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Enzymatic electrochemical detection coupled to multivariate calibration for the determination of phenolic compounds in environmental samples

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ABSTRACT

An approach based on the electrochemical detection of the horseradish peroxidase enzymatic reaction by means of square wave voltammetry was developed for the determination of phenolic compounds in environmental samples. First, a systematic optimization procedure of three factors involved in the enzymatic reaction was carried out using response surface methodology through a central composite design. Second, the enzymatic electrochemical detection coupled with a multivariate calibration method based in the partial least-squares technique was optimized for the determination of a mixture of five phenolic compounds, i.e. phenol, p-aminophenol, p-chlorophenol, hydroquinone and pyrocatechol. The calibration and validation sets were built and assessed. In the calibration model, the LODs for phenolic compounds oscillated from 0.6 to 1.4×10^{-6} mol L^{-1}. Recoveries for prediction samples were higher than 85%. These compounds were analyzed simultaneously in spiked samples and in water samples collected close to tanneries and landfills.

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1. Introduction

The phenolic compounds are characterized by having at least one aromatic ring with one or more hydroxyl groups or other atoms and/or functional groups attached. They are widely present in the environment due to their application in the production of drugs, fragrances, polymeric materials, synthetic detergents, plasticizers, dyes, papers, pesticides, among others [1]. They have significant detrimental effects on water quality or animals as well as on some plants, even at very low levels, due to their toxicity and carcinogenic activity. For these reasons, some of them have been included in the lists of priority pollutants [2]. Many analytical techniques have been used for monitoring phenols, such as colorimetry [3], gas chromatography [4], liquid chromatography [5], and capillary electrophoresis [6]. However, some of these techniques are expensive, time consuming and sometimes require complex sample pre-treatment such as pre-concentration and extraction steps that increase the risk of sample loss. Therefore, the development of sensitive and fast methods for simultaneous determination of phenol and its derivatives is very important in environmental control and in the control of phenolic compound biodegradation in industrial wastewater [7]. Grosso et al. [8] reported that the initial concentration and the kind of compound have great influence on the efficiency of this biodegradation, at high concentrations (>435 mg L^{-1}) these compounds are rapidly reduced, while at low concentrations and in the presence of substituted phenols, the process becomes slower [9]. On the other hand, according to local regulations, the maximum allowed levels for phenol in drinking water should not exceed 2 μg L^{-1}, while other phenolic compounds should be absent [10].

Phenolic compounds can be easily analyzed by using electrochemical detection, due to the fact that they can generate electroactive products in the presence of horseradish peroxidase enzyme (HRP). The operating principle is based on a ping-pong mechanism (see Fig. 1). The first stage involves the oxidation of a ferric native enzyme to a ferryl-oxo porphyrin radical intermediary (compound I or CI) by H_2O_2. In the second stage, the compound I is reduced to the ferryl-oxo intermediary (compound II or CII) by a phenolic molecule. In the third stage, another phenolic molecule is necessary to reduce the compound II to the native enzyme. The second and third stages also involve the oxidation of phenolic compounds to quinines or free radicals. The oxidation products can be electrochemically reduced on the surface of the electrode and so an electrochemical signal directly proportional to the concentration of the phenolic compound is obtained when the H_2O_2 is present in excess [11–14].

The methods based on univariate calibrations determine one compound by using only one analytical response; while methods that use the multivariate regressions assess simultaneously several compounds from various responses. For the environmental

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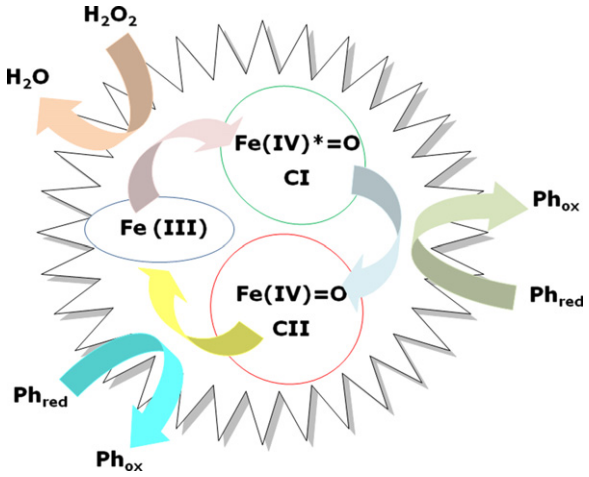

Fig. 1. Schematic representation of HRP enzymatic behavior with H_2O_2 substrate and phenolic compounds (Ph).

samples analysis the multivariate calibration would be the most appropriate method, since it can give information about many analytes in shorter times.

