

Application of a Biomimetic Sensor Based on Iron Phthalocyanine Chloride: 4-Methylbenzylidene-Camphor Detection[#]

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A construção e a aplicação de um sensor biomimético para a determinação de 4-metilbenzilideno cânfora (4-MBC), um protetor de radiação ultravioleta (UV), são descritas. O sensor foi preparado pela modificação de um eletrodo de pasta de carbono com um complexo de cloreto de ferro(III) com ftalocianina, FePcCl. As medidas amperométricas conduzidas com o sensor, sob um potencial aplicado de 0,0 V vs. Ag|AgCl em uma mistura de tetraidrofurano e 0,1 mol L⁻¹ H₂SO₄ (30:70 em volume), mostraram uma resposta linear no intervalo de 1,8×10⁻⁴ to 9,0×10⁻⁴ mol L⁻¹. Uma investigação detalhada da seletividade da resposta frente a outros nove filtros UV também foi realizada. Um mecanismo de resposta do sensor foi proposto e os resultados para a quantificação de 4-MBC em protetores solares comerciais e em águas de piscinas e de rios são apresentados.

The construction and application of a biomimetic sensor for determination of 4-methylbenzylidene camphor (4-MBC), an ultraviolet (UV) radiation protector, are described. The sensor was prepared by modifying a carbon paste electrode with iron(III) phthalocyanine chloride (FePcCl). Amperometric measurements carried out with the sensor under an applied potential of 0.0 V vs. Ag|AgCl in a mixture of tetrahydrofuran and 0.1 mol L⁻¹ H₂SO₄ solution (30:70 volume ratio) showed a linear response range from 1.8×10⁻⁴ to 9.0×10⁻⁴ mol L⁻¹. A detailed selectivity investigation for other nine UV filters was also performed. A sensor response mechanism was proposed and the results for 4-MBC quantification in commercial sunscreens and in water from swimming pools and rivers are presented.

Keywords: iron phthalocyanine, amperometric sensor, methylbenzylidene camphor

Introduction

Chemical ultraviolet (UV) filters have been introduced in 1889, when an acidified quinine sulfate solution was used to block UV radiation,¹ and thereafter sunscreen has become an everyday cosmetic with continuously increasing use.

The positive effect exerted by UV filters is to block the UV light radiation damage from sunlight, sunlamps

and in tanning parlours; moreover, they have color protective abilities and therefore may prevent premature fading of hair color and damage to hair cuticle. On the other hand, it has been recently reported that commonly used chemical UV filters may cause endocrine disrupting effects in both aquatic and terrestrial animals, as well as in human skin cells.¹⁻⁴ In addition, five of the most commonly UV filters used in cosmetic formulations, *i.e.*, oxybenzone, octyl methoxycinnamate (OMC), homosalate, *p*-aminobenzoic acid (PABA), octyldimethyl-PABA (ODP), and 4-methylbenzylidene camphor (4-MBC), exhibited oestrogenic effects in MCF-7 (Michigan

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[#]Dedicated to the memory of our friend Professor Ícaro Sousa Moreira, DQOI-UFC, Brazil

Cancer Foundation-7) breast cancer cells. Furthermore, oxybenzone, 4-MBC and OMC were confirmed to be oestrogenic by classical *in vivo* uterotrophic assays with rats dosed in the feed. For the most potent oestrogenic UV filter 4-MBC, the effect could be even replicated by application on rat skin, resembling human exposures with sunscreen cream. *In vitro* studies have shown other hormone disruption responses (anti-oestrogenic, androgenic, anti-androgenic and progestogenic activities) for almost all the chemical UV filters used in sunscreen formulations.^{5,6}

Recent publication¹ has shown that, in Denmark, urban water is the primary recipient of six UV filters, including oxybenzone, avobenzene, 4-MBC, octyl methoxycinnamate, ODP and homosalate, with estimated 8.5-65 tonnes and 7.1-51 tonnes in wastewater and surface water, respectively. In North of China, it has been observed that the levels of benzophenone-3 (BNP), 4-MBC, OMC and octocrylene (OC) in different units of wastewater plants were in the concentration range 2.13-34.0 ng L⁻¹, with high concentrations in hot seasons.⁷

