

# Colorimetric Solid-Phase Extraction Method for Cu(II) Ion Determination Using 2-Hydroxybenzaldehyde Benzoylhydrazone as Sensing Reagent

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A new sensor based on the use of 2-hydroxybenzaldehyde benzoylhydrazone as a colorimetric reagent immobilized onto styrenedivinylbenzene disks has been carried out for the determination of Cu(II) ions within several minutes. The sensor is designed on a rapid and easy two-step procedure: (1) the extraction of Cu(II) ions onto a disk loaded with the copper-selective colorimetric reagent and (2) the determination of the complexed analyte directly on the surface of the disk using diffuse reflectance measurements at 400 nm. The color of the disk changed from white to green in the presence of Cu(II) ions. The work herein details the optimization of the sensing system employing a fractional factorial design  $3^{3-1}$  considering three variables (pH, immobilization time, and amount of ligand immobilized onto the disk). The Pareto chart and response surfaces in a spherical domain indicated that the optimum conditions for the sensing of copper ions were pH = 7, with a ligand immobilization time of 10 min and 6.25 mg of reagent loaded onto the disk. Under the optimum conditions, the analytical parameters of the proposed method were determined. The calibration graph was linear over the range of 0 to 2.5 mg L<sup>-1</sup> of Cu(II) with a detection limit of 0.21 mg L<sup>-1</sup>. The relative standard deviation for six measurements of 1 mg L<sup>-1</sup> of Cu(II) was found to be 4.87%. The interference from inorganic salts and other metals was found not to be of major concern when monitoring copper ions in water samples. The simplicity and rapidity of this technique make it convenient and amenable for on-site and routine analysis.

Index Headings: **Copper; Sensing; Colorimetric solid phase extraction; 2-Hydroxybenzaldehyde benzoylhydrazone; Diffuse reflectance spectroscopy; Green chemistry.**

## INTRODUCTION

Copper plays an important role as an essential trace element for human beings in various physiological processes. Indeed, many clinical disorders can be associated with the deficiency of copper, such as anemia, gastrointestinal disturbances, or even heart failure.<sup>1,2</sup> However, this metal represents an environmental concern when it is present in uncontrolled amounts exceeding the nutritional requirements. The allowed limit of copper in drinking water is set to 2 mg L<sup>-1</sup> by the European Commission,<sup>3</sup> 1.3 mg L<sup>-1</sup> as primary standard (maximum contaminant level goal), and 1.0 mg L<sup>-1</sup> as

secondary standard (non-enforceable guidelines but with cosmetic effects or esthetic effects) by the U.S. Environmental Protection Agency (EPA).<sup>4</sup> The U.S. National Oceanic and Atmospheric Administration screening quick reference tables present a secondary maximum contaminant level of 1.3 mg L<sup>-1</sup> applicable to ground water.<sup>5</sup> A high concentration of copper in water originates mainly from fungicide residues containing this metal used in agriculture, corrosion of household plumbing systems, or erosion of natural deposits. For this reason, aquatic sites contaminated with this metal must be monitored regularly.

Conventional methods for measuring copper in aqueous environments are usually time consuming, the sample handling and processing steps are labor intensive, and the water sample is often processed days or even weeks after collection. This time lag between sampling and analysis necessitates the addition of some preservatives for sample storage. Furthermore, traditional analytical procedures require considerable quantities of chemical compounds and frequently involve the formation of large amounts of pollutants during the preparation of the samples. Thus, the incorporation of green chemistry rules in the laboratory is desirable.<sup>6,7</sup> One of the best strategies to solve these problems is the development of on-site and real time analysis with environmentally friendly devices that are also highly miniaturized.<sup>8</sup> The use of chemical sensors has made an important contribution to this field, allowing the analyst to bring the laboratory to the sample instead of taking the sample to the laboratory.<sup>9</sup> Optical sensors that make use of polymer membranes containing a reagent that reacts with the analyte to produce a distinctive color change have been well established for the trace analysis of heavy metal ions, among other applications.<sup>10</sup> As mentioned earlier, miniaturization is one way to avoid some of the negative side effects that are often encountered in analytical methods, and miniaturization has been the subject of a significant number of research efforts. In this respect, a combination of a modern analytical technique with miniaturization allows the development of powerful analytical devices for the effective control of processes and pollution.<sup>11</sup>

Colorimetric solid phase extraction (C-SPE) has received special attention over the last few years because it meets many of the requirements mentioned earlier.<sup>12</sup> This methodology involves the extraction of

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**TABLE I. Review of some recent reflectance sensor systems for the determination of metal ions.**

Reference	Reagent <sup>a</sup>	Metal	Support matrix
Gazda et al., 2004 <sup>14</sup>	DMG	Ni(II)	SDB-XC
Filik et al., 2009 <sup>16</sup>	PHZ	Mo(VI)	XAD-16
Arena et al., 2003 <sup>17</sup>	DMABR	Ag(I)	SDB-XC
Mahendra et al., 2003 <sup>18</sup>	Cupron	Cu(II)	XAD-2
Fritz et al., 2003 <sup>19</sup>	Zn DTC	Cu(II)	SDB-XC
Filik and Yanaz, 2009 <sup>20</sup>	PAN	V(V)	XAD-16
Hill et al., 2010 <sup>21</sup>	DMABR	Ag(I)	SDB-XC
Yanaz et al., 2010 <sup>22</sup>	ASA III	Pb(II)	XAD-16
Bradley et al., 2011 <sup>23</sup>	PVP	As(III)	SDB-XC
This work	2-HBBH	Cu(II)	SDB-XC

