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Enhanced photoelectrochemical detection of an analyte triggered by its concentration by a singlet-oxygen generating fluoro photosensitizer

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ABSTRACT: The use of a photocatalyst (photosensitizer) which produces singlet oxygen, instead of enzymes for oxidizing analytes creates opportunities for cost-efficient and sensitive photoelectrochemical sensors. We report that perfluorooisopropyl-substituted zinc phthalocyanine (F6OyPcZn) interacts specifically with a complex phenolic compound, the antibiotic rifampicin (RIF), but not with hydroquinone (HQ) or another complex phenolic compound, the antibiotic doxycycline. The specificity is imparted by the preconcentration of RIF in the photocatalytic layer, as revealed by electrochemical and optical measurements, complemented by molecular modeling that confirms the important role of a hydrophobic cavity formed by the iso-perfluoroisopropyl groups of the photocatalyst. The preconcentration effect favorably enhances the RIF photoelectrochemical detection to nanomolar (ppb) concentrations, LOD = 7 nM (6 ppb) and the sensitivity to 2.8 A·M−1·cm−2. The selectivity to RIF, retained in the photosensitizer layer is further enhanced by the selective removal of all unretained phenols via a simple washing of the electrodes with pure buffer. The utility of the sensor for analyzing municipal wastewater was demonstrated. This, first demonstration of enhanced selectivity and sensitivity due to intrinsic interactions of a molecular photocatalyst (photosensitizer) with an analyte, without use of a biorecognition element may allow the design of related, robust, simple and viable sensors.

The use of enzymatic catalysis in electrochemical detection of phenols is well known.1–3 However, enzymes-based sensors have limitations due to thermal and chemical instability of enzymes, relative short shelf life of component biomolecules and limited reproducibility of enzymatic activity in using different biocatalyst batches.4,5

We have recently introduced a new, bioinspired detection strategy and have demonstrated it for the detection of phenols. Specifically, we have replaced a biological oxidation catalyst, Horse radish peroxidase (HRP) that uses H2O2 to oxidize phenols, with a heme-like photosensitizer that photo generates singlet-oxygen, 1O2, using air under visible light illumination.6 The photosensitizer, a zinc complex belonging to the class perfluoroalkyl-substituted perfluoro phthalocyanines,6 lacks C-H bonds and thus exhibits high thermal and chemical robustness, thereby overcoming the shortcomings of the enzymatic biosensors. The photodynamic effect, Type II mechanism of generating singlet-oxygen is efficient and leads to high detection sensitivity.6,8

While the photosensitizer catalyzes the oxidation of phenols by oxygen, thus, mimicking the reactivity pathway of a biocatalyst (e.g., peroxidase or phenol oxidase) in biosensors,1–3, its molecular mechanism is different, namely an initial [4+2] cycloaddition of 1O2 followed by the formation of the corresponding quinone.7 The quinone is then electrochemically reduced under mild conditions back to a phenol (e.g., hydroquinone (HQ) or its derivative) thus completing the electrocatalytic cycle similar to the action of peroxidases.3 Importantly, the photocatalytic response occurs only under illumination, allowing the detection and quantification of the baseline in the presence of an analyte by simply switching the light off.8

Although the new method does not have an intrinsic specificity (in contrast to biosensors), a limited selectivity can be reached based on the differences in the oxygenation kinetics by 1O2 for different phenols at different pH values.8,9 For example, the highest photocurrent responses were obtained for HQ and p-aminophenol at neutral pH, in agreement with their fast oxidation kinetics. Under the same conditions, signal intensities obtained from more complex compounds containing a phenolic moiety, such as antibiotics amoxicillin and cefadroxil, were three times lower.8,9 Other multifunctional phenolic compounds tend
to show even lower responses, likely due to steric hindrance and slow diffusion in solution and through the layer of the supported photocatalyst casted onto an electrode surface.

