BIOSENSORS WITH FLUORIDE ION SENSITIVE FIELD EFFECT TRANSISTORS

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SUMMARY

Enzyme field effect transistors for glucose and lactate were developed by coupling a new transducer type, the fluoride ion sensitive FET, with the peroxidase-catalyzed reaction liberating fluoride ions from organo-fluoro compounds in the presence of hydrogen peroxide which was produced in coupled oxidase reactions. Glucose oxidase or lactate oxidase and peroxidase were coimmobilized with human serum albumin or polyurethan at a multigate pF-FET. 4-fluoroaniline and pentafluorophenol were used as organo-fluoro compounds. The main functional parameters are given.

INTRODUCTION

A serious problem of ENFET measurements based on common pH-FETs is the dependence of the buffer capacity espcially for the determination of physiological samples. In the present work is shown the fluoride ion sensitive FET coupled with immobilized fluoride producing enzyme systems which allow measurements in

strongly buffered solutions thus beeing independent from buffer capacity changes caused by the sample.
According to the reaction scheme given in figure 1., in a first enzymatic reaction oxidases catalyze the analyte conversion consuming oxygen and producing hydrogen peroxide. In the second, peroxidase catalyzed reaction this hydrogen peroxide is consumend in the cleavage of covalent C-F-bonds in certain organo-fluoro compounds ($R-F_n$).

FIGURE 1. Reaction scheme of fluoride producing enzyme systems

$$\underset{\text{(GLUCOSE)}\ \text{(LACTATE)}}{\text{analyte}} + O_2 \xrightarrow[\text{(GLUCOSE OXIDASE)}\ \text{(LACTATE OXIDASE)}]{\text{OXIDASE}} \text{ox. analyte} + H_2O_2$$

$$\underset{\text{(4-FLUOROANILINE)}\ \text{(PENTAFLUOROPHENOL)}}{R-F_n} + H_2O_2 \xrightarrow{\text{PEROXIDASE}} R-F_{(n-1)} + F^- + H_2O$$

RESULTS AND DISCUSSION

The fluoride sensitive transistor elements have been produced from pH-sensitive FETs with silicon dioxide as gate insulator and a 100 nm silicon nitride layer by covering with 100 nm lanthanum fluoride (see figure 2). The resulting fluoride ion sensitivity was 52.9±0.4 mV/lg$c_{fluoride}$ from 1 µmol/l to 100 mmol/l with a response time of about 6 s.

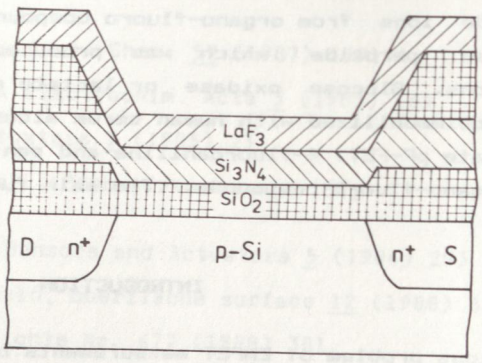

FIGURE 2.
Cross section of the fluoride ion sensitive FET
D - Drain, S - Source

Six single pF-FET elements with an active gate area of each 0.018 mm^2 have been arranged in a multigate layout. For covering with enzyme membranes a area of about 3 mm^2 was excluded by the encapsulation with epoxy resin.

Two methods for covering the pF-FET with enzyme mebranes were tested:

Firstly, glucose oxidase (GOD) and peroxidase (POD) were coimmobilized in a human serum albumine (HSA) layer crosslinked by glutardialdehyde. 4-fluoroaniline was used as organo-fluoro cosubstrate for the peroxidase catalyzed reaction. A glucose sensitivity of 40.1 $mV/lgc_{glucose}$ was observed for a concentration range from 0.05 to 1 mmol/l. Relatively thick membranes produced by this immobilization method caused a long steady state response time of about 3 minutes. A life time of 4 days only were observed.