<sup>a</sup> DMABR, 5-(p-dimethylaminobenzylidene) rhodanine; Cupron,  $\alpha$ -benzoinoxime; Zn DTC, zinc diethyldithiocarbamate; DMG, dimethylglyoxime; PAN, 1-(2-pyridylazo)-2-naphtol; PHZ, phenylhydrazine; ASA III, 2,7-bis(2-arsenophenylazo) 1,8-dihydroxynaphthalene-3,6-disulphonic acid; PVP, poly(vinylpyrrolidone); 2HBBH, 2-hydroxybenzaldehyde benzoylhydrazone; SDB-XC, polystyrene-divinylbenzene; XAD-2 and XAD-16, Amberlite polymeric adsorbent.

analytes onto a proper support impregnated with a colorimetric reagent and then quantified directly on the surface using diffuse reflectance spectroscopy (DRS). DRS is a valuable alternative for the study of the chemical changes that occur on the adsorbent surface of a solid by analyzing the radiation that returns from the sensing region.<sup>13–15</sup> Thus, this technique can be an alternative for achieving green chemistry ambitions, because the elution step, necessary in the SPE, which requires high purity solvents, is eliminated from the process, and the amount of analyte is measured directly on the solid surface. In addition, the simplicity and rapidity of this technique make it convenient and amenable for on-site and routine analysis.

The sensor described in this paper relies on the selective SPE of Cu(II) ions from a water sample onto a disk loaded with a copper-selective colorimetric reagent. Then, the color change of single-use SPE membranes measured by DRS was used to determine the amount of copper extracted using the disk.<sup>16</sup> To date, different organic reagents and solid supports have been applied to the development of the optical sensor employing diffuse reflectance measurements. Some of the most relevant and recent works related to this field are given in Table I.<sup>14,16–23</sup> As can be seen here, papers based on the determination of copper have been rarely reported. The use of cupron ( $\alpha$ -Benzoinoxime) and zinc(II) dithiocarbamates as selective ligands for the determination of copper have been described previously.<sup>18,19</sup>

Hydrazones have shown attractive potentialities for C-SPE applications because of their donor properties in coordinating metals and their ability to behave as polydentate ligands. In our previous paper, 2-hydroxybenzaldehyde benzoylhydrazone (2-HBBH) solution has been described as a selective chemosensor for the determination of copper ions.<sup>24</sup> To the best of our knowledge, this ligand has not been used previously as diffuse reflectance sensing layer for the determination of metal ions. Styrenedivinylbenzene (SDB-XC) membrane disks were employed as the sorbent material for 2-HBBH immobilization. This type of sorbent for disks is useful for compounds poorly extracted by octadecylsiloxane-bond-

ed silica sorbents and soluble in water. Also, SDB disks have the important advantage of being stable in the whole range of pH, and the selectivity of the disks can be improved with the immobilization of suitable reagents onto the solid sorbent.<sup>25</sup>

Hence, the goal of this work was the development of a novel, simple, highly selective, portable, and cost-effective sensor disk for the detection of copper ions in the aqueous environment with the combination of a rapid preconcentration technique and colorimetric chemistry detection using diffuse reflectance measurements. The Cu(II) sensor based on C-SPE was developed with aroylhydrazone as the selective reagent immobilized onto styrenedivinylbenzene polymer disks and a fiber optic to guide the light to and from the sensing area.

Preliminary studies of the influence of variables in the determination of copper ions were carried out with a flame atomic absorption spectrophotometer (FAAS) as the measurement technique after acid elution of metal ion from the disk. Once parameters had been evaluated, reflectance detection was used for the optimization process. A fractional factorial design 3<sup>3-1</sup> considering three variables (pH, immobilization time, and amount of ligand immobilized onto the disk) was employed for the optimization of the sensing system. Fluorescence measurements were also investigated as a comparison with the reflectance signals.

## EXPERIMENTAL

**Reagents.** All reagents were of analytical-reagent or Suprapur grade, and all solutions were prepared with Millipore Milli-Q (USA) deionized water or extrapure ethanol.

The 2-hydroxybenzaldehyde benzoylhydrazone<sup>26</sup> was synthesized by the reaction between 2-hydroxybenzaldehyde (2-HB) and benzoylhydrazine (BH), purchased from Sigma-Aldrich (Steinheim, Germany; see Fig. 1). Ethanolic solution of 2-HB (1 mL in 20 mL) and aqueous solution of BH (1.11 g in 30 mL) were mixed. The complex formed from condensation was filtered and subsequently crystallized three times from 1:1 ethanol/water. The last yellow product obtained was dried in the oven at 50 °C for 6 h. The stock solution of 2-HBBH in extrapure ethanol (Scharlab, Spain) was prepared weekly and stored at 4 °C in darkness. For the preliminary studies utilizing FAAS, 2.5 mg of 2-HBBH in 2.5 mL of ethanol was prepared. For diffuse reflectance studies, 6.25 mg of 2-HBBH in 6.25 mL was used. As previously evaluated,<sup>24</sup> a 1:1 stable complex is formed between copper and 2-HBBH in aqueous ethanolic solution. The ligand coordinates to the copper ion as a monoanionic, tridentate ligand through the phenolic oxygen, the imine nitrogen, and the amide oxygen. A square planar geometry can be proposed for this complex.

