PhD theses

Investigations inside the reaction layer of biosensors with scanning electrochemical microscopy

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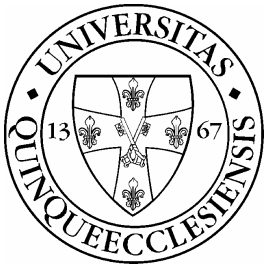

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Introduction

Scanning Electrochemical Microscopy (SECM) is one of the probe microscopy techniques; developed by electrochemists. It was first published by Bard and coworkers in 1989. SECM scans over the target surface a micro- or even nanometer size sensor with a precision positioning device, and collects analytical signal at different locations. The SECM images are created from the location co-ordinates and the measured signal values. The method is being developed continuously; its applications range is widening. One can conclude that scanning electrochemical microscopy is a powerful technique to gather information with high spatial resolution about concentration profiles or local electrochemical reactions.

Based on different electrochemical methods a great number of measuring set-up has been developed. Two main measuring groups can be distinguished: in one hand the analytical signal just reflects the local concentration or ionic activity of a component (passive measuring tip). On the other hand the analytical signal can originate from the interaction of the measuring tip and the target surface (active measuring tip). The active measuring tips are amperometric microelectrodes.

Using an amperometric microdisc electrode that is polarized to a potential where redox process is running, the steady-state current in the bulk of the solution can be calculated:

$$i_{T,\infty} = 4nFDCa$$

where n is the number of the electrodes taking part in the redox reaction, F is the Faraday constant, D a diffusion coefficient, C concentration of the electrochemically active species in the solution, a is the radius of the electrode.

Comparing the electrochemical behavior of the microelectrodes with the conventional ones important differences can be found. The reason for the differences originates from the mass transport properties: using conventional size electrodes the mass transport is linear diffusion dominantly. In case of microelectrodes the diffusion has a hemispherical character during amperometric measurements. Owing to the hemispherical diffusion the steady-state current evolves within some tenths of a seconds, and the convection within the cell can only slightly influence this value. All of these microelectrodes are well applicable for SECM investigations, where the electrochemical information is collected by amperometric mode doing continuous scanning over the target.

As the electrode is moving towards an insulating surface, in the presence of an electrochemically reversible mediator the amperometric current begin to decrease from the steady-state bulk solution value, because of the diffusion of the electroactive component to the electrode surface is hindered. This phenomena is called negative feedback. As the electrode is moving closer to a conducting surface, however the species generated at the tip is regenerating at the conducting surface and can reach the electrode again. As a result the local concentration of the detected species is increasing. Reaching a small electrode – target distance (smaller then 3 times the radius of the electrode) the current is increasing, that is called as “positive feedback” effect.

In the practice of the SECM different measuring set-ups can be use:

- The electrochemical measurement cell contains a constant concentration of an electrochemically reversible compound (called as mediator) and an appropriate constant tip-potential is set, the amperometric current can be measured during scanning over or approaching the target. This is the feedback mode that has been described previously. It can be used to gather information chemical information about target. It can well distinguish surface patches or insulating or conducting nature.
- Substrate generating – tip collecting mode (SG/TC): the measuring tip indicates the local concentration of the species generated by the substrate.
- Penetration mode: using this method the tip is penetrated into a microstructure (polymer film, immobilized enzyme layer) and measuring information is gathered about the concentration distribution of electrochemically active compound or investigations are carried out the transport processes.
- Surface modification mode: the reactants generated at the electrode surface can locally modify the target surface using the high resolution of SECM.

The biosensors are well known analytical tools. They can be used to measure concentration of different species in complex matrices selectively. The selectivity is provided by the biological nature of their working principle. As it is well known their measuring function is based on a chemical reaction that takes place in a catalytic reaction layer. The interaction of this reaction and the mass transport processes produce changes inside the reaction layer. In order to describe the function of the different enzyme sensors or to design

efficient ones, the distribution of the different species inside the differently made, sized and formed reaction layers during their operation is necessary to be known.

Considering the complex interaction of different processes, (distribution of different species between the sample and the reaction layer, mass transport inside the sample phase and in the reaction layer, enzyme catalysis) very complicated spatial and time dependencies of concentration of different chemical compounds can be expected.

