive index (CI). PF was obtained by slopes ratio of the calibration graphs obtained with ($y = 0.025 + 1.31 \times 10^6$ [chloroguaiacol]) and without ($y = 0.048 + 11.894$ [chloroguaiacol]) preconcentration step, whose value achieved was 110.1. CE defines the preconcentration factor attained by a sorbent flow preconcentration system during 1 min of preconcentration. Thus, as the entire time of preconcentration step was 4 min, CE calculated was found to be 27.5 min^{-1}. Consumptive index, on the other hand, establishes the sample volume (in millilitres) required for achieving a unit of PF, thus it is calculated by equation CI = sample volume/PF. Hence, considering the sample volume employed in the preconcentration step (10 mL) the resulting CI was found to be 0.09 mL. From these attained results, it can be concluded that the proposed sorbent flow preconcentration system provides a better or similar performance when compared with the traditional flow preconcentration systems, mainly those developed for metals determination [17–20].

3.5. Application of the proposed method for chloroguaiacol determination in water samples

In order to assess the performance of the proposed method for the samples analyses, tap and river water were used. Prior to analyses, river water samples were filtered under vacuum through 0.45 μm cellulose acetate membranes. The accuracy of the method was evaluated through the recovery test after spiking samples. As can be seen in Table 6, the efficiency of the method was assured by recoveries values between 93.3 and 112% for water samples. It is important to stress that although the chloroguaiacol was not detectable in these samples with the flow preconcentration system, the limit of quantification reached (78 nmol L^{-1} or 12.4 μg L^{-1}) allows us to evaluate the chloroguaiacol contamination in river waters nearby from industry according to commonly levels detected at μg L^{-1} [21].

Table 6
Chloroguaiacol determination in spiked samples using the proposed method

Samples	Chloroguaiacol added (μmol L^{-1})	Chloroguaiacol found[a] (μmol L^{-1})	Recovery[b] (%)
Tap water	–	<LQ	–
	1.0	1.12 ± 0.07	112
	3.0	2.80 ± 0.20	93.3
River water	–	<LQ	–
	1.0	0.95 ± 0.09	95
	3.0	2.9 ± 0.32	96.6

[a] The results are expressed as mean value ± S.D. based on three replicates. Confidence interval of 95%. LQ = limit of quantification.

4. Conclusions

In this work, the adsorption of chloroguaiacol onto molecularly imprinted polymers using a flow preconcentration system coupled to amperometric detection has been clearly demonstrated. This novel method developed as a first approach showed that other structurally related phenolic compounds can also be adsorbed onto MIP; however, the adsorption profile of chloroguaiacol was higher than those phenolic compounds. From this behaviour, the satisfactory chloroguaiacol determination was easily achieved even in the equimolar presence of investigated analogous phenolic compounds. The concomitant adsorption of several phenolic compounds can be attributed due to the application of the proposed method in water samples, in which is less compatible with solvent of synthesis (acetonitrile). Although this drawback, the method can be successfully applied for chloroguaiacol determination in real samples without including a washing step. The good performance of MIP was assured by application of method in river water that contains humic substances, and of course, could interfere in the preconcentration step.

In relation to the features of the method it was showed to be sensitive, selective and precise and obviously fast due to the mechanization process. Among other interesting features of the method, the parameters related to performance of the flow preconcentration system including preconcentration factor, concentration efficiency and consumptive index were better or comparable to the other traditional flow preconcentration systems. Finally, from our point of view, the on-line coupling of a preconcentration system to amperometric detection offers a new alternative for expanding the application of MIP.

Acknowledgements

The authors are grateful to FAPESP and CNPq for their financial support.

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