Stock aqueous solutions of Cu(II) were prepared weekly by the dilution of Cu(II) standard solution of 1000 mg L<sup>-1</sup> (Merck, Darmstadt, Germany) in 0.06 mol L<sup>-1</sup> HNO<sub>3</sub>.

Hydron phosphate Chemvelope buffers purchased from Sigma-Aldrich were used to adjust the pH (range 3–9) by adding to 500 mL of Milli-Q deionized water. This buffer and other different buffer solutions (listed below)

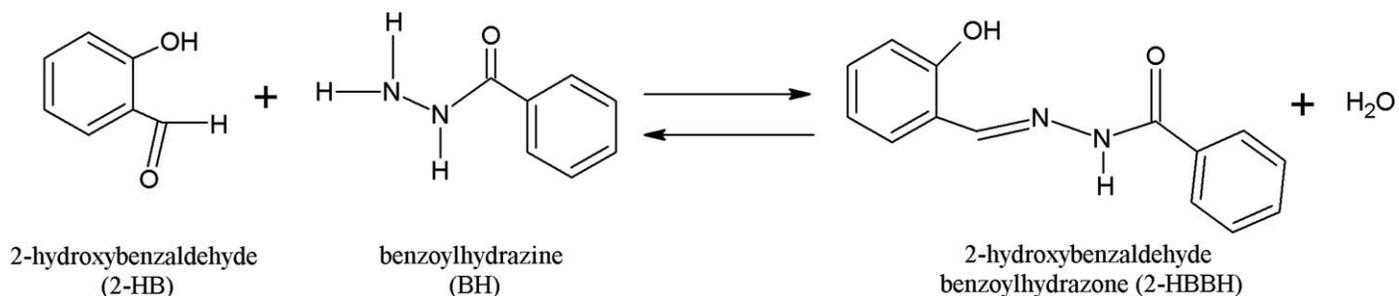


Fig. 1. The synthetic route of 2-hydroxybenzaldehyde benzoylhydrazone (2-HBBH).

were prepared and adjusted to pH 7, which was chosen as the optimum pH: 0.5 mol L<sup>-1</sup> HEPES from *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid (Sigma-Aldrich) and suprapur ammonia (Merck); 0.05 mol L<sup>-1</sup> from piperazine-*N,N'*-bis(ethanesulfonic acid) (PIPES) and suprapur ammonia (Merck); 1 mol L<sup>-1</sup> suprapur ammonia (Merck), and 1 mol L<sup>-1</sup> suprapur nitric acid (Merck); and 0.5 mol L<sup>-1</sup> potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) and 1 mol L<sup>-1</sup> NaOH.

**Apparatus.** The FAAS used for the initial measurements of copper concentration using SPE was a Solaar M Series (Unicam, UK), operating under recommended conditions for Cu(II). Diffuse reflectance measurements were recorded using AvaSpec-PC2000 High UV-sensitivity CCD Spectrometer and an FCR-7UV200-2-45 reflection probe (Avantes), controlled using the software Spectra-Win 4.2(c) 2000 (Top Sensor Systems). Fluorescence measurements were recorded with an LS 55 PerkinElmer Luminescence Spectrometer (Waltham, MA) with excitation and emission slit widths set at 10 and 20 nm, respectively. A Hanna Turtle pH meter was used for pH measurements.

**Preparation of Modified Extraction Disks and Extraction Procedure.** Empore SDB-XC (3M) polystyrene-divinylbenzene 47 mm extraction disks were used as the extraction membranes. The membrane disk was placed in the filtration apparatus (Millipore glass 47 mm vacuum filter holder assembly with 300 mL funnel with a glass fritted base) and washed with 10 mL of 1.0 mol L<sup>-1</sup> nitric acid solution, 10 mL of methanol, and 10 mL of acetonitrile in order to remove the contaminants from the disk. After all the solvents had passed through the disk, it was dried using passing air for several minutes. Then, the membranes were cut into 13 mm disks with a cork borer. The small disk was placed on a 13 mm plastic Fisherbrand SyringeSwinnex Filter Holders (Millipore). A solution of 2-HBBH prepared by dissolving 6.25 mg of reagent in 6.25 mL of ethanol was passed through the disk using a 5 mL plastic syringe attached to the holder (for diffuse reflectance measurements). After impregnation with 2-HBBH, the disk was dried for a few minutes and washed with 5 mL of water to pre-wet the surface of the disk. Then, 50 mL of the sample adjusted to pH 7 with 0.05 mol L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>/NaOH buffer was passed through the modified membrane disk. The solution was pushed manually using the syringe at a rate of approximately one drop per second. This step required about 10 min. After the extraction, 10 mL of air was passed through the holder to remove remaining sample solution. The holder

was detached from the syringe and unscrewed, and the disk was removed from the holder. Finally, the optical fiber was placed in direct contact onto the disk in the dark to acquire the diffuse reflectance spectrum. It is important to highlight that no copper recovery was obtained without employing reagent onto the disk.

