AUSTRIAN INSTITUTE OF TECHNOLOGY

BioSensor Technologies

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Report on the dissertation "Plasma-assisted deposition of functional bioactive nanostructured films" prepared by Mgr. Petr Sezemsky

Doctoral thesis of Mgr. Petr Sezemsky builds up on his experimental work carried out at the Faculty of Science, University of South Bohemia in Ceske Budejovice. The work presents a wide range of original results in the field of preparation of plasma-deposited thin films and their utilization in combined optical and electrochemical biosensors. The cumulative high-level thesis is written in very good English, it is logically structured, and provides brief introduction to the field, clearly formulates research goals, provides a summary of achieved results, and includes published scientific papers that constitutes the thesis. The work of the PhD candidate was published in 17 papers, from which 15 are collaborative projects and 2 are the first author papers. All these papers were published in peer reviewed journals that are important for the scientific community of optical sensors and materials including those assumed to be in the best category (e.g. Biosensors and Bioelectronic, Sensors and Actuators B).

The results are organized in three groups dealing with (A) preparation of thin indium tin oxide (ITO) films on the surface of cleaved optical fibers with controlled optical and electronic properties, (B) development of an sensor instrument that allows for bi-modal sensing through optical refractometric measurement and cyclic voltametry, and (C) implementation of the sensor for detection of biomolecular species.

The most valuable results can be summarized as follows:

a) Successful preparation of ITO thin films with tailored grain size, resistivity and optical characteristics that allow for optimum operation of electrochemical (EC) and refractometric readout by using optical lossy mode resonance (LMR) or guided wave spectroscopy through coupling with long period gratings (LPG).
b) Implementation of the sensor system that allows for *in situ* real time monitoring of its surface based on parallel EC cyclic voltammetry and LMR measurements. The operation of such bi-modal sensor was characterized by using refractometric experiments and through the monitoring of a formation of in *in situ* electropolymerized films. The key interesting features (the cross-sensitivity of the optical readout to driving the cyclic voltammetry operation and the differences in the mechanisms and effects these methods are sensitive to) are discussed.
c) Employing the developed platform for the monitoring of biofunctionalization steps and affinity binding events. These include using model biotin – streptavidin interaction that (interestingly) shows that EC modality provides relatively better sensitivity compared to LMR to the binding of low molecular weight analytes, while for higher molecular weight molecules the effect is opposite. Steps towards a functional immunoassay for detection of bacterial pathogens are made.

Dissertation of Mgr. Petr Sezemsky presents a great deal of high-quality results that are important for the research community of optical and electrochemical sensor development and that were published in best journals of the field. Mgr. Peter Sezemsky proved the ability to design, carry out and evaluate his experimental work that combined fiber optics, electrochemistry, thin film technologies, optical spectroscopy, and biofunctionalization for bioanalytical applications. In addition, he obviously managed a range of collaborative projects and successfully operate in multidisciplinary research domain. The evaluator fully recommends accepting the thesis for the defense and supports granting the candidate PhD title in strongest terms.

Possible questions for discussion after the defense presentation:

1. In order to utilize the prepared ITO coatings on the cylindrically shaped fiber optic element, the homogeneity of the thickness over the fiber surface may be a challenge. What would be the effect of varying the thickness over the sensing area to the performance of the LMR or LPG – based sensor and how it was mitigated?
2. Concenptually, what is the difference between the used LMR sensing principle compared to e.g. so called reflectometric interference spectrometry (RiFS) that as introduced by the Tuebingen group of Prof. Gauglitz. How does the monitored optical features in LMR relate to Fabry Perot interference?
3. How were the analyzed liquid samples exchanged / transported through the reaction chamber comprising the optically active working electrode with ITO, reference electrode and counter electrode? What would be the impact of diffusion of analyte molecules to the affinity binding kinetics and how it affects the measured sensorgrams?
4. Figure 39 summarizes the measured response to binding of streptavidin to the immobilized biotin moieties depending on its concentration. For the readout signal in equilibrium, this response should exhibit Langmuir isotherm like trend with response saturating at concentrations above the dissociation equilibrium constant K_d (which is at 10^{-15} M for this high affinity pair). Why is it not the case?
5. In general, the optical signal LMR or LPG respond to changes occurring only on the surface of ITO layer in the zone where the optical fiber cladding was cleaved. However, the EC may be affected also by binding of the molecules on the surface of reference and counter electrodes. If it is so, can that be mitigated to assure that both modalities probe the same sensing area and thus the same biomolecular processes?
6. The mentioned, potentially competing, technique of surface plasmon resonance (SPR) was also implemented by using fiber optic probes. Recently, this approach method was also utilized for dual optical and electronic (field effect transistor-based) measurement (see 10.1021/acssensors.1c02313). Would there be an advantage using the ITO-based system in this case and are there some phenomena that could be resolved by such sensor that the combination with SPR cannot do?

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