A Capacitive Immunosensor for Atrazine-Detection

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Statement of Purpose: There is a high demand of sensor systems which directly detects antigen bonds to immobilized antibodies in environmental analytics. A sol-gel based capacitive immunosensor for the detection of the low molecular carcinogenic pesticide atrazine was developed. It featured a combined high specificity of antigen-antibody interaction with a fast analytic and an extended storage time.

Methods: The principal function of the capacitive immunosensor was based on the influence of the dielectric of a capacitor caused by the highly specific molecular recognition of antigens by antibodies. [1] The capacitance values were measured by an HP Analyzer HP 4192 ALF with an alternating voltage of 50 mV. The immunosensor was submerged in 300 µl sodium-phosphate buffer solution (pH 7) at room temperature during the measurement. The frequency ranged from 1 kHz to 0.005 kHz.
Two of the used antibody-antigen-combinations should be presented. The target system was the rabbit polyclonal antibody anti-atrazine (α-atrazine) and the s-triazines-herbicide atrazine. The combination of the mouse monoclonal anti-alkaline phosphatase (APB3) and alkaline phosphatase (AP) was used as a model system for testing the enzyme activity in a enzyme-linked immunosorbent assay (ELISA) and directly on the sensor surface. The antibodies were immobilized by protein A on the gold electrodes [2]. The electrodes were sputtered (Emitech K550; V=12 V; $I_{deposition}$=50 mA; $t_{deposition}$=4 min; vacuum 7x10-² mbar, N_2-atmosphere) on an aluminium oxide wafer of 5x35 mm (Keral 96). A sol-gel of tetramethyl orthosilicate (TMOS) [3] was coated over the protein and the antibodies that were deposited on the electrodes. The wafers were re-used after each measurement; nitrohydrochloric acid was used for the recycling process.

Results / Discussion: The antibody-antigen-systems exhibited reproducible decrease in capacity after binding of the antigen as illustrated in Fig. 1. [4]

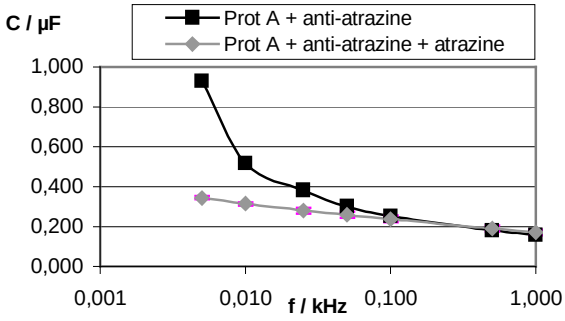

Fig. 1: Decrease in capacitance after atrazine recognition

The specificity of the capacitance decrease was confirmed by the use of the unspecific antibody rabbit IgG, which resulted in only slight decrease of capacitance.
The specificity of the capacitance decrease in the model system (APB3 / AP) was confirmed by the spectroscopic proof of the enzymatical activity of the AP.
The coating with the TMOS-gel increased the capacitance difference in comparison to the direct absorption of the antibody on the gold surface. The sensors which were coated with the protein-antibody-complex protected by the sol-gel-film could be stored in a dry state at 4°C up to 72 h (Fig. 2).

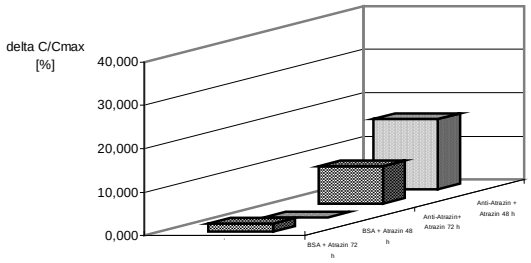

Fig. 2: Detection of atrazine after storage up to 72 h at f = 0.05 kHz

Conclusions: An antibody based capacitor was developed which detected low molecular substances like atrazine. Furthermore, a capacitor with TMOS-gel coating provided higher capacitance values and an increased stability of the protein A-antibody-complex. The immunosensor was proven to be applicable for fast detection of low molecular substances for environmental analytics.

References:

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