Since the use of UV filters in countries like Brazil is expected to continuously increase, and the disposal of such compounds in aquatic environments is still not regulated by any government agency, it seems worthwhile to apply the knowledge in biomimetic sensors based on metalophthalocyanines and metaloporphyrins for detection of UV filters, more specifically 4-MBC, which is commonly used in sunscreens for children.

Metalophthalocyanines and metaloporphyrins have been widely employed in sensor construction with different transducer systems, such as optical,⁸⁻¹³ chemoresistive,¹⁴⁻¹⁸ impedimetric,¹⁹ piezoelectric^{20,21} and mainly electrochemical,²²⁻³⁰ since they present high electrocatalytic activities for many redox reactions. In addition, enzymeless sensors^{31,32} using these macrocycles³³⁻³⁸ as biomimetic active sites of redox enzymes, as peroxidase,^{34,37} dopamine monooxygenase³⁸ and P450^{35,36} have been described, offering a real and an alternative tool to official analytical methods. Furthermore, it is well known that established methods for identifying pollutants usually require preparative steps and expensive equipments, such as high performance liquid chromatography (HPLC) and mass spectrometry (MS), and the development of alternative methods for selective, sensitive, quicker and cheaper analyses would be welcome.

Experimental

Chemicals and solutions

All chemicals used were of analytical or HPLC grade. Iron(III) phthalocyanine chloride (FePcCl),

p-aminobenzoic acid (PABA), octyldimethyl-PABA (ODP), 4-methylbenzylidene camphor (4-MBC), [3-(4-methylbenzylidene)bornan-2-one], isoamyl *p*-methoxycinnamate (IMC) and 2-phenyl-5-benzimidazolesulfonic acid (PBS) were acquired from Aldrich. Benzophenone-3 (BNP); octylmethoxycinnamate (OMC); menthyl anthranilate (MA); octyl salicylate (OSA) and avobenzene (AVB) were purchased from Fluka. Tetrahydrofuran (thf), acetonitrile (MeCN) and sulfuric acid were obtained from Synth, Brazil.

The 4-MBC standard solutions were prepared in thf for the electroanalytical experiments and in the respective mobile phase for the HPLC experiments.

Sensor construction

In order to obtain homogeneously modified carbon pastes, the sensor was prepared by mixing an adequate amount of FePcCl with graphite powder and 1.0 mL of 0.1 mol L⁻¹ phosphate buffer solution (pH 7.0). After drying at room temperature, 50 μ L of mineral oil were added and mixed to obtain a homogeneous paste, which was placed into the cavity of a glass tube (4 mm internal diameter and 1 mm depth) with a platinum slide inserted for electrical contact with the paste. The effect of the FePcCl content on the sensor response was evaluated by preparing five different pastes with the macrocycle weight percentage varying from 5 to 15%.

Electrochemical measurements

The electrochemical experiments were conducted, at room temperature, in solutions containing a 70:30 volume ratio of 0.1 mol L⁻¹ H₂SO₄ and thf, respectively, and using a conventional three-electrode cell with the modified carbon paste electrode as working electrode, Ag|AgCl electrode and platinum wire as reference and counter electrodes, respectively. All experiments were carried out in air-equilibrated solutions, since preliminary experiments have shown that dissolved oxygen did not interfere in the sensor response. The data acquisition was performed with a PalmSens potentiostat (Palm Instruments BV, Netherlands) interfaced with a microcomputer.

