

Optical and Electrochemical Sensing with Gold Nanoparticles

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Abstract— Thin-film structures containing gold nanoparticles can be used to detect contaminants and target molecules in liquids by both optical and electrochemical means. An important stage in developing such sensors is forming a layer of gold nanoparticles with appropriate properties on the sensor surface. For this process, preparative cyclic voltammetry and chronoamperometry were used in our work, and continuous optical monitoring was applied using an ellipsometer. In the future, we aim to develop a heavy metal detection sensor to evaluate liquid samples conveniently at the sampling site. Such technology would allow us to reduce the logistical problems and time loss associated with the analysis of drinking water.

Keywords—electrochemistry, ellipsometry, gold nanoparticles, protein filaments, drinking water.

I. INTRODUCTION

In developing countries, drinking water contaminated with heavy metal ions is a global health problem. Table I shows the limits for some heavy metal ion concentrations in drinking water as set by the World Health Organisation (WHO). The available state-of-the-art testing methods are not suitable for routine field testing. A portable electrochemical sensor set would not only result in higher sensitivity than currently used test strip methods but would also be less subject to the human error factor.

TABLE I. HEAVY METAL ION CONCENTRATION LIMITS AND THEIR HEALTH RISKS [1].

Heavy metal ion	Limit (mg/l)	Health risk
Cu(II)	2	Anaemia, kidney diseases.
Ni(II)	0.07	Oncological diseases, allergic reactions.
Cd(II)	0.003	Compromised renal function, hypertension and lymphocytosis.
Pb(II)	0.05	Can permeate the blood-brain barrier and cause neurodegenerative diseases (e.g. Alzheimer's disease).
Fe(III)	3	Haemochromatosis, cell damage of the liver and heart.
Hg(II)	0.001	Neurodevelopmental disorders, digestive and immune system disorders, hypertension.

II. METHODS

Among the laboratory methods required to develop a sensor for on-site contamination detection, the electrochemical-voltammetric background and ellipsometric measurement technique for continuous optical monitoring will be discussed in the next section.

A. Steps of the development of the sensor structure

As a first step of sensor development, gold nanoparticles were deposited on silicon wafers previously covered with a gold thin film by thermal evaporation. These allow the binding of specially modified protein filaments (prepared at the University of Pannonia's Faculty of Bio-Nanotechnology) on the surface (second step). The modified protein was dissolved in 0.1 M HEPES buffer (pH=7.1) and deposited onto the surface using preparative voltammetric cycles. These proteins allow the binding of heavy metal ions (especially nickel) on the surface and thus the detection of their concentration in ppm range by voltammetric methods. Details of the protein modification can be found in our previous article. [2].

Both the gold nanoparticles and the proteins are deposited on the surface by electrochemical methods (preparative chronoamperometry and cyclic voltammetry), and continuous optical monitoring of them was performed by ellipsometry.

B. Electrochemical processes

Gold nanoparticles were deposited on a gold-covered silicon wafer from a 5.14 mM hydrogen tetrachloroaurate(III) stock solution, thus increasing the specific surface area and promoting the binding of the sensor protein to the surface. The technique used to investigate the mechanisms of the redox processes is voltammetry, also known as amperometry, of which there are several forms (we used chronoamperometry and cyclic voltammetry in this project). Based on our knowledge from previous measurements and the relevant literature, we assumed that most bonds are formed during the initial cycles, so we used 2-cycle measurements to study protein adsorption on the surface [3].

Voltammetric methods, which are still commonly used today, consist of applying a known voltage to the electrodes and measuring the current flowing through the

electrochemical cell, resulting in a so-called voltammogram, which provides visual feedback on the process. Relevant information can be obtained by examining the correlation between the voltage applied to the electrodes and the time dependence of the measured current.