The enhanced selectivity and sensitivity of detection of phenolic analytes could be based on their interactions with the photosensitizer or its supporting matrix e.g., TiO2 or SiO2. The interaction of TiO2 surface with the vicinal phenols 1,2-dihydroxybenzene and 1,8-dihydroxynaphthalene has been reported. In our case, the photosensitizer F64PcZn exhibits a strong Lewis acidic, hydrophilic metal center and a highly hydrophobic ligand cavity, both opposite features being simultaneously imparted by the fluorine substituents: the higher the number of fluorine groups, especially aliphatic ones, the more hydrophobic the ligand is, but also the more electron withdrawing, thus enhancing the metal electron deficiency and, consequently, its affinity for functional groups bearing electronic lone pairs. Indeed, single-crystal X-ray structures of F64PcM complexes, M = Zn, Co, V=O, have revealed the presence of axial N and O atoms coordination, including the O of H2O. The hydrophilic Zn center may thus contribute to the binding of an analyte via coordination bonds, in addition to van der Waals interactions, the latter being observed for non-stERICally hindered phthalocyanine materials that adsorb polycyclic aromatic rings. The role of supported photosensitizers in photochemically-induced electroosensing is complex. The layer of the supported photosensitizer is not electrically conductive, the photocurrent response measured by the sensor being the result, inter alia, of a complex interplay of the adsorption/desorption kinetics and diffusion of the analyte. Despite this complexity, the interactions between an analyte and the supported photosensitizer could be a viable, rational approach for improving the detectability of complex phenolic compounds, for example the antibiotic rifampicin (RIF).

The rise of antibiotic resistance, including towards RIF has become a worrying problem for public healthcare, as resistant bacteria can be induced in the environment under selection pressures of antibiotics present in effluents and the environment. Thus, monitoring potentially contaminated effluents and wastewaters is essential to estimate levels of antibiotics discharged in the environment. In particular, RIF, introduced for human use in 1960s remains alongside isoniazid an essential first-line antitubercular drug worldwide.

We report here that molecular interactions between the supported F64PcZn photosensitizer deposited on an electrode and RIF result in the selective preconcentration of the latter at the electrode, greatly enhancing the sensitivity of the photoelectrochemical sensor towards RIF. The preconcentration is selective, as it was not observed for simple phenols such as hydroquinone (HQ) or catechol (CTH) and more complex OH-reach phenols such as antibiotics doxycycline (D XC) and oxytetracycline (OTC).

To the best of our knowledge this is the first demonstration of an enhanced selectivity and sensitivity of a 02-based photocatalytic sensor, which is ascribed to intrinsic interactions of the photocatalyst with an analyte, without the use of any biorecognition element.

Experimental

Materials. The perfluorinated phthalocyanine zinc complex F64PcZn was synthesized and deposited on TiO2 (Degussa, P25) as described previously result in the supported photosensitizer abbreviated F64PcZn/TiO2. HQ, CTH and resorcinol (RES) were purchased from Sigma-Aldrich (Belgium), RIF (>95% purity), DXC (98% purity), and OTC (>95% purity) were purchased from Across Organics. The measuring buffer (phosphate buffer pH 6) consisted of 0.1 M KCl and 20 mM KH2PO4 dissolved in ultrapure water (18.2 MΩ, Millipore Simplicity) and adjusted to pH 6. Measuring buffers with a different pH or ionic strength are specified in the text.

Instruments. The photoelectrochemical measurements were conducted using a PalmSens2 potentiostat from PalmSens BV (Utrecht, The Netherlands) and a red 655 nm diode laser (Roithner Lasertechnik) operating at 30 mW power and adjusted to a spot diameter of 4 mm. A power supply (Arduino Uno) was used to switch on and off the light beam at preprogrammed times. An AvaSpec-2048L UV-Vis spectrophotometer from Avantes (Netherlands) equipped with an Avalight-DH-S-BAL light source was used for spectrophotometric measurements.

Electrodes. Screen-printed electrodes (SPE, DRP-110, DropSens, Spain) with carbon working area of 4 mm in diameter were typically, unless stated otherwise, modified by depositing a single 5 µl droplet of an aqueous suspension of 10 mg/ml F64PcZn/TiO2 (3% wt) followed by overnight drying. The modified electrodes are abbreviated F64PcZn/TiO2/SPE. Both the volume and concentration of F64PcZn/TiO2 suspensions were varied in order to optimize the amount of deposited materials.

