Immunoassay for Estrogens in the Environment: Multi- Analyte- Detektion based on Fluorescence

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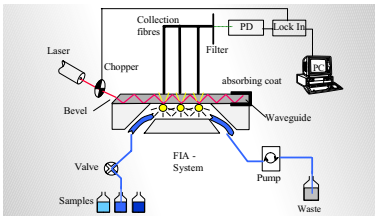
Introduction

- There is a rising number of substances which verifiably show ***estrogenic activity***. They contaminate the environment, especially the surface water. A ***water monitoring system*** is needed.
- The objective is the establishment of an ***immunoassay*** for estrogens in environment. The instrument should be simple, low-cost and small-sized to use it for the monitoring of water quality.
- We present a method to detect ***several estrogenic substances simultaneously*** by a heterogeneous phase immunoassay. Analyte specific antibodies are labelled with a fluorescence dye (Cy 5.5) and react with the analyte in the sample. The unoccupied antibodies can bind to the modified surface of a glass transducer. The detection is based on ***TIRF***.

Set-up for TIRF

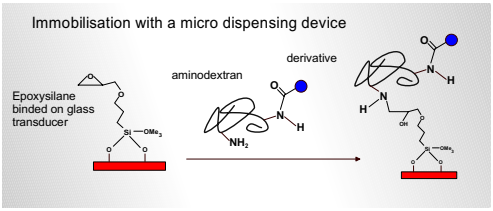
(Total Internal Reflection Fluorescence)

The River Analyser (RIANA) is based on specific immunoassays for analyte recognition and Total Internal Reflection Fluorescence as transducer principle. The device consists of an optical detection unit, a flow cell, and an integrated fluid handling based on flow injection (FIA).



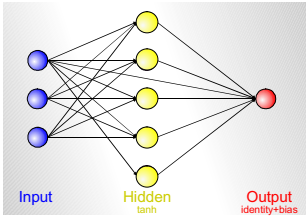
Transducer Modification

For the spatially resolved surface modification the glass transducer is silanised with an epoxysilane.
Subsequently a conjugate of aminodextran with analyte derivative is immobilised with a micro-drop dispenser on pre-defined detection spots.
Covalent bonds ensure a stable surface modification.



Data Analysis

- Application of a calibration function for removing any time dependency of measurements
- Normalization and Centering and Scaling of data
- Analysis by neural networks:
  - One neural network for each analyte
  - Application of pruning algorithms during training
  - Independent data for testing the networks

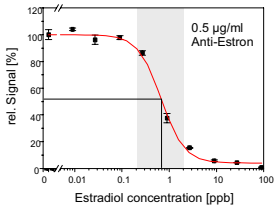


Results

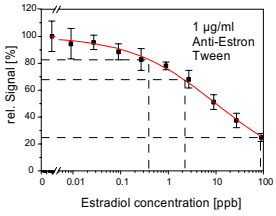
- **single analyte measurement**

Transducer modified with a single analyte derivative

Laboratory Conditions:



Waste Water Samples:

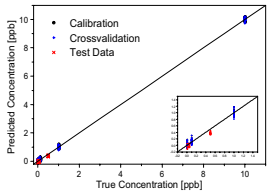


Sample	Concentration [ppb]	
waste water	measured	true
1	0.4	1
2	2	3
3	91	90

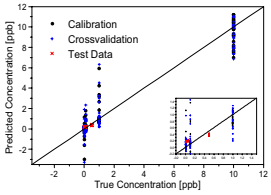
- **multiple analyte measurement**

Mixture of estradiol and ethynylestradiol simultaneously analysed

Estradiol True-Predicted-Plot



Ethynylestradiol True-Predicted-Plot



Summary and Outlook

- The limit of detection for Estradiol as single analyte is 0.2 ppb. The test midpoint is 0.7 ppb.
- TIRF assay for the measurement in waste water gives a good prediction.
- The assay for simultaneous detection of two analytes with a cross reactive antibody is established.
- Multivariate data can be analysed by neural networks. Improvement will be obtained by enlarging the dataset.
- Simultaneous assays for Bisphenol A, Estradiol and Ethynylestradiol will be established. Next step is the measurement of Nonylphenol and Chrysene.

Acknowledgement

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