**Diffuse Reflectance Measurement.** The optical signal recorded using the detector as counts is indicative of diffuse reflectance of the incident light using the sensing membrane. The reflectance measurements of the disk surface were measured at 400 nm, which was the maximum reflectance signal. The diffuse reflectance standard (a white material) used in the experiments was a bare polystyrene-divinylbenzene disk. For optical isolation, the fiber optic and the membrane disk were kept in a black box to minimize any interference from ambient light. Spectra data were transferred to the computer and downloaded to a Microsoft Excel worksheet to plot reflectance data.

Note that the diffuse reflectance ( $R$ ) at 400 nm was used to calculate the Kubelka–Munk function  $F(R)$  using the expression:

$$F(R) = \frac{(1 - R)^2}{2R} \quad (1)$$

$F(R)$  can be directly related to the concentration of the complex in the membrane disk to obtain a linear calibration plot for quantitative measurements<sup>15</sup> using the expression:

$$F(R) = \frac{\varepsilon \cdot C}{S} \quad (2)$$

where  $\varepsilon$  is the molar absorptivity of the sample and  $S$  is the scattering coefficient of the disk surface, both of which can be considered as constants in this work. The diffuse reflectance equipment employed in this work converts reflectance data to the Kubelka–Munk function, which is directly proportional to absorbance. For the remainder of this manuscript, the value of the Kubelka–Munk function will be referred to as reflectance signal.

## RESULTS AND DISCUSSION

Some preliminary experiments were carried out in order to investigate the quantitative retention of copper ions using the membrane disk in the absence and presence of 2-HBBH. It was found that the bare membrane disk did not show any tendency for the

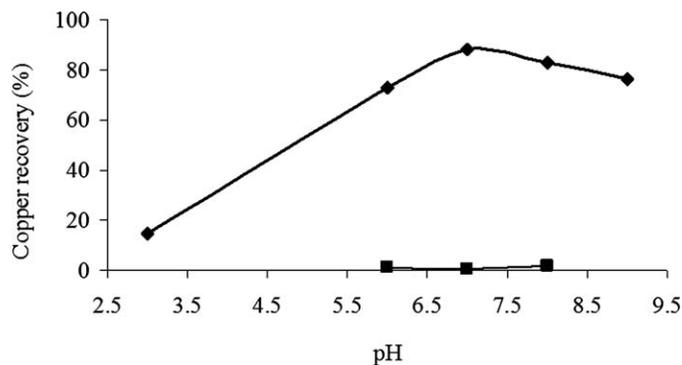


FIG. 2. Effect of pH on the extraction of  $40 \mu\text{g L}^{-1}$  Cu(II) using the disk: (a) with immobilized reagent ( $\blacklozenge$ ); (b) without reagent ( $\blacksquare$ ) ( $V_{\text{sample}} = 500 \text{ mL}$ ;  $V_{\text{eluant}} = 10 \text{ mL}$  of  $1 \text{ mol L}^{-1} \text{ HNO}_3$ ; FAAS measurements).

retention of copper ions, while the modified SDB-XC disks with 2-HBBH were capable of retaining Cu(II) ions on the surface.

The optimization process was developed using a two-stage strategy. First, previous studies of chemical parameters such as pH, amount of reagent, and type of buffer were carried out using the univariate method in order to achieve high efficiency and good selectivity. Retained Cu(II) ions extracted in these experiments were determined using FAAS. Then, to select the optimum conditions for pH, amount of ligand immobilized on the disk and time for immobilization, a reduced factorial design (fractional factorial design  $3^{3-1}$ ) was employed. With this kind of design, the factors can be efficiently evaluated using a small fraction of the experiments of the full factorial design. For the optimization experiments, retained Cu(II) ions were determined using diffuse reflectance measurements.

**Preliminary Studies for the Solid Phase Extraction of Cu(II) Using Flame Atomic Absorption Spectrophotometer (FAAS).** Preliminary studies of the influence of variables on the copper ions extraction were carried out with FAAS as the measurement technique after acid elution of metal ion from the disk.

#### Effect of pH on the Solid Phase Extraction of Cu(II).

The pH of the medium plays a key role in the extraction procedure improving the ligand-metal complex reaction efficiency.<sup>3</sup> The influence of the pH on the recovery of copper was studied in the range 3 to 9 by passing 500 mL solutions of  $20 \mu\text{g Cu(II)}$  through the modified membrane disk. The pH values of the solutions were adjusted with 50 mL of Hydrion<sup>®</sup> buffer. A solution of 2-HBBH was passed through the disk. We used 10 mL of  $1 \text{ mol L}^{-1}$  nitric acid for the acid elution step, obtaining a preconcentration factor of 50. The plot of copper recovery versus pH showed pH values close to 7 as the optimum condition (Fig. 2). A neutral pH value is considered appropriate for biological and environmental applications.