Prior to photoelectrochemical measurements, 80 µl of RIF solution was placed on F64PcZn/TiO2/SPE and the electrodes where kept in the dark in a closed vessel. Chronoamperometric measurements were carried out using either a drop of the same RIF solution or pure buffer (after additional rinsing with pure buffer). For experiments involving sequential illuminations lasting a total of 4 hours the drop was refreshed after each illumination. For the hydrodynamic setup, four F64PcZn/TiO2/SPE were immersed in 16 ml of RIF solution and stirred magnetically at 300 rpm for 2 hours. Following the bulk equilibration, the electrodes were removed from the solution, connected to a potentiostat and covered by a single drop of the same RIF solution.

UV-Vis quantification of RIF adsorbed on solid TiO2 and F64PcZn/TiO2. 1, 2, 3 or 4 mg of solid TiO2 or F64PcZn/TiO2 were weighed in Eppendorf tubes, washed 3x with phosphate buffer pH 6 using a vortex and an ultrasonic bath for resuspension (for 10 min) and then centrifuged at 7000 rpm (for 5 min) to isolate the powders. Finally, the powders were resuspended in 1ml of 10 µM RIF solution in phosphate buffer pH 6 and left in dark for 2 hours on a mechanical agitator. Afterwards, the suspensions were centrifuged at 14000 rpm for 30 min to remove all solid particles and UV-Vis spectra of the supernatants were recorded using a 0.5 cm quartz cell to assess the change in the RIF concentration from the initial 10 µM.
Molecular modeling of RIF-F₄PcZn interactions. The docking of RIF onto F₄PcZn were performed using the GOLD v. 2020.1 program²⁰, RIF was designated as the “ligand” while F₄PcZn were the “protein”. RIF conformers were initially generated via Mercury CSD Conformer Generator v. 2020.²¹ to minimize conformational biases during docking calculations. A coordination binding site with a 10 Å radial distances from the Zn center was selected given the likely propensity of RIF oxygen-based functional groups for binding to the electron deficient metal. Docked RIF-PcZn structures were visualized with Hermes v. 2020.¹² A fine grid of fitting points, keeping the vdW interactions below -2.5 kcal/mol was incorporated to illustrate enthalpic interaction energies below -2.5 kcal/mol. Additional dockings were performed using Zn-O bond distance constraints of approximately 2.1, 2.3, and 2.5 Å consistent with statistical data on zinc-oxygen interactions, Figure S2. Rough RIF-F₄PcZn enthalpic interaction energies were obtained using MOPAC2016 with the PM7 Hamiltonian. Geometry minimizations were performed until potential energy gradients were <10 kcal/mol/Å for RIF complexes and <1 kcal/mol/Å for F₄PcZn. A dielectric constant, ε = 78.4 was introduced to mimic the aqueous environment in phosphate buffer saline.

Municipal wastewater analysis. Samples of effluents obtained from the Cluj-Napoca Wastewater Treatment Plant (Cluj, Romania) were spiked with a solution of RIF in phosphate buffer pH 6 and passed through a 0.45 μm pore size membrane filter (Chromafil AO-45/25, Macherey-Nagel, Germany) to give a final RIF concentration of 1 µM. Samples were diluted 10 times with the measuring buffer before being analysed.