The amperometric measurements were carried out at a previously optimized potential of 0.0 mV vs. Ag|AgCl, and the current was continuously monitored and registered until a steady state was reached (approximately 3 s). Right after, 50 μ L of 2.5 \times 10⁻² mol L⁻¹ UV filter standard solution was added to the electrolytic solution and stirred for 10 s for solution homogenization. Then the current was monitored in the quiescent solution for 10 s. Thus, successive additions

of the UV filter standard solutions were performed, in order to obtain the analytical curve.

HPLC analyses

The chromatographic analyses were performed in a Shimadzu® model 20A liquid chromatograph coupled with SPD-20A UV/Vis detector, SIL-20A auto sampler and a DGU-20A₅ degasser, and monitored by a microcomputer. The column used was a C18 (250 4.6 mm, Shim-Pack CLC-ODS) fixed inside a Shimadzu® CTO-10AS oven to keep the temperature constant.

Due to the lack of an official analytical procedure for 4-MBC quantification, the procedure reported in the literature³⁹ was adapted and optimized by preparing the mobile phase with a mixture of acetonitrile and water in a volume ratio of 93:7. The flow rate was 1.0 mL min⁻¹ and the injection sample volume was 30 µL. The column temperature was maintained at 30 °C. The measurement wavelength was 321 nm and the analysis time was 15 min for each standard and sample.

Sunscreen analyses

Child commercial sunscreens and a Lanette anionic gel-cream sample were analyzed. Before each analysis, the sample was pre-treated according to the procedure established by the Brazilian sanitary surveillance agency (ANVISA - Agência Nacional de Vigilância Sanitária).⁴⁰ For this, 2.0 g of the sunscreen was mixed with 4.0 mL of methanol and 250.0 µL of 1.0 mmol L⁻¹ H₂SO₄, heated at 40 °C for 10 min and finally sonicated for 3 min. After this pre-treatment, the solution was filtered and injected in the chromatograph or introduced in the electrochemical cell for the measurements.

Aquatic sample analyses

Two water samples from swimming pools and five samples collected from rivers near to the city of Araraquara, in São Paulo state, were enriched with 4-MBC and analyzed with the proposed sensor, in order to evaluate the matrix effects.

Results and Discussion

The electrochemical behaviors of the 4-MBC on the carbon paste electrode unmodified or modified with FePcCl are illustrated in Figure 1. The response of the unmodified paste electrode did not present any difference before and after addition of 4-MBC to the electrolyte. On the other

hand, the sensor based on carbon paste modified with the iron(III) complex showed an increase in the reduction current for potentials less positive than 0.12 V, with a maximum at around 0.0 V vs. Ag|AgCl. In addition, in absence of the analyte in the electrolyte, it was possible to observe a peak at approximately 0.1 V, which can be associated to the Fe³⁺/Fe²⁺ redox couple and recognized as the catalytic site for the sensor response.

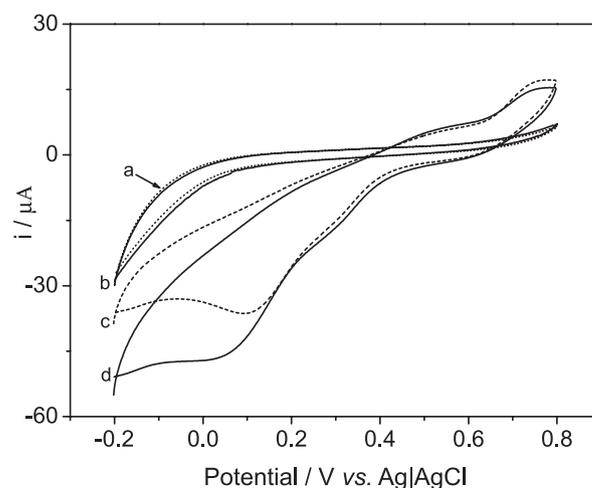


Figure 1. Voltammetric profiles for unmodified (a and b) and modified carbon paste electrodes with FePcCl (c and d) in 0.1 mol L⁻¹ H₂SO₄/thf 70:30 (v:v) solutions in presence (solid line) and absence (dashed line) of 1.0×10⁻⁴ mol L⁻¹ 4-MBC. Potential scan rate: 20 mV s⁻¹.