Secondly, GOD and POD were immobilized in crosslinked polyurethane (PU). Because the reaction product formed by POD from 4-flouroaniline changed diffusional properties of the enzyme/polyurethane-membrane, pentafluorophenol was used as organo-fluoro POD-cosubstrate. The PU/GOD-FET responds 4 times faster than the glucose ENFET with HSA-immobilization. The glucose sensitivity of this ENFET was $41.3/lgc_{glucose}$. A linear range from 0.01 to 0.2 mmol/l and a life time of at least 10 days were obtained. The fluoride sensitivity with and without membrane and the hydrogen peroxide sensitivity resulting only from the peroxidase catalyzed reaction were given in figure 3.

For comparison an equivalent amount of GOD containing gluconolactonase was immobilized in crosslinked PU onto a pH-FET. Than a lower sensitivity of 24.5 $mV/lcc_{glucose}$ was observed in a usual buffer solution of very low buffer capacity. A faster response was obtained probably caused by the faster transducer response and by a lower diffusional resistance of this only one enzyme containing membrane. The life time of this glucose ENFET was about 50 days.

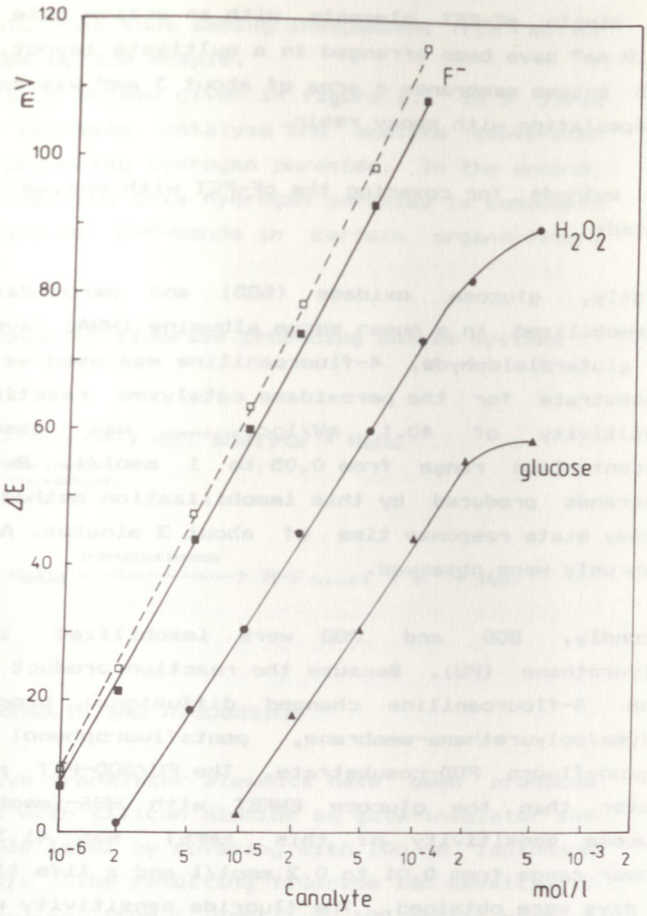

FIGURE 3.

Calibration curves of glucose-ENFET (GOD and POD in crosslinked PU)

sensitivities: ($mV/lgc_{analyte}$)

fluoride	
– without membrane	52.5
– with membrane	49.6
H_2O_2	42.6
glucose	41.3

Using also the PU-immobilization method, the pF-FET was covered with a lactate oxidase / POD – membrane. This relatively dense membrane showed a response time of about 65 s. After the enzyme coverage a sensitivity of 37.5 $mV/lgc_{fluoride}$ was found . The lactate sensitivity with 19.2 $mV/lgc_{lactate}$ from 0.005 to 0.5 mmol/l was also relatively low compared with the glucose sensitivity of the GOD/POD/PU-FET and could not be improved with an additional LOD/PU- membrane.

In conclusion the coupling of fluoride ion sensitive FETs with fluoride producing enzyme systems extends the use of FET-based biosensors. Because of the detection of fluoride ions measurements in strongly buffered solutions are possible without interferences of pH- or buffer capacity changes caused by the sample.