Results and discussion

Photoelectrochemical detection of RIF. The molecular structures of studied compounds exhibiting at least one phenol group are shown in Figure 1. RIF, a zwitterion at physiological pH contains three phenolic hydroxyls grafted on its naphthalene core structure. Two of them, at positions 1 and 4 form a hydroquinone-like moiety. The third one, position 8, is deprotonated while a piperazine N is protonated (pKₐ = 3, pKₖ = 7.5).²²²³ RIF is slowly oxidized by oxygen in aqueous solutions to form rifampicin quinone RIF-Q (within hours at pH 7.5 and 37°C).²⁴ The ¹O₂-based photoelectrochemical detection of phenols, including RIF is shown in Figure 2A. The detection of RIF (or HQ) relies on two main processes: (1) the oxidation of RIF by ¹O₂ that is generated by F₄PcZn under illumination from air oxygen, and (2) the electrochemical reduction at an electrode of a formed product or products. These two processes create the redox cycling that enhances sensitivity of the detection method.⁶ The method has been proven to work for antibiotics containing a phenolic moiety such as amoxicillin, cefadroxil and doxycycline.⁸⁻⁹ RIF contains a hydroquinone (HQ) moiety that can be reversibly oxidized into the corresponding quinone RIF ↔ RIF-Q, Figure 2A. Thus, RIF is expected to be well-detectable via oxidation with ¹O₂. The zwitterionic and oxidized forms of RIF, however, are large molecules with multiple functionalities and charges that may interact with F₄PcZn/TiO₂ and the electrode material (carbon ink) complicating the detection. Indeed, the profile of the amperometric response drastically differs for RIF in comparison with HQ, Figure 2B. In the first few seconds after the light triggered the response, the photocurrent was similar for both HQ and RIF, but decayed for RIF while remaining constant for HQ. The steady-state photocurrent for RIF was reached in about 10 min resulting in a four times lower value compared to the steady-state current for HQ.

Figure 1. Molecular structures of studied compounds with at least one phenol group and the FnPcZn phthalocyanines, n = 1, 6, 16. The F atoms are depicted as vdW spheres. The Zn is represented as a small sphere; the remaining atoms are depicted as capped sticks. Color code: Zn, orange; F, green; C, gray; N, blue. The arrows point toward the –OH and =O groups found via dockings to be closest to the Zn centers of F₄PcZn and F₆PcZn, respectively. See also Figures S3 and S4.

Figure 2. (A) Schematic representation of the mechanism for the photocurrent response of RIF using the F₄PcZn/TiO₂/SPE electrode. (B) A typical photoelectrochemical response at −0.1 V potential of 10 µM HQ (blue) and 10 µM RIF (red) in phosphate buffer pH 6.
The shape of the current response as a function of time suggests that RIF and RIF-Q may exhibit poor diffusion in the \( \text{F}_{64}\text{PcZn/\text{TiO}_2} \) layer, leading to the corresponding decay of the photocurrent measured by \( \text{F}_{64}\text{PcZn/\text{TiO}_2}/\text{SPE} \). The slow diffusion may result in slow equilibration of the electrode in the RIF solution, a hypothesis that was examined by using longer equilibration times combined with (1) multiple short illuminations, or (2) a single illumination at the end of the long equilibration time, Figure 3. Considering the significant decay of the signal, Figure 2B, the illumination time was shortened to 10 s.

**Figure 3.** Photoelectrochemical measurements of 10 µM RIF (A, C, E) and 10 µM HQ (B, D, F) in phosphate buffer pH 6 using a series of cycles of dark (290 s) and light (10 s) periods (A, B); a long preconditioning in dark for 70 min followed by a short illumination for 10 s at a continuously polarized electrode (C, D); and a long equilibration step (65 min) of a disconnected electrode followed by a measurement with a short illumination time of 10 s (E and F). Potential applied, −0.1 V. Coating, 4 µg/mm². Sequential illumination/dark cycles, 10/290s, Figure 3A revealed that the photocurrent for RIF increased 1.8 times during first 10 – 12 illumination/dark periods and stabilized afterwards, i.e. after about 1 hour. In contrast, the HQ photocurrent showed a small decrease by 17% using the same illumination protocol during the first 5 – 6 periods, Figure 3B, reaching after about 30 minutes a stable value identical to the response of RIF.