The dependence of the sweep rate-normalized current density ($\Delta i_p v^{-1/2}$) with the potential sweep rate (v) is shown in Figure 2. The plot shape is characteristic of a typical electrocatalytical reduction process,^{35,36,41} indicating that the sensor response is carried out first by a chemical reaction between the analyte and the iron complex (catalyst) and further by an electrochemical process involving the

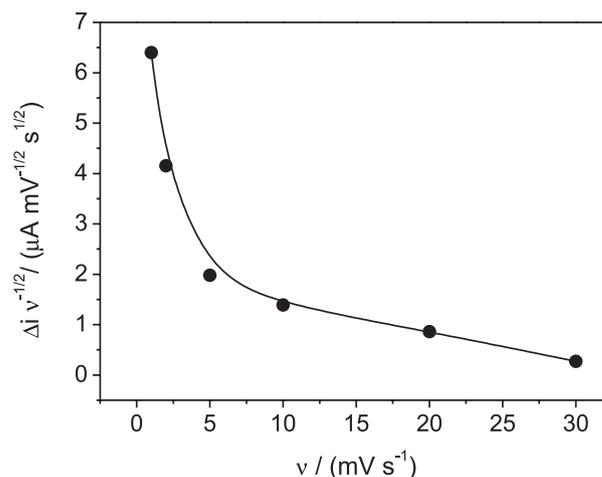


Figure 2. Plot of the sweep rate-normalized current density ($\Delta i_p v^{-1/2}$) versus the sweep rate (v). [4-MBC] = 2.0×10⁻⁴ mol L⁻¹ in 0.1 mol L⁻¹ H₂SO₄/thf 70:30 (v:v) solution.

catalytic site regeneration ($\text{Fe}^{3+}/\text{Fe}^{2+}$ redox process), with the current being proportional to the 4-MBC concentration and associated with the reduction of ferric to ferrous ions.

On the other hand, when the response profile was investigated beyond the sensor linear range, the curve presented a hyperbolic shape (data not shown), as expected for enzymatic biosensors and biomimetic sensors that follow a Michaelis-Menten kinetic relationship.^{31,36} Thus, in order to evaluate the apparent Michaelis-Menten constant ($K_{\text{MM}}^{\text{app}}$) for the 4-MBC sensor, the Lineweaver-Burk graph was constructed (Figure 3). The obtained $K_{\text{MM}}^{\text{app}}$ value was of $3.1 \times 10^{-4} \text{ mol L}^{-1}$, which is appropriate for enzymatic and biomimetic catalysts with high analyte affinity.³¹

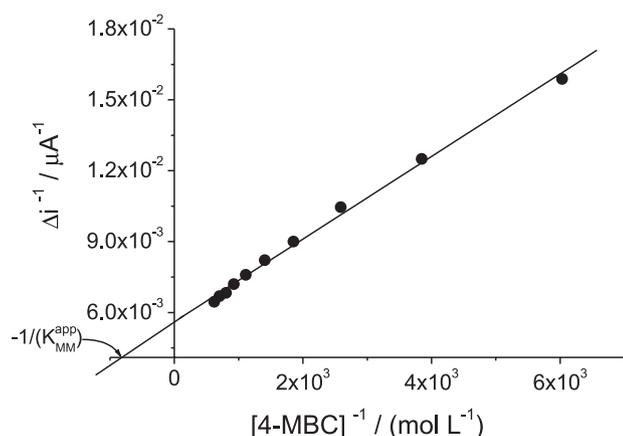


Figure 3. Lineweaver-Burk plot for the 4-MBC reduction catalyzed by the FePcCl-based sensor.

