



Proceedings Enzymatic Sensor Based on Dye Sensitized TiO₂ Electrode for Detection of Catechol in Water ⁺

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1. Summary

The aim of our work is to show a proof of principle of a new sensor concept. The sensing material consists of TiO₂ nanoparticles combined with selective enzymes and dyes, which are able to detect phenolic molecules in water. A light induced excitation is employed to induce electron transfer from dye/enzyme to TiO₂ layer. The generated electrical signal amplitude is related to the analyte concentration in water [1]. With this newly designed sensor in combination to well-chosen nanomaterial, we are able to detect a low concentration of phenolic molecules in drinking water, within the accepted maximum concentration defined by the European Commission. We study the sensing of catechol as a prominent molecule of the phenolic chemical compounds.

2. Materials and Methods

2.1. Cell Construction

The used setup in this work is a modification of a photochemical/electrochemical cell [2], consisting of an illuminated working electrode, a counter electrode and an aqueous electrolyte, containing the analyte. Figure 1 shows the schematic representation of the sensor cell. It consists of working and counter electrodes. The signal is measured as a voltage drop across a working load, which is related to the reaction rate. The working electrode is composed of a glass substrate, coated with a layer of fluorine doped tin oxide (FTO) and a porous, sintered TiO₂ layer. Depending on the type of experiment to be conducted, additional dyes and enzymes are bound to the nanoporous TiO₂ layer.



Figure 1. (a) Basic sensing element with modified nanoporous-TiO₂-layer. (b) Sintered TiO₂-nanopartikles on top of crystalline FTO (at imperfectly covered location).

2.2. Nanoporous-TiO2 Film Sintering

Titania with a high surface area was synthesized by a sintering process. A 2 μ m layer of TiO₂ nanoparticle was spun coated onto FTO/glass substrate. The spin coating solution was prepared as follow: 720 μ L (750 mg, 16.7 mmol) acetic acid were mixed with 2.5 mL ethanol and 3 g TiO₂ paste (MANSOLAR) to obtain a 5 M solution containing about 20 wt% nanoparticles of 20 nm diameter. To ensure a homogeneous distribution of TiO₂ nanoparticles in an even layer, the solution was sonicated for 20 min before spin coating process. A 400 μ L of the prepared solution was applied statically onto the substrate surface of the electrode and dispensed at 1000 rpm. Preliminary solvent evaporation was achieved at 100 °C for 2.5 min using a hot plate. For thermal sintering of the TiO₂ nanoparticles, a first annealing process of the electrodes were done at 400 °C under nitrogen for 1 h with a heating ramp of 4 °C min⁻¹ (Yield Engineering Systems, Inc., Livermore, CA, USA). So far the electrodes were handled under clean-room conditions. After cooling down, the electrodes were again annealed at 500 °C under atmosphere for 1 h and a ramp of 4 °C min⁻¹.

The illumination setup uses light sources from selected spectral ranges to excite also solely the dye or enzyme molecule. Even though a laser with monochromatic light would be more convenient to study excitation effects at a single wavelength, LED's open up new opportunities in the general illumination market and have the advantage of being inexpensive for the development of a sensor system. The used LEDs were provided by ledxon and integrated to fit in the electrochemical setup. In this work four different high intensity LED's were used, to generate light in the red, green, blue and UV-A spectral region. It was important to set the output optical power equally for all four LED's. Therefore, an optical power measurement device (Texas Instrument, Texas, USA) was employed to set the electrode illumination power to 50 W/m². Figure 2 shows the emission spectral ranges of the used LED's (355.8 nm, 417.2 nm, 466.3 nm, 522.6 nm, 625.8 nm) and the corresponding absorption characteristic of TiO₂-nanoporous layer. It is obvious, that excitation with 355 nm generates electron/hole pairs within the bandgap of TiO₂.



Figure 2. (**a**) TiO₂ absorption (grey area) and spectral ranges of LED light emission. (**b**) Measure signal with TiO₂ in water at different excitation wavelengths (100 kOhms load resistor).