**Effect of Amount of Ligand 2-Hydroxybenzaldehyde Benzoylhydrazone (2-HBBH).** The extraction of copper at pH 7 was studied with each SDB disk modified with different quantities of ligand (Fig. 3). The minimum quantity of 2-HBBH required to achieve the maximum recovery of copper (around 85%) was 1.25 mg. In order to

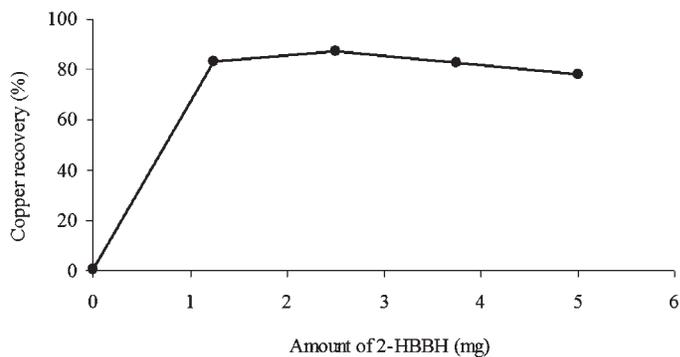


FIG. 3. Effect of the amount of ligand on the extraction of  $40 \mu\text{g L}^{-1}$  Cu(II) using the modified disk ( $V_{\text{sample}} = 500 \text{ mL}$ ;  $V_{\text{eluant}} = 10 \text{ mL}$  of  $1 \text{ mol L}^{-1} \text{ HNO}_3$ ; FAAS measurements).

assure that there was enough ligand to form a complex with copper ions, 2.5 mg was selected as the adequate amount of 2-HBBH for FAAS measurements.

**Effect of Buffer Composition Used to Adjust the Sample pH Value.** In order to choose the proper buffer for the extraction of copper ions, the pH of the solution was adjusted using five different buffers (Table II). For this experiment, 500 mL of solution containing  $20 \mu\text{g}$  of Cu(II) was adjusted to pH 7 by adding 50 mL of each buffer solution. Each solution was passed through a disk containing 2.5 mg of 2-HBBH. We used 10 mL of  $1 \text{ mol L}^{-1}$  nitric acid for the acid elution step, obtaining a preconcentration factor of 50. The best recoveries were achieved using both  $\text{KH}_2\text{PO}_4/\text{NaOH}$  and Hydrion buffers.

**Optimization of Variables Using Diffuse Reflectance Measurements.** Using the values of suitable parameters obtained in the preliminary studies, the new colorimetric-solid phase extraction method was investigated using diffuse reflectance measurements. The largest difference in reflectance before and after reaction with Cu(II) was observed at 400 nm and adjusting the pH with  $\text{KH}_2\text{PO}_4/\text{NaOH}$ . The color of the disk changes with the concentration of copper from white to green, being detected with diffuse reflectance measurements using optical fiber.

Due to its relevance and important effect in optical sensors, the pH, amount of ligand immobilized onto the disk, and time for immobilization were evaluated. In order to determine the influence of these factors, a fractional factorial design  $3^{3-1}$  was performed. It has the advantages of identifying and isolating the significant factors with a minimum number of experiments.<sup>27</sup> To the best of our knowledge, this statistical method has never been applied to C-SPE processes.

TABLE II. Influence of the composition of buffer solution used to adjust the sample pH value on copper recovery ( $V_{\text{sample}} = 500 \text{ mL}$ ;  $V_{\text{buffer}} = 50 \text{ mL}$ ).

Buffer solution added (pH 7)	Recovery (%)
Hydrion-buffer	88.06
$\text{HNO}_3$ ( $1 \text{ mol L}^{-1}$ )/ $\text{NH}_3$ ( $1 \text{ mol L}^{-1}$ )	74.35
HEPES ( $0.5 \text{ mol L}^{-1}$ )	55.15
$\text{KH}_2\text{PO}_4/\text{NaOH}$ ( $0.5 \text{ mol L}^{-1}$ )	88.73
PIPES ( $0.05 \text{ mol L}^{-1}$ )	67.27

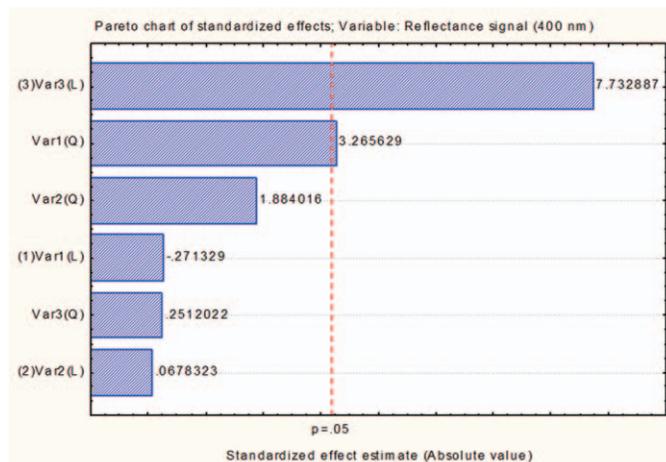
**TABLE III. Variables and levels of the fractional factorial design  $3^{3-1}$  ( $V_{\text{sample}} = 50 \text{ mL}$ ;  $V_{\text{buffer}} = 5 \text{ mL}$  of  $0.5 \text{ mol L}^{-1} \text{ KH}_2\text{PO}_4/\text{NaOH}$ ;  $C_{\text{Cu(II)}} = 2.5 \text{ mg L}^{-1}$ ).**

Variable	-1	0	+1
pH	5	7	9
Immobilization time (min)	5	10	15
Amount of ligand immobilized (mg)	0.25	3.25	6.25