Therefore the experimental data suggests that the FePcCl complex acts as a biomimetic catalyst according to the mechanism presented in Figure 4. In this mechanism, the reduction of the 4-MBC ketone group to alcohol⁴¹ via one electron in acid media promotes the oxidation of Fe^{2+} to Fe^{3+} , and is followed by the regeneration of Fe^{2+} on the electrode surface. The mechanism also suggests that the substrate (4-MBC) reduction is favored if the ketone is a α,β -unsaturated group, as demonstrated with the 10 (ten) UV filters (Figure 5) investigated in the selectivity experiments (Figure 6). It was observed that higher

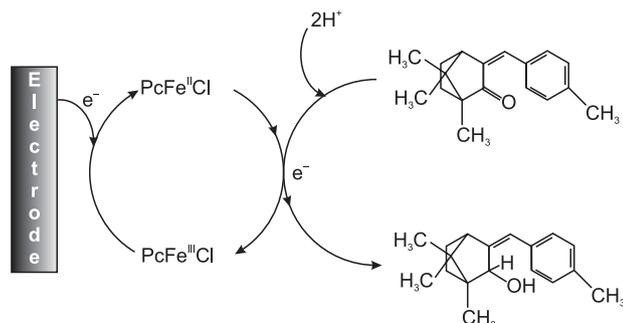


Figure 4. Mechanism proposed for the 4-MBC sensor.

sensitivities are obtained with α,β -unsaturated ketones, and as the unsaturation in the ketone group diminishes the sensitivity also decreases.

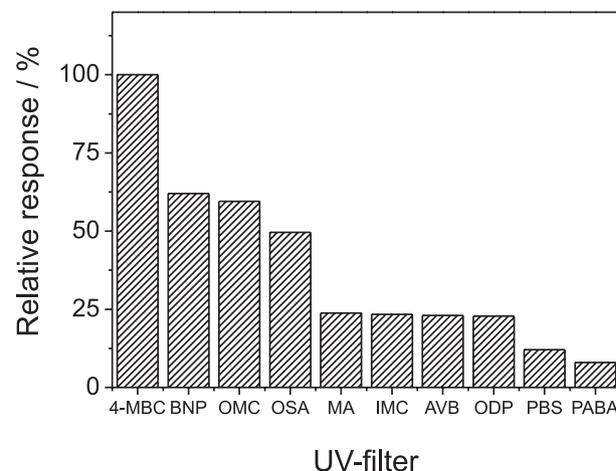


Figure 5. Relative response (%) obtained with the FePcCl-based sensor for ten different UV filters. The parameter was calculated considering the sensor response for 4-MBC as 100%.

