

Proceedings

# Sensing of Copper(II) by Immobilized Ligands: Comparison of Electrochemical and Surface Plasmon Resonance Transduction <sup>†</sup>

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**Abstract:** Two sensors for the detection of the metal ion Cu(II), based on the same sensing layer, are compared. They rely on different transduction methods, i.e., electrochemistry and surface plasmon resonance (SPR). D,L-penicillamine was used as the specific receptor since it is a strong ligand for copper(II). Moreover it is easily immobilized on a gold layer by a self assembling procedure by contacting the gold layer overnight with water/EtOH (80/20) containing D,L-penicillamine, taking advantage of the spontaneous interaction of thiols with gold surfaces. Both the electrochemical and SPR platforms were derivatized in the same way. In the first case a gold disc was used as working electrode, and in the second one a thin gold layer (60 nm thick) was deposited by sputtering over the exposed core of a plastic optical fiber (POF), after removing the cladding along half circumference.

**Keywords:** Electrochemical sensors; surface plasmon resonance sensors; copper(II); L,D-penicillamine; plastic optical fibers (POFs)

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## 1. Introduction

In the present investigation, the possibility of using the same receptor for copper(II), D,L-penicillamine, in connection with different transduction methods for sensing purposes is examined. In particular, sensors with electrochemical and SPR transduction are considered. In both these devices the D,L-penicillamine can be easily deposited over the transducer (gold surface chip) by formation of a self assembled monolayer (SAM). Chemical sensors are based on the strict integration of a receptor with an instrument able to generate a signal upon the combination of the receptor with the substrate. Both the characteristics of the binding reaction, in particular the affinity constant, and that of the transducer, for example the sensitivity, are of overwhelming relevance for determining the performance of a sensor. In this work we compare two different transduction methods, combined with the same receptor, D,L-penicillamine, for the detection of the metal ion Cu(II).

## 2. Sensing Platforms

Electrochemical sensors are chemically modified electrodes (CME), at the surface of which some species capable of specifically interacting with the desired analyte are fixed. A signal (a potential, a current or an impedance variation) is generated by the transducer upon this combination, which can be used to determine the concentration of the analyte, or the characteristics of the combination reaction [1–3].

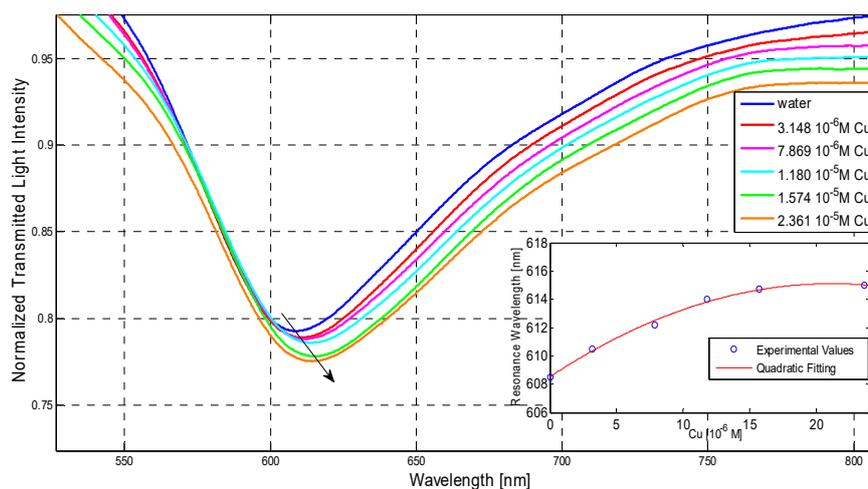
In this work, the working electrode was a gold disk with a cross-section exposed of 2.0 mm. It was abraded with successively finer grades of alumina (from 1 to 0.05  $\mu\text{m}$ ) and then rinsed with water and briefly cleaned in an ultrasonic bath to remove trace alumina from the surface. SAMs were prepared by dipping the cleaned gold electrode in 5 mM ethanolic solution of D,L-penicillamine for 12 h. The electrode was then rinsed with ethanol and water before use, as reported in [1]. Measurements were carried out with an Amel 433/W polarographic analyser equipped with a standard three-electrode cell with an Amel gold electrode as working electrode (2.0 mm diameter), a platinum wire as auxiliary electrode and an Ag/AgCl/KCl (4 M KCl saturated with AgCl) reference electrode. All measurements were carried out in 10 mL 0.1 M acetic acid buffer at pH = 4 in a concentration range of copper(II) from 0.04 to 0.4  $\mu\text{M}$ . The determination of copper(II) is based on the fixation of copper(II) at the electrode surface by complexation with the sulfide groups, with reduction to copper(I), and subsequent oxidation [1]. The signal is the peak current ( $i_p$ ) obtained by linear sweep stripping voltammetry (LSSV) at the following conditions:  $E_{\text{dep}} = -400$  mV, deposition time ( $t_{\text{dep}}$ ) = 60–120 s,  $E_f = +600$  mV, scan speed 50 mV/s.