Surprisingly, an extended equilibration step without illumination, about 70 min, similar to the time of the first 12 dark/illumination periods (60 min) resulted in a further enhancement of the photocurrent for RIF, Figure 3C, compared with both the response after 5 and 60 min, Figure 3A. Under prolonged equilibration, the photocurrent response for RIF, Figure 3C, was even higher than that for HQ, Figure 3D. Moreover, the equilibration step was more efficient for RIF even without the potential applied, compare Figure 3C and 3E. To conclude, the photocurrent response for RIF (−1.2 µA) was 2x the response of HQ (−0.62 µA) obtained after equilibration of disconnected electrodes in RIF or HQ solutions, respectively, while the response of RIF after the equilibration was 5x the response of RIF without the equilibration step (the first illumination in Figure 3A).

**Figure 4.** (A) The photoelectrochemical response to 10 µM RIF (red) and 10 µM HQ (blue) as a function of incubation time. (B) Effect of \( \text{F}_{64}\text{PcZn/\text{TiO}_2} \) coverage (layer thickness) on the photoelectrochemical response after 2 h incubation in 10 and 0.5 µM RIF. Each data point was measured using three separate electrodes incubated individually in a drop of RIF or HQ solution for a given time. The error bars equal the standard deviations.

Taken together, RIF can be preconcentrated at the surface of the modified electrode during the equilibration period, resulting in an enhanced sensitivity to RIF in solution compared with HQ. Given the above, a protocol that includes the preconcentration step of RIF without electrode polarization was employed. This protocol insured that the highest response was obtained and allowed the simultaneous equilibration of several electrodes.

**Kinetics of RIF preconcentration on \( \text{F}_{64}\text{PcZn/\text{TiO}_2} \text{SPE} \).** The diffusion of RIF is slow and thus the saturation of the interfacial \( \text{F}_{64}\text{PcZn/\text{TiO}_2} \) layer may require hours. Hence, the maximum current response depends on the initial concentration of RIF in solution and the thickness of the \( \text{F}_{64}\text{PcZn/\text{TiO}_2} \) layer on the electrode. The general picture of RIF accumulation kinetics was obtained using a batch of electrodes incubated separately for a given time in RIF solution. For the first 120 min a near constant photocurrent was measured for HQ, whereas for RIF a steep increase in the photocurrent, that reached −1200 nA, was observed, Figure 4A. Decreases, up to 30% were observed after additional 120 min, attributed to the worsening of adhesion of the \( \text{F}_{64}\text{PcZn/\text{TiO}_2} \) coating with time, and, thus, its mechanical instability during electrode manipulations.
The effect of F$_{64}$PcZn$_2$/TiO$_2$ coating amount was examined next. A set of electrodes with coatings ranging from 0.08 to 8 µg/mm$^2$ pre-equilibrated in RIF solution for 120 min were tested, Figure 4B. For 10 µM RIF concentration, the current increases roughly proportionally up to 2 µg/mm$^2$ when the entire electrode seems to be coated, see Figure S1A. Then the current intensity drops with increase loading reaching 50% of its maximum value at 8 µg/mm$^2$. This can be explained by accumulation of RIF in the upper part of the layer which slows equilibration of F$_{64}$PcZn$_2$/TiO$_2$ in the part of the layer close to the electrode. This additionally confirmed by decrease in gradient of the time-dependent signal with the amount of F$_{64}$PcZn$_2$/TiO$_2$, Figure S1B.

Interestingly, the effect of the layer thickness was much more pronounced for 0.5 µM RIF with the maximum photocurrent at 1 µg/mm$^2$ (in contrast to 2–4 µg/mm$^2$ for 10 µM RIF) followed by 5-fold drop in the response then the thickness of the layer increased 8 times (from 1 to 8 µg/mm$^2$), Figure 4B. Thus, thinner coatings seemly improve the detection of RIF in the sub-micromolar concentration range by facilitating the saturation of the photosensitizer at close proximity to the electrode but may limit the sensitivity at higher concentrations due to saturation of the F$_{64}$PcZn$_2$/TiO$_2$ layer.