The SECM technique gives the possibility to investigate concentration profiles in aqueous solutions or in gel phases. In some cases the local concentration of the species produced in an enzymatic reactions could be measured in the solution (SG/TC mode), as it is diffusing away from the surface containing the immobilized biocatalyst. In other cases the local decrease of the reactants could be detected. The reaction generated local pH change can be detected, as well. SECM studies with ultramicro tip can penetrate and measure in elastic thin films.

Since the early times of biosensor research several attempts have been made to describe the concentration profiles inside biocatalytic layers or to explain the function of different kinds of enzyme sensors. However, to give an explicit equation with measurable variables and parameters is a difficult task. Often equations derived are applicable only in special limiting cases, or their use often ends with giving qualitative explanation on experimentally observed behaviour.

The other approach is to solve the equations numerically. The well established finite difference method can also be well used to simulate numerically concentration profiles inside catalytic reaction layers. The availability of simulation for determining the optimal physical and chemical parameters of the reaction-layer (e.g. thickness, viscosity, permeability, catalytic activity) is limited, it can be used only for describing tendencies or interpret some phenomena. The equations derived to describe enzyme sensor response or the dependencies obtained by digital simulations for the different cases should be verified experimentally.

As measuring the diffusion coefficient by “flight time” method a very small dose of the investigated material is introduced into the measurement cell. The detector electrode is in a given distance from the source. The introduced species is spreading away by spherical diffusion and reaching the detector surface, where a maximum shape concentration – time transient can be measured. The diffusion coefficient can be calculated as one knows the “flight distance” and the “flight time” until reaching the maximum of the transient.

The accuracy of the method is determined by the uncertainty of the distance determination. Using SECM, distances can be set with high precision. This allows to measure the “flight time” at several distances, which results in a higher accuracy for this simple technique.

Novel scientific results

Theses

1. A well working Scanning Electrochemical Microscope was built together. The necessary software for operating the device was written and tested. Microelectrodes with different shapes and sizes were prepared. Control measurements were done to check the operation of the instrument.

2. Oxygen and hydrogen-peroxide concentration distribution was measured within the reaction layer of working biocatalytic sensors. The optimal reaction-layer thickness was stated as 200 μm in case of a glucose measuring biosensor based on glucose-oxidase enzyme. Similar results were obtained as tests were done with different substrate concentrations and enzyme activities. Checking the influence of pH on the concentration profiles of oxygen and hydrogen-peroxide, buffer with pH 7.0 gave the most significant local changes inside the biocatalytic layer signal height.

3. Concentration profiles were investigated in a sequential multi-enzyme system containing invertase, mutarotase and glucose-oxidase co-immobilised. Sucrose quantitative analysis can be done with a biosensor based on this sequential reaction layer. Concentration distribution was measured in such a reaction layer. As a result I was able to state that the analytical signal has a maximum value – similarly to the glucose measurements – at a distance of 200-300 μm from the dialysis membrane. On the bases of the results it could be concluded that the role of the mutarotase enzyme within the sequential enzyme catalysed reaction is very important; without it the inversion of glucose from α to β – a quite slow reaction – would be the rate-determining step. Therefor the electrode response would be small.

4. Eliminator layer has been prepared and placed in front of the sucrose-measuring layer. This sensor gives the opportunity to measure the concentration of sucrose in the presence of glucose too. In order to get rid of the interfering effect of glucose, an optimal glucose-oxidase – catalase enzyme ratio was chosen based on the results obtained with eliminator layer. Using both enzymes in 100 U/ml concentrations the proposed aim is attainable.

   In the reaction layer of the complex sucrose measuring sensor provided with the eliminator layer hydrogen-peroxide and oxygen concentration profiles were checked in solutions with and without glucose. The observed, very low hydrogen-peroxide concentration at the border of the eliminator and a sucrose-measuring layer means, that the eliminator layer works well.

5. Measuring the oxygen concentration in glucose-oxidase containing reaction layer it turned out, that in case of a layer, which gets oxygen influx from both directions, the oxygen concentration profiles reaches a minimum. In order to get a more realistic sensor structure the oxygen diffusion from the back direction should be eliminate. To do this a paraffin oil layer was poured on top of the immobilized enzyme containing gel. Doing investigations in such layers at high glucose sample concentrations significant oxygen depletion could be observed. It can be expected that the enzymatic processes slowed down in the deeper regions of the reaction layer owing to the lower oxygen concentrations there.