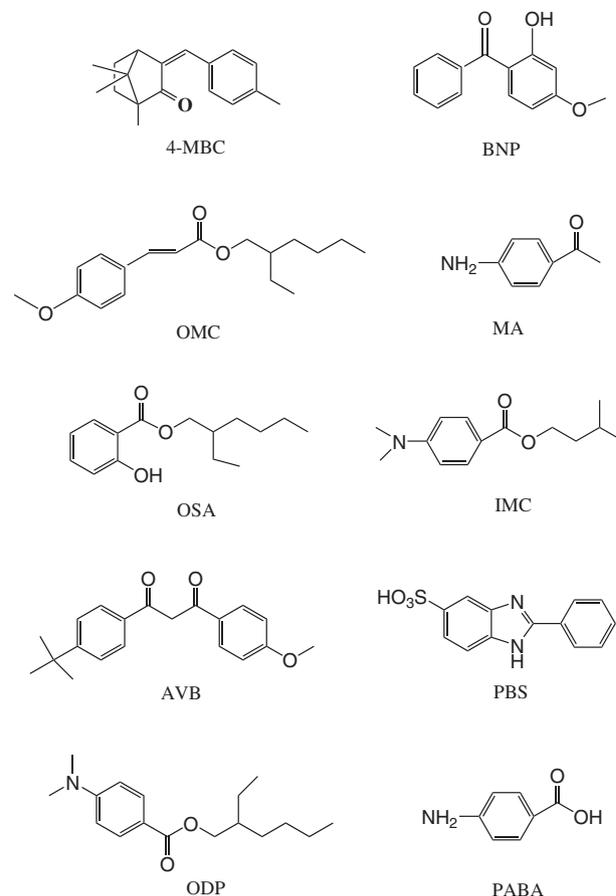


Figure 6. Chemical structures of ten UV filters evaluated in the selectivity studies. 4-MBC: 4-methylbenzylidene camphor; BNP: benzophenone-3; OMC: octyl-methoxycinnamate; OSA: octyl salicylate; MA: menthyl anthranilate; IMC: isoamyl *p*-methoxycinnamate; AVB: avobenzone; ODP: octyldimethyl-PABA; PBS: 2-phenyl-5-benzimidazolesulfonic acid and PABA: 4-aminobenzoic acid.

Typical chronoamperometric signals and the corresponding analytical curve for 4-MBC are shown in Figure 7. The applied potential of 0.0 V vs. Ag|AgCl was prior optimized in the potential range from -0.1 up to +0.1 V vs. Ag|AgCl at intervals of 0.025 V (data not shown). The higher sensitivity obtained at 0.0 V is in agreement with the voltammetric profile (Figure 2), in which the higher current variation was observed at 0.0 V vs. Ag|AgCl. It is important to emphasize that, for sensor construction, potentials close to this value are preferred in order to minimize possible interferences from other electrochemically active compounds.

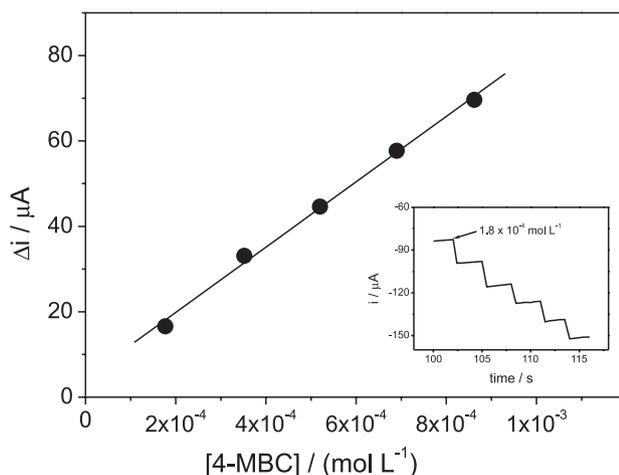


Figure 7. Typical analytical curve profile of the FePcCl sensor for 4-MBC under optimized conditions. Inset: chronoamperometric measurement for successive additions of the analyte.

The electrolyte solution consisted of a mixture of 0.1 mol L⁻¹ H₂SO₄ and tetrahydrofuran (thf) with a 70:30 optimized volume ratio. The experiments showed that, for 7.00 mL of this electrolyte, at least ten successive additions of 500 μL of the standard UV filter solution (solubilized in thf) could be carried out without phase separation. In addition, when other acid or buffer solutions were tested no satisfactory responses were obtained, indicating the important role of H⁺ ions in the sensor response, as suggested in the proposed mechanism (see Figure 4).

The influence of the FePcCl content in the carbon paste was also evaluated for the analytical sensor response and the results (Table 1) showed that 12.5% gives the highest sensitivity for 4-MBC detection.

After optimization of the parameters involved in the sensor preparation and chronoamperometric measurements, the analytical parameters for 4-MBC quantification were evaluated and are summarized in Table 2. The detection and quantification limits were calculated as suggested by the IUPAC recommendations.⁴³ The repeatability of the measurements was estimated by plotting seven successive

Table 1. Dependence of the sensor response on the amount of FePcCl in the carbon paste. Measurements carried out in 0.1 mol L⁻¹ H₂SO₄/thf solutions with 70:30 volume ratio. Applied potential: 0.0 V vs. Ag|AgCl

FeClPc amount (%)	Sensitivity (μA L mol ⁻¹)
5.0	3.5 × 10 ³
7.5	8.4 × 10 ³
10.0	6.3 × 10 ⁴
12.5	7.6 × 10 ⁴
15.0	2.6 × 10 ⁴

analytical curves in the corresponding linear range of the sensor. In this case, the repeatability was defined in terms of the relative standard deviation (RSD) from seven sensitivities. The reproducibility of the sensor was estimated in terms of the RSD for sensitivities calculated for five sensors prepared and used on different days by two experimentalists in different laboratories.