The three variables studied were coded at three levels, high, medium, and low, denoted using (+1), (0), and (-1), respectively. Selected levels of each variable were chosen according to the data from preliminary experiments (Table III). In total ten experiments were carried out, including two repetitions of the central point. The response variable evaluated with the fractional factorial design was the reflectance signal of Cu-2-HBBH complex. No significant parameters were kept fixed: volume of sample solution, 50 mL; buffer concentration ( $\text{KH}_2\text{PO}_4/\text{NaOH}$ ), 0.5 M; and copper concentration,  $2.5 \text{ mg L}^{-1}$ . Table IV shows the matrix of the experimental design and the respective response variable. Statistical analysis was performed using Statistica 7.0 for the fractional factorial design  $3^{3-1}$ .<sup>28</sup> The interpretation of the factorial design using the Pareto chart (Fig. 4) demonstrated that pH (Var 1) and amount of ligand (Var 3) showed significant effects (at 95% confidence level). On one hand, amount of ligand immobilized showed a linear positive effect over the reflectance signal. On the other hand, pH showed a quadratic positive effect. Due to its nonsignificant effect, the immobilization time (Var 2) was fixed at an intermediate value so that the experiments were not long. Response surfaces were represented graphically in a spherical domain. Diffuse reflectance intensity was taken as the response signal in the three response surfaces, keeping one variable at its optimum level and the other two varying within the experimental ranges (Fig. 5). The effect of the variables pH and immobilization time over the reflectance is shown in Fig. 5a, where the amount of 2-HBBH was held at 6.25 mg, according to the results of the Pareto chart. The effect of pH and amount of ligand at a constant immobilization time (10 min) is shown in Fig. 5b. Finally, Fig. 5c shows the effect between immobilization time and amount of ligand using pH as the medium value of the interval (pH

**TABLE IV. Design matrix and the results of the fractional factorial design  $3^{3-1}$  ( $V_{\text{sample}} = 50 \text{ mL}$ ;  $V_{\text{buffer}} = 5 \text{ mL}$  of  $0.5 \text{ mol L}^{-1} \text{ KH}_2\text{PO}_4/\text{NaOH}$ ;  $C_{\text{Cu(II)}} = 2.5 \text{ mg L}^{-1}$ ).**

Experiment	pH	Immobilization time	Amount of ligand	Reflectance signal (400 nm)
2	-1	0	+1	0.80
5	0	0	0	0.81
3	-1	+1	0	0.57
8	+1	0	-1	0.43
4	0	-1	+1	0.87
7	+1	-1	0	0.53
6	0	+1	-1	0.47
9	+1	+1	+1	0.80
1	-1	-1	-1	0.43
10	0	0	0	0.80



**FIG. 4. Pareto chart of standardized effects for fractional factorial design using reflectance signal as response variable (Var 1, pH; Var 2, immobilization time; Var 3, amount of ligand).**

7). Such plots were helpful in determining the optimal experimental conditions. The results indicated that the higher the amount of ligand, the higher the reflectance signal in the studied experimental domain. As far as immobilization time and pH are concerned, both showed better results at the medium values of the interval ranges studied.

Hence, the optimum conditions selected for the sensing of copper ions were pH = 7, 10 min as immobilization time, and 6.25 mg for the amount of ligand. As can be seen, the optimum amount of ligand is higher than the quantity employed in the preliminary studies. This can be explained using the lower sensitivity of the diffuse reflectance measurements compared with atomic absorption.

**Lifetime and Reusability of the Sensor.** The study of lifetime of the sensor was accomplished and no significant differences in the optical properties of the disk were found over several days. The diffuse reflectance signal was recorded at a wavelength of 400 nm as a function of time when the sensing layer with 6.25 mg of 2-HBBH was exposed to  $1 \text{ mg L}^{-1} \text{ Cu(II)}$  at pH 7.0. It was observed that there was no decrease in the reflectance intensity of the C-SPE disk after repetitive measurements together with no leaching of 2-HBBH. Thus, the results obtained showed a high degree of stability of the sensing membrane. For this reason, the proposed sensor can be used to extract and retain copper ions on-site and then analyze the disk in the laboratory several days after collection without losing its characteristics.

The reversibility of the sensor in solution was reported in our previous paper.<sup>24</sup> It was demonstrated that the binding between 2-HBBH and Cu(II) is really chemically reversible after addition of ethylenediaminetetraacetic acid (EDTA) to the solution. However, for trace analysis in a solid support, the use of a new modified disk is recommended for each measurement in order to avoid subsequent contaminations.<sup>20</sup>

**Analytical Features of the Sensor.** Under the optimum experimental conditions, the analytical parameters of the proposed method were determined. The calibration graph for Cu(II) was linear over the range of 0 to  $2.5 \text{ mg L}^{-1}$  of