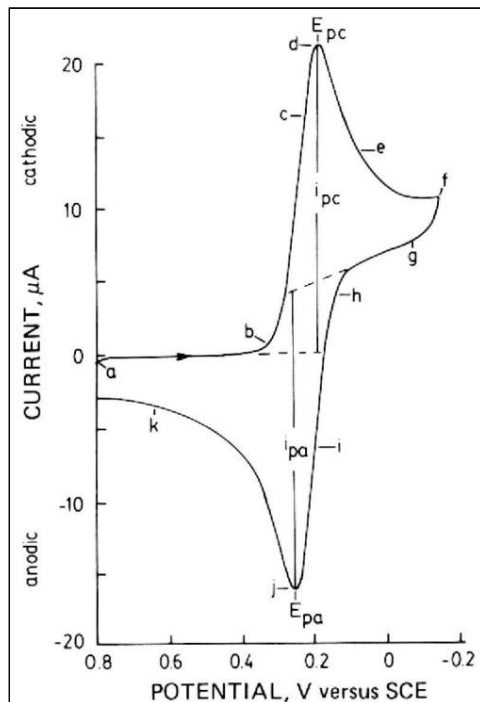


Fig. 1. A characteristic curve of a typical cyclic voltammogram for CV measurement with relevant quantities [4].

For the typical cyclic voltammogram shown in Figure 1, peaks of the anode current and cathode current are observed, and correspondingly, the anodic and cathodic potentials belonging to these current maxima are also observed. The graphical analysis of the voltammogram allows us to distinguish the potential at which the substance under investigation is oxidized or reduced; such effects are known as redox peaks. The first derivative concerning the time of the applied voltage gives the scanning rate, which is an important parameter in cyclic voltammetry, since at lower scanning rates, there is a risk of losing the useful signal. In general, the success of the specific application of the cyclic voltammetry technique is greatly influenced by the number of cycles that can be predefined by the user, by the potential range applied and the sampling rate [5].

C. Ellipsometry

Nanotechnology needs test methods that provide information on the material composition, surface roughness and thickness of thin films created by different methods in a non-destructive, inexpensive and fast way, without requiring complicated sample preparation. Ellipsometry, which detects the change in relative and absolute phase shift due to the reflection of polarised light from a given sample, has been used as a process tracking technique for the sensor development because:

- it is highly sensitive,
- non-invasive,
- easily reproducible,
- it has a wide range of applications.

As a first step of the evaluation of the properties of the gold nanoparticle depositions, the ellipsometer software CompleteEASE was used to develop an ellipsometric model of the gold nanoparticle surface, as shown in Figure 2, in order to fit the real layer structure as accurately as possible. The substrate in the model is the evaporated gold layer, with a gold nanoparticle layer on top represented by a mixture of gold and aqueous solution. Refractive indices of gold and water characterized by the Sellmeier refractive index were used to model this binary sublayer, and Bruggemann effective index method was used to determine the effective refractive indices. Thickness and composition values were determined by fitting the model to the measured data.

Following the protein deposition, a further sublayer containing protein filaments represented by a Cauchy dispersion model with a refractive index of 1.45 was introduced, and the aqueous phase reference values were modified to HEPES refractive indices.

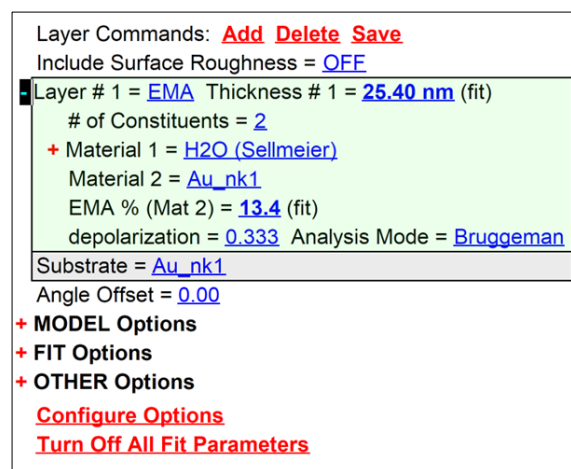


Fig. 2. Example of the single-layer model used for ellipsometric evaluation for the deposition of golden nanoparticles with chronoamperometry parameters -0.45 V; 0.25 mM; 10 min.

