Visualization of Micro-Structured Enzyme Patterns Using Scanning Electrochemical Microscopy (SECM)

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Miniaturisation of biosensors is important for their possible future application especially for continuous monitoring in medical analysis. Obviously, in order to guarantee high spatial resolution and good reproducibility non-manual deposition of the sensor compounds on top of the transducer surface is indispensable. In this respect, the electrochemical-induced formation of sensing layers exclusively on the surface of (micro)electrodes has found increasing interest in the past years [1]. In addition, micro dispensing using ink-jet technology has been proposed for the formation of enzyme-containing spots with dimensions of several 10 µm [2].

Moreover, complex enzymatic interaction is in principle possible by combining different enzymes at a single immobilisation site. The enzymes have to be chosen in that way that either productive interaction (product of enzyme 1 is substrate of enzyme 2) or destructive interaction (product of enzyme 1 is consumed by enzyme 2) occurs.

Here, we want to report on two different approaches to build up multi-enzyme micro-structures using either electrochemical formation of polymer layers with integrated biological recognition elements or ink-jet deposition of multi-enzyme structures. The described film formation processes allow us in principal to vary the sensor architecture, to design compex interaction schemes between different enzymes, to integrate redox-relays to ensure fast electron-transfer rates between the active site of the enzymes and the electrode surface, and to add additional polymer layers in order to reduce the influence of interfering compounds.

In the first approach, an electrochemically induced pH-modulation at the immobilisation site non-conducting polymer films are deposited on a conducting surfaces controlling their lateral dimensions by means of scanning electrochemical microscopy (SECM). The polymer film can be chemically modified by introducing redox relays, charged side-chains, and additional polymer layers with defined size-exclusion properties. Results concerning the formation of such micro-structured biosensors will be presented.

The second approach is based on the deposition of micro-structured enzyme-containing layers using a micro-dispenser with a typical micro-droplet size of 100 pL. Complex enzyme patterns can be obtained which are visualised using SECM. Besides quantification of an enzyme's substrate the microstructures allow to compensate for interfering compounds, and to simultaneously detect several substrates of different enzymes avoiding crosstalk effects (Fig. 1).

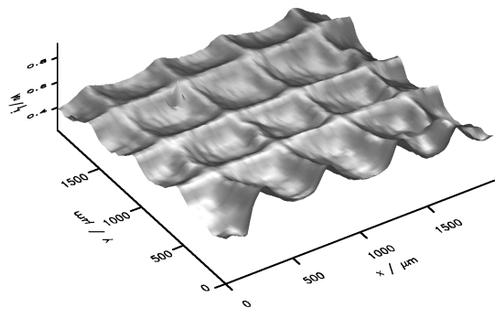

Fig. 1 Glucose oxidase grid structure visualied by means of SECM.

[1] a) P. N. Bartlett, P. R. Birkin, *Synth. Met*. **61** (1993) 15-21 b) W. Schuhmann. *Microchim. Acta.* **121** (1995) 1-29. c) S. Cosnier, *Biosens. Bioelectron*. **14** (1999) 443-456

[2] a) T. Laurell, L. Wallman, J. Nilsson, *J. Micromech. Microeng*. **9** (1999) 369-376. b) D.P. Little, T. J. Cornish, M. J. O'Donnell, *Anal. Chem.* **69** (1997) 4540-4546.

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