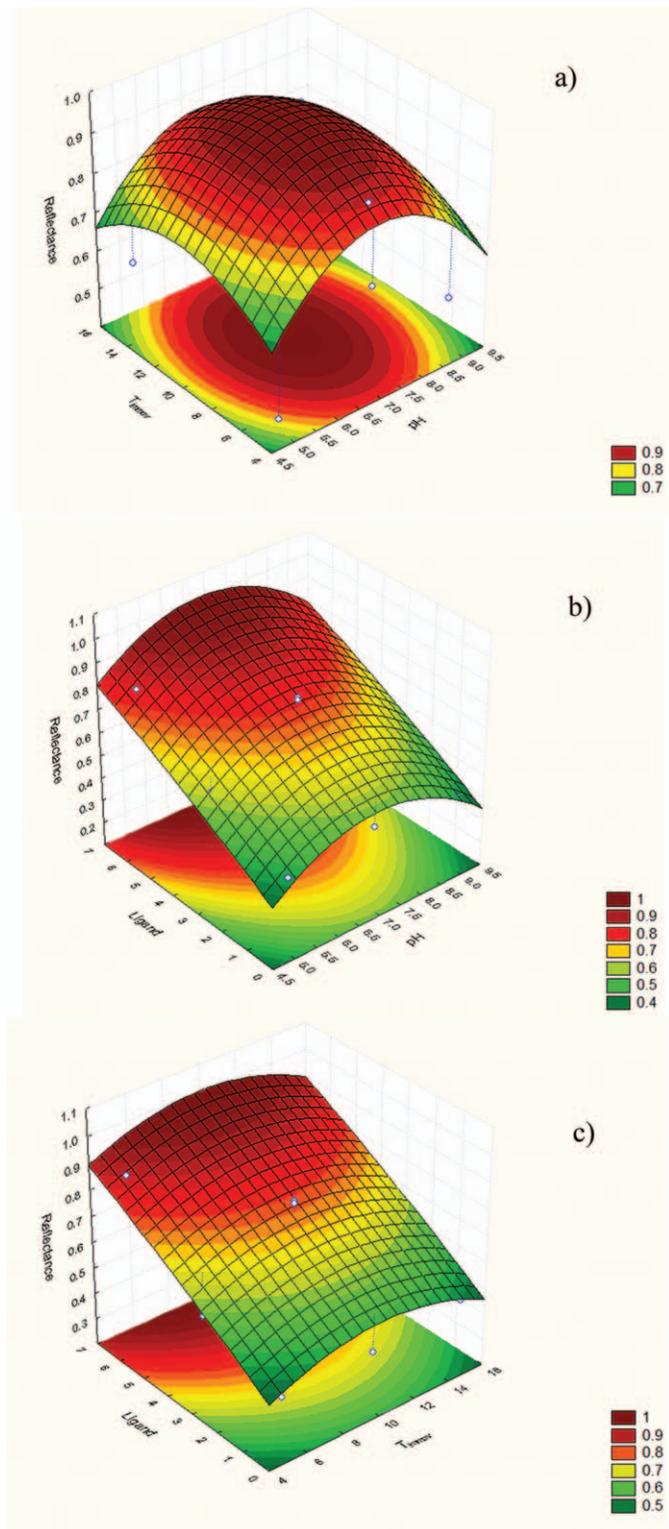


FIG. 5. Response surface plot obtained using fractional factorial design: (a) Reflectance signal vs. pH and immobilization time (amount of ligand, 6.25 mg); (b) Reflectance signal vs. pH and amount of ligand (immobilization time, 10 min); (c) Reflectance signal vs. immobilization time and amount of ligand (pH value, 7).

Cu(II) with a correlation coefficient of the linear regression being  $R^2 = 0.976$  (Fig. 6). The response function for copper concentration was  $[Cu] \text{ (mg L}^{-1}\text{)} = (y - 0.156)/0.259$ . The detection limit (DL), calculated according to  $DL = 3 \sigma/m$ ,<sup>29</sup>

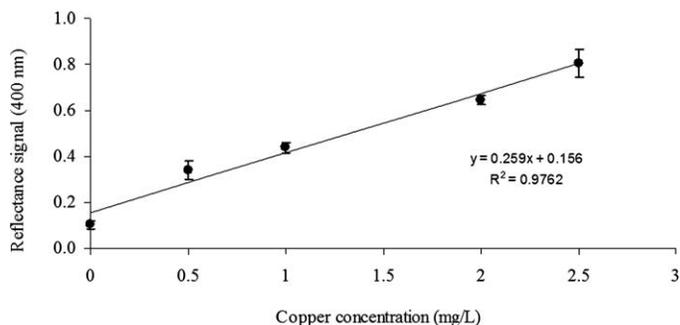


FIG. 6. Calibration curve for different Cu(II) concentrations using the optimum conditions at 400 nm using diffuse reflectance measurements ( $V_{\text{sample}} = 50 \text{ mL}$ ;  $V_{\text{buffer}} = 5 \text{ mL}$  of  $0.5 \text{ mol L}^{-1} \text{ KH}_2\text{PO}_4/\text{NaOH}$  at pH = 7; 6.25 mg 2-HBBH).

where  $\sigma$  is the blank standard deviation and  $m$  is the slope of the calibration graph, was found to be  $0.21 \text{ mg L}^{-1}$  of Cu(II) ( $n = 7$ ). The repeatability of the C-SPE sensing layer was also evaluated through the relative standard deviation (RSD) of six replicate measurements of a solution containing  $1 \text{ mg L}^{-1}$  of copper ion. The RSD was found to be 4.87%, and the precision of the method was 4.01% at a significance level of 95% ( $n = 6$ ).

**Interference Study.** Under optimum conditions, the effects of various foreign ions on the determination of Cu(II) were examined using the proposed C-SPE method. Possible interference by matrix of water samples was investigated by adding different inorganic salts and metals present in seawater to a solution containing  $1 \text{ mg L}^{-1}$  of Cu(II). The possible interferences of inorganic salts, such as KCl,  $\text{NaHCO}_3$ , NaCl, NaF,  $\text{MgCl}_2$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{H}_3\text{BO}_3$ , KBr, and  $\text{SrCl}_2$  were evaluated using the normal concentration found in seawater.<sup>30</sup> On the other hand, the possible interference of some inorganic cations, such as Ca(II), Cd(II), Zn(II), K(I), Mg(II), Na(I), Pb(II), Fe(II), and Fe(III) were evaluated at  $1 \text{ mg L}^{-1}$ . It was found that the method was free from interference of the saline matrix, being only affected by Fe(III) and Zn(II) at  $1 \text{ mg L}^{-1}$ .