2.3. Sensitation with Dye Iron Phthalocyanine (FePc), Nafion and Enzyme Laccase from Trametes Versicolor

Several dyes have been selected due to their absorption in the visible spectral range, suitable for the LED characteristics. A serial of experiments showed no significate correlation between the catechol concentration and the measured signal by the cell. However, Iron Phthalocyanine (FePc) dye (Figure 3a) fulfilled all the criteria. It has a relatively strong absorption in the red spectral range. Compared to other investigated dyes, FePc enabled to measure a signal dependent the catechol concentration. This means that the FePc dye fits well to the internal energy levels of the laccase (Figure 3c) and transfers the electrons from the laccase catechol reaction to the conductive band of TiO₂. The Nafion polymer (Figure 3b) acts as an ion conductor and provides a biocompatible interface to the water. It shows a hydrophobic characteristic and therefore improves the selectivity of electrochemical biosensors due to the electrostatic repulsion of unwanted species. In our study, we coated the TiO₂ layer by nafion molecules to avoid a direct contact between the catechol and the TiO₂ nanoparticles. Laccase from Trametes Versicolor [3] offers spatially separated reaction centers. It oxidizes catechol at one end and transfers the gained electrons to the other end of the molecule. Thus, the oxygen reduction in of water molecules take place. Only when the enzyme molecule contacts the TiO₂-surface with the reducing reaction center, the sensing mechanism works properly. In general, this enzyme possesses a functional group specificity and a stereochemical specificity, so it reacts with phenols in general but also distinguishes between their molecule sizes.



Figure 3. (a) FePc-dye molecule, (b) Nafion molecule, (c) Laccase molecule from Trametes Versicolor and reaction scheme with catechol [4].

3. Results

Measurements of the presence of catechol molecule in water were done using unmodified TiO₂electrodes (Figure 4a), FePc/Nafion-primed/TiO₂ (Figure 4b) as well as with additional enzyme adsorption. For all the presented measurements, the working electrode excitation was done with blue LED-light emission (466.3 nm).



Figure 4. Measured electrical signal versus time for different catechol concentrations; under blue LED-light excitation (**a**) with untreated TiO₂-electrode (**b**) with FePc-Nafion sensitized TiO₂-nanoparticles.

Unmodified electrodes offer the highest signal change by increasing catechol concentration. Unfortunately, there is a signal saturation for catechol concentration above 10 mmol/L. The saturation is avoided by coating the TiO₂ nanoporous layer with FePc/Nafion-priming, even if the monitored signal change is smaller. The smallest signal spreading is achieved with the combination of the three compounds FePc-Laccase-Nafion. In Figure 5b, the concentration of catechol in water was varied from 0.01. mmol/L to 50 mmol/L. The recorded electrical current is plotted against catechol concentration in water. The values of the deionized water measurement are placed at a concentration of 0.001 mmol/L. The data points are connected with an unmeaningful fit, as a guide to the eye. For

the bare TiO₂-electrode the concentration value at 50 mmol/L was not included in this tentative fit because the signal is getting saturated.



Figure 5. (a) Electrical signal versus time using FePc-Nafion-Laccase treated TiO₂-electrode for different catechol concentrations; blue LED-light excitation (b) calibration curves from all three types of coatings dependent on Catechol concentration (mean value of the reached signal value after 200 s for each Catechol concentration).

4. Discussion

Catechol shows a ligand-to-metal charge transfer mechanism, which make possible a correlation between the catechol concentration and the measured signal under light excitation of the unmodified TiO₂-electrode. Independent of the use of laccase, catechol molecules are oxidized and electrons are transferred to the conduction band of TiO₂ (Figure 6). All measured signal values in the presence of catechol are above the signal value of pure deionized water. Due to the limited locations of this complex formation, a saturation of the signal is observed above 10 mmol/L. To avoid this complex formation, TiO₂ was successfully protected with nafion polymers. As a result, only catechol could couple to FePc-dyes and/or the enzyme molecules. The available site in dye and enzyme for catechol is larger than the existing sites in the nanoporous TiO₂ layer. Therefore, a signal saturation for catechol concentration above 10 mmol/L is overcome. The reason is that FePc, with and without enzyme, fits perfectly to the internal energy levels of either the laccase and/or the catechol. In both cases, FePc transfers the resulting electrons from the reaction of catechol to the conductive band of TiO₂. Although the combination FePc-Laccase-Nafion delivers the smallest signal change, the advantage should be the selectivity between different phenols. In the future work, we will deal with the selectivity problem.



Figure 6. Scheme of energy levels for the combination of TiO₂ and FePc (**left**) as well as with added laccase (**right**). Catechol molecules are oxidized and electrons are transferred to the conduction band of TiO₂. The depicted energies values are relative to the vacuum level. FePc is aligned with its mean value of Homo and Lumo energy levels versus the TiO₂-fermi energy.

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Conflicts of Interest: The authors declare no conflict of interest.

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