   As the sucrose-measuring layers were checked, the oxygen level showed a very low value, as higher substrate concentrations were applied. This remarkable lack of oxygen could also cause a lower reaction rate of the enzyme catalysis.

6. Model calculations were done on the basis of the experimentally observed biosensors. Measured and calculated concentration profiles showed good correlation. Useful information was collected about concentration distribution of experimentally not investigated species (e.g. glucose, sucrose) as well. These data helped to refining the experimental work.

7. Method based on Scanning Electrochemical Microscopy has been worked out for the measurement of the diffusion coefficient of electrochemically active compounds. Using this method diffusion coefficient of several species has been studied in aqueous solutions (in solvents and in gels) and in ionic liquids. The values obtained were in the range of that

in the literature. To my opinion these values are fairly exact and reliably for the media investigated.

8. The effect of the polyelectrolytes on the velocity of diffusion has been investigated. Diffusion coefficient measurements were carried out in agarose gel containing or not containing Nafion®, which has a cation exchanging property. The diffusion coefficient value measured for $[Ru(NH_3)_6]^{3+}$ decreased by about 15% in the presence of 0.3% Nafion®.

List of publications

Publications connected to the dissertation

Csóka B., Kovács B., Nagy G.: Bioszenzorok katalitikus rétegének vizsgálata pásztázó elektrokémiai mikroszkópiás méréstechnikával
Magyar Kémiai Folyóirat, 2002 (108) 4, 185 - 194.

B. Csóka, B. Kovács, G. Nagy: Investigation of concentration profiles inside operating biocatalytic sensors with Scanning Electrochemical Microscopy (SECM)
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B. Csóka, B. Kovács, G. Nagy: Scanning Electrochemical Microscopy inside the biocatalytic layer of biosensors. Investigation of a double function complex multienzyme reaction layer
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B. Csóka, G. Nagy: Determination of diffusion coefficient in gel and in aqueous solutions using Scanning Electrochemical Microscopy (SECM)
Journal of Biochemical and Biophysical Methods, 2004, 61(1-2), 57 - 67.

Other papers written during the PhD studies

B. Kovács, **B. Csóka**, G. Nagy, I. Kapui, R. Gyurcsányi, K. Tóth: Automatic Target Location Strategy, a Novel Approach in Scanning Electrochemical Microscopy
Electroanalysis, 1999, 11(5), 349 - 355.

B. Kovács, **B. Csóka**, G. Nagy, A. Ivaska: All-solid-state surfactant sensing electrode using conductive polymer as internal electric contact
Analytica Chimica Acta, 2001, 437, 67 - 76.

M. Södergård, **B. Csóka**, G. Nagy, A. Ivaska: Lowering the Detection Limit of Solvent Polymeric Ion-selective Membrane Electrodes. An Experimental Study with Calcium-selective Micropipette Electrodes
Analytical Letters, 2003, 36(14), 2909 - 2923.

Cumulative impact factor of the papers: ***11.166***
Citations: ***21*** *(17 without self-citations)*

Conference lectures

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2nd international workshop on Scanning Electrochemical Microscopy – Southampton, UK, 2001. június 29.- július 2.

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G. Nagy, **B. Csóka**, B. Kovács: Application of microelectrodes in biosensor research
Mátrafüred 2002 – 2002. október 13-18.

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XXV. Kémiai Előadói Napok – Szeged, 2002. október 28-30.

Csóka Balázs, Nagy Géza, Kovács Barna: Bioszenzorok vizsgálata pásztázó elektrokémiai mikroszkópiával
VIII. Nemzetközi Vegyészkonferencia – Kolozsvár, 2002. november 15-17.

Csóka Balázs, Kovács Barna, Nagy Géza: Biokatalitikus szenzorok működésének tanulmányozása Pásztázó ElektroKémiai Mikroszkópiával
Analitikai Napok 2003 – Budapest, 2003. január 29-30.

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Teh 3rd Bi-national France-Israeli Workshop on Biosensors, Biochips and Nanobiotechnology – Eilat, Izrael, 2003. november 30. - december 4.

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Vegyészkonferencia 2004 – Balatonföldvár, 2004. június 30. - július 2.

Posters

B. Csóka, G. Nagy: Methods for Determination of Diffusion Coefficinets of Electrochemically Active Species Using Scanning Electrochemical Microscopy (SECM)
7th International Symposium on Instrumental Analysis – Pécs, 2003. szeptember 21-24.