**Evaluation of Fluorescence Quenching Using Cu-2-Hydroxybenzaldehyde Benzoylhydrazone (Cu-2-HBBH) Sensor.** Due to the fact that fluorescence detection methods can be more selective and sensitive over other light-based methods such as reflectance,<sup>31,32</sup> fluorescence analysis was performed as an alternative form of measurement of the analyte on the solid surface (for copper analysis) and compared with the reflectance measurements. In this manner, the optical fiber was placed in direct contact onto the disk under darkness to acquire the fluorescence spectrum. The fluorescence intensity was recorded at 447 nm using an excitation wavelength of 360 nm. Under the optimum conditions, the fluorescence quenching data were observed, and the data were analyzed using the Stern–Volmer relationship, given below:

$$F_0/F = 1 + K_{SV}[Q] \quad (3)$$

where  $F$  and  $F_0$  are the fluorescence intensities of the reagent in the presence and absence of copper ion, respectively.  $[Q]$  is the copper ion concentration

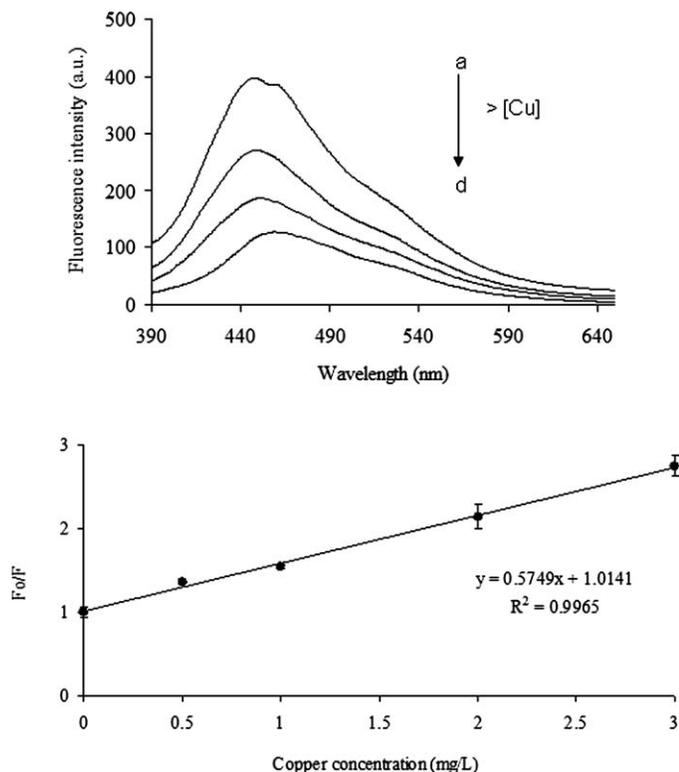


FIG. 7. Top: Fluorescence emission spectra of 2-HBBH in the presence of different concentrations of Cu(II) ions ( $\text{mg L}^{-1}$ ): (a) 0; (b) 1; (c) 2; (d) 4; Bottom: Stern–Volmer plot of 2-HBBH with increasing concentrations of Cu(II) ( $V_{\text{sample}} = 50 \text{ mL}$ ;  $V_{\text{buffer}} = 5 \text{ mL}$  of  $0.5 \text{ mol L}^{-1} \text{ KH}_2\text{PO}_4/\text{NaOH}$  at  $\text{pH} = 7$ ;  $6.25 \text{ mg}$  2-HBBH). Excitation and emission wavelengths were 360 and 447 nm, respectively.

(quencher) and  $K_{\text{SV}}$  is the Stern–Volmer quenching constant. The calibration graph for Cu(II) was linear over the range of 0 to  $3 \text{ mg L}^{-1}$  of Cu(II) with a correlation coefficient and equation of the linear regression being  $R^2 = 0.996$  and  $F_0/F = 0.575 Q (\text{mg L}^{-1}) + 1.014$ , respectively (Fig. 7). Detection limit evaluated, as indicated previously, was found to be  $0.28 \text{ mg L}^{-1}$  of Cu(II). It can be concluded that the combination of SPE onto a modified membrane disk with reflectance or fluorescence spectroscopy can be applied with good results.

## CONCLUSION

A simple, easy, and inexpensive sensor based on the immobilization of 2-HBBH onto SDB-XC disks for the sensing of Cu(II) in waters has been described. The sensor is characterized using the rapidity of the analysis and the possible portability of the system.

The detection limit and the calibration curve can allow the use of the sensor to analyze water samples that are reasonably contaminated with copper. Thus, successful applications could be in using the sensor as an alarm for rapid monitoring of drinking water or in the waste stream of a copper-based industry where the concentration of copper is not allowed to exceed a maximum level. The combination between the C-SPE method and diffuse reflectance or fluorescence spectroscopy for the determination of copper presented in this paper has been shown to be a useful tool for further analytical

applications. This field opens extensive possibilities for the determination of metal ions in aqueous samples, and several studies in this direction are now in progress in our laboratory.

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