**RIF adsorption by F$_{64}$PcZn$_2$/TiO$_2$ and F$_{64}$PcZn$_2$/TiO$_2$|SPE**

The adsorption of RIF was probed by using electrodes pre-equilibrated in a solution of RIF, washed in pure buffer and then used for detection of the photocurrent. Figure 5 shows the responses of RIF and, for comparison, HQ, with and without washing with pure buffer. Following a single washing the initial signal, Figure 5A, trace (a) loses about 60% of its intensity, Figure 5A, trace (b) but this value remains constant at least after the 3rd washing, Figure 5A, trace (c). In contrast to RIF, the photocurrent response for HQ dropped to the level of blanks after washing with pure buffer as shown in Figure 5A, traces (d)-(g). These data indicate that even after rigorous washing RIF remained at the electrode, whereas HQ was easily and completely removed, thus suggesting that preconcentration imparts detection selectivity, simple phenols being prevented from interfering by washing. Further proof of RIF adsorption was obtained by measuring directly its concentration decrease in solution in the presence of F$_{64}$PcZn$_2$/TiO$_2$, Figure 5B. Following incubation of a 1 ml 10 µM RIF solution with 1 mg/ml suspension F$_{64}$PcZn$_2$/TiO$_2$, a composition roughly corresponding to conditions of amperometric analysis with F$_{64}$PcZn$_2$/TiO$_2$|SPE (50 µl drop of 10 µM RIF, 4 µg/mm$^2$ F$_{64}$PcZn$_2$/TiO$_2$ or 50 µg per electrode), a decrease in UV-Vis absorbance at 474 nm after 2 h incubation corresponding to loss of 27% of RIF was observed. Increasing the amount of F$_{64}$PcZn$_2$/TiO$_2$ fourfold resulted in the near complete removal of RIF from the solution. In average, 3.0 ± 0.6 mmol RIF are adsorbed on 1 mg F$_{64}$PcZn$_2$/TiO$_2$, which is comparatively large. A layer of 4 µg/mm$^2$ can create deficiency of RIF over 1 mm distance from F$_{64}$PcZn$_2$/TiO$_2$|SPE already for 10 µM RIF un unstirred solution or even larger distances for more diluted RIF solutions, which correlates well with slow kinetics of RIF preconcentration.

Since TiO$_2$ alone does not absorb RIF, Figure 5C, the preconcentration of RIF by F$_{64}$PcZn$_2$/TiO$_2$|SPE is ascribed to the supported fluorinated photosensitizer, F$_{64}$PcZn. Theoretical modeling provides insights into this binding.

**Molecular modeling of RIF-F$_{64}$PcZn interactions.** RIF, Figure 1, exhibits aromatic rings and N- and O-based functional groups that could contact the phthalocyanine scaffold via stacking, vdW or metal-coordination interactions. The absence of adsorption effects for HQ and 4-aminophenol$^8$ suggest that the interactions of amino- or phenol groups alone cannot explain the observed RIF-F$_{64}$PcZn interactions. Notably, HQ and 4-aminophenol are relatively small and thus the coordination of their O and N groups to the Zn centers of phthalocyanines, Figure 1, is not sterically hindered. Aromatic stacking interactions are also not precluded for F$_{16}$PcZn, but they are for F$_{64}$PcZn by the iso-perfluoro alkyl groups (iso-R$_{f}$). On the other hand, the bulky, peripheral iso-R$_{f}$ groups form a hydrophobic cavity around the metal raising the possibility of vdW interactions, including with RIF. Insights into the role of iso-R$_{f}$ groups, the structural chemistry of F$_{64}$PcZn was compared with that of F$_{16}$PcZn, an electronically similar complex, but with all iso perfluoro alkyl groups replaced by aromatic F groups, Figure 1. The hydrophobic cavity is thus eliminated, rendering F$_{64}$PcZn sterically unhindered. A histogram of the Zn-O bond length in F$_{64}$PcZn-O complexes, Figure S2, with the oxygen belonging to water, ether, ketone, alcohol, ester, amide, sulfoxide, phosphine oxide and nitrito functional groups revealed a quasi-Gaussian distribution. The Zn-O distances vary between 1.949 and 2.178 Å, with a mean of 2.100(9) Å for the crystal structures with R < 15% reported in the Cambridge Structural Data Bank.