Table 2. Analytical parameters for 4-MBC determination on the proposed sensors based on carbon paste modified with FePcCl

Parameter	Response
Linear range (mol L ⁻¹)	1.8 to 9.0 × 10 ⁻⁴
Sensitivity (μA L mol ⁻¹)	76308 ± 2505
Correlation coefficient	0.9984
Detection limit (μmol L ⁻¹)	14
Quantification limit (mol L ⁻¹)	0.5 × 10 ⁻⁴
Response time (s)	0.5
Measurement repeatability (%) (RSD, n = 7)	1.9
Sensor reproducibility (%) (RSD, n = 5)	1.8

The proposed sensor was tested for UV filter quantification in three commercial sunscreen formulations (denoted as 1, 2 and 3) used by children in Brazil. The concentrations were determined by the sensor using the external calibration method and compared with those obtained with the chromatographic method (Table 3). One can see that the results are not significantly different at a confidence level of 95%, and it is possible to emphasize that both methods offer similar relative standard deviation values. Such data suggest an excellent sensor precision and the methodology as an efficient and rapid alternative for sunscreen analyses.

In order to verify if the sensor could be applied to aquatic environments, water samples from two private swimming pools (samples A and B) and five rivers close to Araraquara city (SP, Brazil) were analyzed after enrichment with 4-MBC. The good recovery results (Table 4), with values close to 100% for the swimming pool samples and lower values but still acceptable for the river samples,

Table 3. Determination of 4-MBC in sunscreen samples using the sensor based on FePcCl modified carbon paste; the chromatographic method was employed as reference

Sample	UV filter concentration in % (m/m)			Error / %
	Label value	Comparative method (HPLC)	Proposed sensor	
Sunscreen 1 ^a	6.0	7.8	8.1	3.8
Sunscreen 2 ^b	6.0	6.9	7.1	2.9
Sunscreen 3 ^c	8.0	8.5	8.9	4.7

Validities: ^aJune 2010, ^bDecember 2009, ^cFebruary 2009.

Table 4. Recovery of 4-MBC using the proposed sensor in water samples from swimming pools and rivers

Sample	4-MBC (mol L ⁻¹)		Recovery (%)
	Added	Found	
Swimming pool A	2.00 × 10 ⁻⁴	1.94 × 10 ⁻⁴	97
Swimming pool B	2.00 × 10 ⁻⁴	1.96 × 10 ⁻⁴	98
Jacaré Pepira river	2.00 × 10 ⁻⁴	1.86 × 10 ⁻⁴	93
Jacaré Guaçu river	2.00 × 10 ⁻⁴	1.80 × 10 ⁻⁴	90
Jau river	2.00 × 10 ⁻⁴	1.90 × 10 ⁻⁴	95
Tietê river in the limit with the Igarapu city	2.00 × 10 ⁻⁴	1.82 × 10 ⁻⁴	91
Tietê river under the bridge in Barra Bonita city	2.00 × 10 ⁻⁴	1.76 × 10 ⁻⁴	88

indicated that the sensor was successfully applied in the direct analyses of such water samples.

Conclusion

This work described an alternative methodology for the determination of the chemical UV filter 4-MBC on a sensitive and selective amperometric sensor based on a carbon paste modified with FePcCl. The sensor was satisfactorily applied in sunscreen and water samples from swimming pools and rivers and it opens up real possibility for UV filter quantification in other aquatic environments, such as sewage effluents and wastewater from treatment plants.

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