The partial least-squares (PLS) technique is often used for the analysis in multivariate calibration methods [15–18]. There are two types of PLS techniques: on the one hand, PLS1 analyzes one analyte at a time and optimizes working conditions for each analyte independently; on the other hand, PLS2 evaluates and predicts the concentrations of several simultaneously analytes [19]. The PLS calibration can be performed by ignoring the concentrations of all other components except the analyte of interest; this is an additional advantage of such multivariate methods. Therefore, these methods are especially interesting for the determination of the analytes in complex samples, whose matrix may show analytical signals, which are severely overlapped with those from the analytes.

This work is focused on the development of methods based on the electrochemical detection of electroactive products generated by the reaction of HRP and phenolic compounds. On the one hand, a systematic optimization procedure of three factors involved in the enzymatic reaction was carried out using response surface methodology through a central composite design. An enzymatic electrochemical detection coupled with univariate calibration was performed and then evaluated to phenol analysis in natural water samples. Finally, a method based on the enzymatic electrochemical detection coupled with multivariate calibration was developed for the quantification of five phenolic compounds and then was applied to the analysis of water samples recollected close to tanneries and landfills.

2. Materials and methods

2.1. Instrumentation

Cyclic voltammetry, square wave voltammetry (SWV), amperometry and chronoamperometry were performed with a voltammetric analyzer Epsilon BAS, Bioanalytical Systems Inc (West Lafayette Indiana—USA) with a three electrode system based on graphite-epoxy composite (GEC) as working electrodes [20–22]; platinum as auxiliary electrode and a (Ag/AgCl) silver/silver chloride electrode in 3 mol L^{-1} NaCl solution as reference electrode (Orion 92-02-00). The effective areas of electrodes were 0.22 cm^2 (RSD%=14%, n=9) by cyclic voltammetry and chronoamperometry with potassium ferricyanide.

2.2. Reagents

Horseradish peroxidase (1310 U mg^{-1}), hydroquinone and pyrocatechol were purchased from Sigma. All other reagents such as hydrogen peroxide, phenol, p-chlorophenol and p-aminophenol were also analytical grade. Phenol, hydroquinone, H_2O_2 and pyrocatechol solutions were prepared with Millipore water, while p-chlorophenol and p-aminophenol solutions were prepared in ethanol:millipore water (50:50). The working buffer solutions were phosphate 0.1 mol L^{-1} and KCl 0.1 mol L^{-1} (for buffering from pH 5.0–7.0) and acetate/phosphate/borate 0.05 mol L^{-1} and KCl 0.1 mol L^{-1} (for buffering from pH 6.0–9.0).

Water samples were collected according to 29012 IRAM norms from different localities close to tanneries and public landfills of the geographic regions of The Littoral and the Mesopotamia in Argentine (see Table 1) and were stored in plastic containers at 4 °C in the dark.

2.3. Enzymatic reaction and electrochemical detection

The cyclic voltammetry (with a scan rate 0.1 V s^{-1}) was independently performed for phenol, hydroquinone, p-chlorophenol, pyrocatechol and p-aminophenol in the range from 1200 to −400 mV. Phenolic compound solutions 2×10^{-3} mol L^{-1} in phosphate buffer 0.1 mol L^{-1} and KCl 0.1 mol L^{-1} at pH 6.00 were used in these assays.

In a 5.0 mL reaction cell, the enzymatic electrochemical detection was carried out with 4.0 mL of buffer and KCl 0.1 mol L^{-1} where small volumes of HRP enzyme, phenolic compound standards and the H_2O_2 solutions were added at different concentration ranges. Then, the analytical signals were obtained by SWV (whose parameters: step width=4 mV, amplitude=25 mV and frequency=15 Hz were not optimized and were set to default values by instrument).

In univariate calibration for phenol, the reduction current peak was obtained by SWV when it was swept from 500 to 50 mV vs. reference electrode.

In multivariate calibration for five phenolic compounds, the analytical signal was the sum of two voltammograms by SWV.

Table 1
Water samples analyzed by enzymatic electrochemical detection coupled to univariate and multivariate calibration.

Samples	Calibration[a]	Type of water	Source nearby to	Location[b]
-1-	UV	Groundwater	At 100 m of tannery	Castellanos, SF
-2-	UV	Groundwater	At 200 m of tannery	Castellanos, SF
-3-	MV	Groundwater	At 100 m of tannery	Las Colonias, SF
-4-	MV	Groundwater	At 200 m of tannery	Las Colonias, SF
-5-	MV	Groundwater	At 100 m of tannery	General Obligado SF
-6-	MV	Groundwater	At 200 m of tannery	General Obligado SF
-7-	MV	Stream water	At 300 m of tannery	General Obligado SF
-8-	MV	Superficial water	At 100 m of tannery	La Capital, SF
-9-	MV	Stream water	At 100 m of tannery	La Capital, SF
-10-	MV	Stream water	At 50 m of landfill	Nogoyá, ER
-11-	MV	Superficial water	At 50 m of landfill	Nogoyá, ER

[a] univariate calibration=UV and multivariate calibration=MV.
[b] Santa Fe, Argentine=SF and Entre Ríos, Argentine=ER.