**Figure 5.** Photoelectrochemical responses (A) of 10 µM RIF (a-c), 10 µM HQ (d-f) and blank buffer (g) obtained after 2 h incubation in the respective solution followed by either testing using the same solution (a, d) or washing and testing in blank buffer (b, e, f). Other conditions are the same as those in Figure 3. The UV-Vis spectra of the RIF solution after incubation with different amounts of F$_{64}$PcZn$_2$/TiO$_2$ (B) and TiO$_2$ (C).
The modeling indicates that the neutral and zwitterionic complexes reveal that the antibiotic is in its neutral form. Nevertheless, based only on Zn--O coordinative interactions RIF cannot be expected to have a much higher affinity for molecular mechanics based on its vdW interactions with the iso-R1 groups, as also illustrated by the 2x more vdW fit points (radial coverage) for F66PcZn-RIF relative to F16PcZn-RIF, Figure S4.

We have established previously for several phenols, that higher pH values increase significantly the photocurrent response due to an increase in the oxidation rate of deprotonated phenols by \( \cdot O_2^- \). However, RIF is chemically unstable at pH above 8 and below 4. Thus, we studied the photocurrent response in this pH range using phosphate, citrate and Tris buffers. As shown in Figure 7B, Tris slightly improves the detection at pH 7 and 8 but the currents were not higher than those observed for phosphate at pH 6. The photocurrent was optimal in phosphate buffer pH 6, which is close to the optimal pH for extraction of RIF into a dimethylpolysiloxane layer used in HPLC assisted by stir bar-sorptive extraction. A decrease in the photocurrent at pH 7 and 8 drastically differs from our previous work with other phenolic analytes where the photocurrent increased at higher pH. This difference is ascribed to better preconcentration and mobility of RIF at pH 6 in the F66PcZn/TiO2 layer.

Figure 7C depicts the effect of F66PcZn wt% loading on TiO2 on the photocurrent response. The photocurrent increased linearly with the loading up to 2wt%, a value consistent with the 3wt% one needed for monolayer coverage on TiO2 P25. Additional F66PcZn layers on top of the layer in direct contact with the support may act as barriers, surface morphology modifier as well as optical light absorbers thus limiting the efficiency of the photooxidation reaction.
Analytical performance. Taken together, the optimization studies led to the selection of 1 and 4 µg/mm² coatings for the construction of calibration curves. Figure 8A shows that the photocurrent response depended linearly on the concentration of RIF in range 0.05 - 2.5 µM and 0.1 - 10 µM for 1 and 4 µg/mm² F64PcZn/TiO₂, respectively. Both ranges span about 2 orders of magnitude, but the one for the thicker coating was larger, congruent with the slow diffusion and accumulation of RIF. Conversely, the sensitivity in the low concentration range was more than three times higher for the thin layer, the slope being 348 nA·µM⁻¹ (2.77 A·M⁻¹·cm⁻²) and 95.3 nA·µM⁻¹ (0.76 A·M⁻¹·cm⁻²) for the 1 and 4 µg/mm² coverages, respectively.

The corresponding limit of detection (LOD), equals 3 standard deviation of blanks divided by the slope of the calibration curve, LOD = 3·SDblank/slope, are estimated to be 7 and 28 nM for 1 and 4 µg/mm² F64PcZn/TiO₂ coatings, respectively. These values, however, are difficult to reach in practice due to the slow preconcentration diffusion of RIF in dilute solutions. The accumulations can be accelerated by stirring, leading to a lowering of the LOD from 28 to 10 nM, as illustrated in Figure S6.

Importantly, the reported analysis method is more sensitive than the spectrophotometric assay (LOD = 0.7 µM)³⁰ as well as chromatographic³¹,³² or electrophoretic³³ methods coupled with a UV-detector (Table S1).