D. Measurement setup

The measuring setup used for gold nanoparticle and protein deposition is shown in Figure 3. The combined three-electrode measuring cell, which allows the simultaneous application of electrochemical processes and optical monitoring, the BioLogic SP-150e potentiostat for electrochemical processes and the J.A. Woollam M-2000 ellipsometer can be observed.

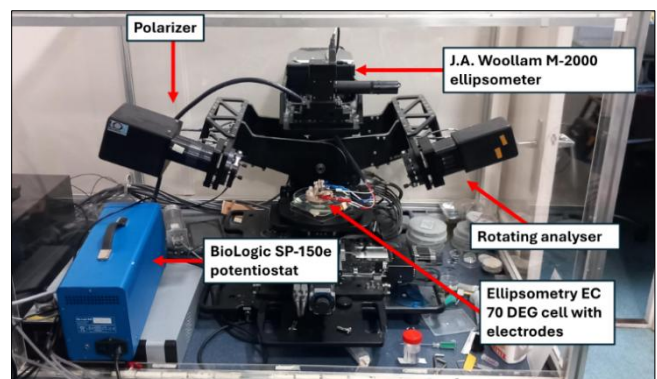


Fig. 3. Measurement setup during protein detection.

In the measurement setup, a three-electrode system is used: the potential is varied between the working electrode and the reference electrode, the voltage applied to the working electrode immersed in the solution under test is linearly increased from an initial value to the turning potential, where it is also linearly increased to the second turning potential, which is usually the same as the starting point of the experiment, by changing the sign. During the cycle, the strength of the current is detected between the measuring electrode and the reference electrode, thus resulting in the voltammogram. The electrodes with the combined measuring cell are shown in Figure 4.

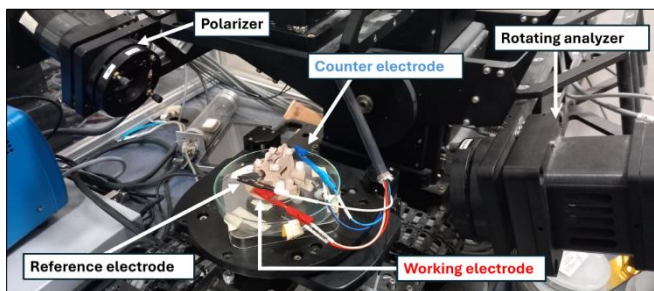


Fig. 4. Electrochemical cell positioning with electrodes during protein deposition.

III. RESULTS

By observing the ellipsometric spectra in real-time during voltammetry and preparative chronoamperometry, we concluded that the deposition of gold nanoparticles and sensor proteins on the surface was successful. This was confirmed by the redox peaks seen in the voltammograms as well. We also determined the optimal concentration and potential values in order to maximize the quantity of adsorbed filaments.

A. Evaluation using scanning electron microscopy (SEM)

In order to confirm the ellipsometric findings by an independent method, secondary electron imaging was performed on the samples to examine the morphology of the sample surfaces. Fig. 5 shows the surface structure of a filament-covered gold nanoparticle surface with a nanoparticle size of about 50 nm, and the presence of protein filaments can be seen in the form of dark fibrous structures in the foreground.

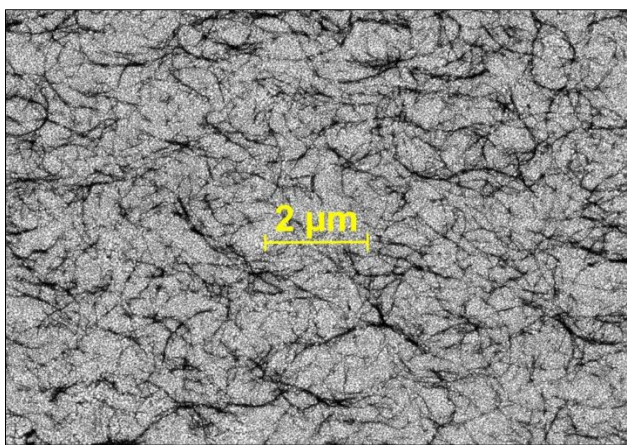


Fig. 5. Protein filaments on the surface of a sample prepared with deposition parameters of 1 mM, -350 mV at 25000x magnification.

