HIGHLY SENSITIVE IMMUNO-SENOSORS FOR ATRAZINE,

BASED ON THE DIRECT AND

COMPETITIVE ASSAY PRO-CEDURES

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The improved piezoelectric immuno-sensors for the determination of atrazine were developed. The piezoelectric quartz crystals (with smooth and rough surfaces) were used in a highly sensitive nanobalance system (EQCN-900, Elchema Co.). All measurements were performed in a flow-through arrangement. To immobilize the biorecognition layers, the surface of gold electrodes of the crystals was modified by self-assembled thiolayers using either cystamine, 4-aminothiophenol or dithiobis(succinimidyl propionate).

Initially, the competitive assay procedure was tested. Atrazine was immobilized on the surface through albumin as a bridge molecule. The competitive assay for atrazine employed the monoclonal anti atrazine antibody D6F3. Mixture of antibody with either standard or sample was pre-incubated for 15 min and then injected to the flow cell with atrazine-modified crystals. The binding curve was recorded continuously and the response after 10 min was evaluated. The calibration curve allowed measuring of atrazine concentrations as low as 0.01 μ g/L, the upper limit of detection was 1 mg/L. To improve the regeneration of the piezosensors, carboxylated atrazine derivative was immobilized directly to the monolayer of thiocompounds thus allowing reliable and complete regeneration of the immobilized ligand using hydrolysis of immunocomplexes with the help of proteinase (pepsin at pH 2).

In the direct piezoelectric immunosensor for atrazine, we have employed the antibody D6F3 and immobilized it covalently on the sensing surface. The oriented immobilization through Protein A was chosen and the sensing surface was stabilized by crosslinking with dimethylpimelimidate. This immunosensor allowed us to directly detect atrazine in samples without using of any labels. The immuno-complex was designed to be stable only in the presence of atrazine. The experiments shown that the complex dissociated quickly and spontaneously when placed in a pure buffer solution. In this way, the reusability of the biosensor was satisfactorily achieved.

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