A surface plasmon resonance (SPR) sensor is an optical sensor based on the plasmonic phenomenon. In particular, the SPR platform used in the present investigation is based on a D-shaped plastic optical fiber (POF), with a planar surface obtained by erasing the cladding and partially the core, over which a multilayer structure (photoresist buffer layer and thin gold film) was realized as previously described [4–6]. On the gold layer of this SPR-POF platform a D,L-penicillamine SAM has been realized. The signal of analytical interest is the variation of the resonance wavelength when copper(II) binds to the ligand D,L-penicillamine fixed at the gold surface. Measurements were carried out in 0.1 M nitric acid solution, in a concentration range from 3 to about 20  $\mu\text{M}$ .

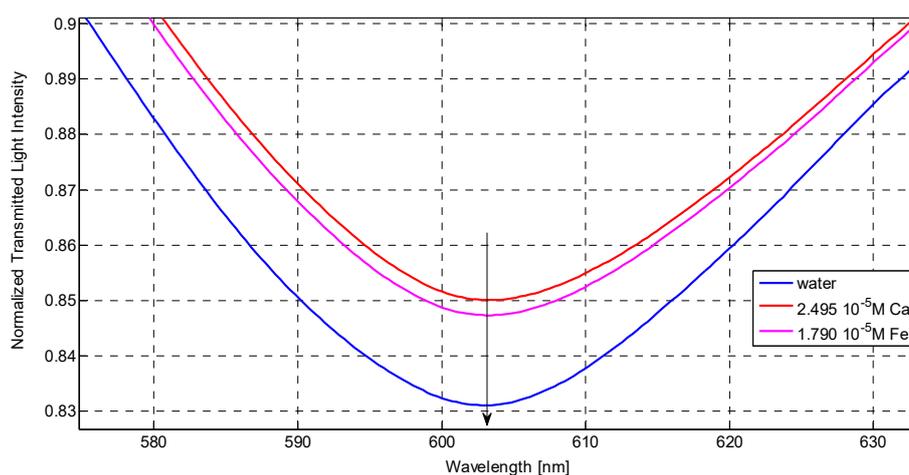
### 3. Experimental Results

The procedure for copper(II) determination by D,L-penicillamine SAM electrochemical electrode at pH = 4 was reported previously [1], obtaining an LOD of  $5 \times 10^{-8}$  M by LSSV at the conditions described above. Instead, the binding at pH = 1 was investigated by the SPR-POF sensor, which is supposed to have a better sensitivity. Nevertheless a response was obtained only at Cu(II) concentrations higher than those considered for the electrochemical sensor [1].

Figure 1 shows the SPR-POF response, i.e., the resonance wavelengths obtained at different Cu(II) concentrations. When the Cu(II) concentration increases, the SPR resonance shifts towards higher wavelengths because the refractive index of the D,L-penicillamine SAM increases when the binding with the Cu(II) takes place. The binding isotherm is reported in the insert of Figure 1. By applying the Scatchard model, the affinity constant has been found to be  $5(1) \times 10^5$   $\text{M}^{-1}$ , and the sensitivity at low concentration  $3.4(7) \times 10^6$   $\mu\text{A}/\text{M}$ . The detection limit, calculated from the error at the lowest concentration, is  $1.39 \times 10^{-7}$  M. Figure 2 shows the SPR spectra obtained with different metals (calcium(II) and iron(III)). Not any shift of the SPR wavelength is observed. This demonstrates the very good selectivity of the receptor layer here considered.



**Figure 1.** SPR transmission spectra, normalized to the air spectrum, for different analyte concentrations [Cu(II)  $3.1 \times 10^{-6}$  M– $2.4 \times 10^{-5}$  M]. Inset: Plasmon resonance wavelength as a function of analyte concentrations, with the corresponding fitting curve.



**Figure 2.** SPR transmission spectra, normalized to the air spectrum, for different analytes: Ca and Fe. For comparison, the spectrum of pure water is reported.

#### 4. Conclusions

The SPR sensor shows an LOD of about  $1.39 \times 10^{-7}$  M for Cu(II) in water, whereas that of the electrochemical sensor is about  $5 \times 10^{-8}$  M. This difference can be ascribed to the different acidity of the working solution, since the binding site is the same (D,L-penicillamine). The higher value of LOD at pH = 1 is due to the lower value of the affinity constant at more acidic pH, where there is a competition by  $H^+$  for the binding to D,L-penicillamine. At pH = 4 the activity of  $H^+$  is lower, and so is the competition, giving an higher value of the affinity constant. This is a well known effect of the metal ion complexation in water. A consequence is a better selectivity at low pH, which has been also found in this work.

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

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