B. Evaluation using Energy-dispersive X-ray spectroscopy (EDS)

Figure 6. displays the result of an elementary composition analysis of the protein-covered surface. The analysis was carried out by sampling the EDS spectra of 2 well-separated parts of the sample surface. The red spectrum represents an area which was not in contact with the deposition solution, while the yellow spectrum belongs to a surface area shown in Fig. 5. Excitation energy had to be kept at 2.5 keV because, at higher excitation energies, the thickness of the investigated layer is not sufficient for the sampling depth to achieve a proper signal-to-noise ratio.

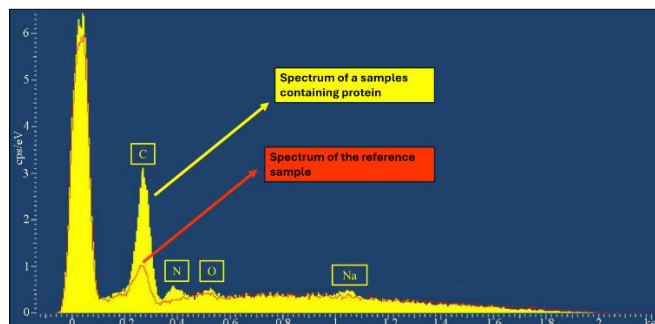


Fig. 6. Comparison of protein-treated and untreated surfaces by energy-dispersive X-ray spectroscopy.

By using the untreated sample as a reference (red spectrum), it can be seen that both carbon and nitrogen are present in higher amounts in the protein-covered part (yellow spectrum). As carbon is often present in hydrocarbon molecules contaminating the surface, their presence does not clearly demonstrate successful protein deposition. However, the nitrogen-related peak is only present in the filament-covered area, so its appearance observed on the protein-treated surface is clear evidence that the fibrous structure in Fig. 5. is indeed deposited protein filament.

C. Characterization of the sensor properties

Protein-covered golden electrodes were tested in HEPES solution containing increasing amounts of dissolved Ni(II) ions. Ni concentration was expressed as multiples of WHO limit (Table I). The samples were tested by cyclic voltammetry, and the area below the observed redox peaks between -350 and -550 mV was determined. These areas which are proportional to the transferred redox charge, are shown in Fig. 7. The horizontal axis shows the multiples of the limit of the allowed nickel ion, while the vertical axis shows the electric charge measured during the redox process.

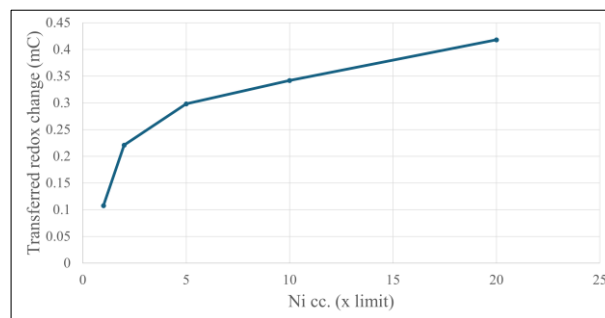


Fig. 7. Nickel sensitivity of the sensor as a function of the health limit value.

Fig 7. shows that the amount of Ni(II) ions observed is in a monotonously increasing relationship with the applied Ni concentration in the solution. The curve shows a saturation character towards higher concentrations, however the measured values are clearly distinct below 5x concentration. Therefore, we can claim that this device is applicable as a semi-quantitative tool for detecting the presence of Ni(II) ions in that concentration range.