The reported method is also comparatively simple to setup and use. Indeed, enhanced sensitivity techniques have been used for the electrochemical detection of antituberculous drugs, mainly by adsorptive stripping voltammetry and various electrode modifications,²⁶,²⁷ including with metallic lead, graphene oxide, gold and silver nanoparticles, carbon dots, metal oxides, molecularly imprinted polypyrrole and cyclodextrines and various combinations thereof. Most of these materials increase the electrode surface area, preconcentrate the analyte and facilitate its oxidation resulting in LOD values from low nanomolar to micromolar range. In contrast, the reported electrode modification requires neither a complex architecture nor modification procedures and can be easily scaled up for mass production while exhibiting one of the lowest LOD demonstrated.

Finally, the practical applicability and validity of the proposed method was tested on a wastewater treatment effluent spiked with RIF. The standard addition method, Figure 8B, yields a 344 nA·µM⁻¹ slope identical to that obtained from the pure buffer, thus suggesting no interference of the effluent matrix. The 99 ± 6 % (RSD = 6.2%, n = 3) recovery value indicates the adequate accuracy and repeatability of the proposed method for real sample analysis.

Additional improvement in selectivity toward phenols that adsorbs in the layer of the photosensitizer can be envisaged due to the retention of the analyte in the layer under washing with pure buffer. Table 1 lists relative response of RIF in comparison to three simplest dihydroxybenzenes (HQ, CTH, RES) and DXC and OTC, two antibiotics bearing several functional groups, including a phenolic moiety. RIF exhibited the highest photocurrent, followed by HQ, CTH and DXC.

**Table 1.** Photocurrent responses of phenols after incubation of electrodes for 2 h in their 10 µM solutions. Average ± SD of three measurements.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Measured in tested solutions</th>
<th>Washed and measured in pure buffer</th>
</tr>
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<tbody>
<tr>
<td>RIF</td>
<td>-1.210 ± 0.010 (100%)</td>
<td>-0.480 ± 0.020 (100%)</td>
</tr>
<tr>
<td>HQ</td>
<td>-0.454 ± 0.003 (38%)</td>
<td>-0.016 ± 0.002 (3%)</td>
</tr>
<tr>
<td>CTH</td>
<td>-0.280 ± 0.050 (23%)</td>
<td>-0.059 ± 0.017 (12%)</td>
</tr>
<tr>
<td>RES</td>
<td>-0.004 ± 0.007 (0%)</td>
<td>-0.004 ± 0.002 (1%)</td>
</tr>
<tr>
<td>DXC</td>
<td>-0.100 ± 0.005 (8%)</td>
<td>-0.017 ± 0.004 (4%)</td>
</tr>
<tr>
<td>OTC</td>
<td>-0.030 ± 0.001 (2%)</td>
<td>-0.003 ± 0.002 (1%)</td>
</tr>
</tbody>
</table>
Remarkably, the washing step widened the gap between the photocurrent responses of RIF and the other compounds. Except for RIF, only CTH was noticeably retained at the electrode after the washings, although showing a 5x loss of signal. In general, the interference of dihydroxybenzenes and both tetracyclines was diminished after the washing step.

Conclusions

We demonstrate for the first time that a functionalized molecular photocatalyst (photosensitizer type II) exhibits analyte selectivity in interacting with a complex phenolic compound, viz. the antibiotic RIF, while showing no significant interactions with HQ or another complex phenolic compound, the antibiotic DXC. Experimental and modeling studies suggest the appearance of a preconcentration effect in a comparatively thick electrically nonconductive layer, which results in a favorable LOD, 7 nM (6 ppb). The preconcentration of RIF, a phenolic analyte in a thick, electrically nonconductive layer at the electrode surface is effective despite of its strong adsorption, resulting in a complex interplay among the diffusion, adsorption/desorption kinetics and photocatalytic oxidation/electrochemical reduction. Thus, functionalizing a robust photocatalyst and a supporting material with the aim of preconcentration of an analyte is an attractive strategy to fabricate cost-effective and ultrasensitive photoelectrochemical sensors.

ASSOCIATED CONTENT

Supporting Information. All materials, reagents, instruments, experimental methods used in this study and more information are described in detail in the Supporting Information.

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