D. Examination of the selectivity of the sensor

In order to determine the selectivity of the sensor structure, the Ni characterization measurement described under the *Characterisation of the sensor properties* section were repeated in the presence of Fe(II), Cu(II) and Sn(II) ions in the HEPES solution. These ions were applied in increasing concentrations, and for each ion, the corresponding Ni values were repeated in the molar concentrations. Figs. 8. and 9. summarize the results.

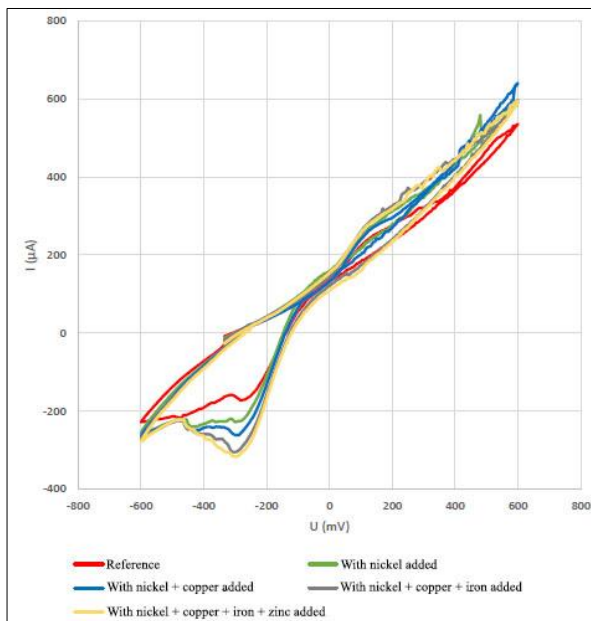


Fig. 8. Cyclic voltammograms of the sensor in the presence of Ni and other ions.

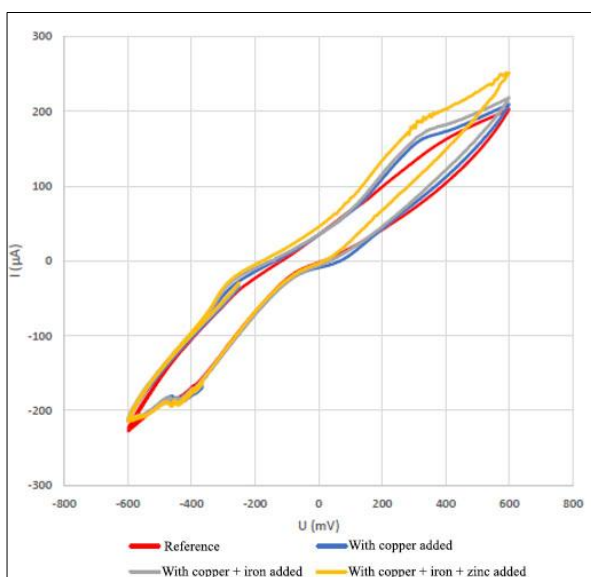


Fig. 9. Cyclic voltammograms of the sensor without the presence of Ni but in the presence of other ions.

Fig. 8 shows the observed voltammograms in the presence of Ni(II) ions, while Fig. 9 shows the effect of applying other metal ions only. The presence of peaks around +350 mV in Fig 8. shows that the response of the sensor for Ni is observable in the presence of foreign ions as well, while Fig. 9. displays that the presence of the examined foreign ions do not result in redox processes in the same potential range.

IV. SUMMARY AND CONCLUSION

The subject of this study is the investigation of the feasibility of a modified protein-based heavy metal sensor structure. Our aim was to build a field-applicable semi-quantitative device for Ni detection in the ppm range.

The sensor is based on modified protein filaments deposited onto a gold nanoparticle-covered surface using preparative electrochemical techniques. Ellipsometry was used as an *in situ* measurement technique throughout the process.

Our results show that golden nanoparticles successfully bonded protein filaments onto the electrode surface. The structure was able to detect cyclic voltammetry peaks in Ni(II) containing solutions and detect the presence of Ni(II) in the range of the WHO health limit. Good selectivity and cross-sensitivity of the device were proven for Fe(II), Cu(II) and Sn(II) ions.

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