UREA BIOSENSOR BASED ON AN AMMONIA GAS PROBE USING AN NH_4^+-SENSITIVE PVC-ISE

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Summary

The low cost urea biosensor presented here is based on the detection of ammonia which is generated by an enzymatic reaction in the presence of urea. Therefore an ammonia gas sensor is used which consists of a potentiometric NH_4-electrode with a modified PVC membrane covered by a gas membrane.

Introduction

Urea is one of the bioproducts, which is monitored in blood and urine as an indication for the renal function. Moreover, urea is a hygienic parameter for instance in swimming pool water.

Earlier attempts for the development of urea biosensors were carried out by immobilizing urease (EC 3.5.1.5) on the surfaces of NH_4^+-sensitive glass electrodes and on silicon-nonactin NH_4^+-sensitive disc membrane electrodes. But these sensors have limited applications when used in physiological samples because of poor selectivities of the transducer for NH_4^+ ions in the presence of potassium and sodium ions, which are normally found in biological fluids as well.

In this poster we present a urea sensor with no interferences from these ions by using a transport via the gas phase.

Development

In the presence of urease urea decomposes according to the following equation:

$$(NH_2)_2CO + H_2O \xrightarrow{urease} CO_2 + 2\ NH_3$$

In aqueous solution an equilibrium will be established between the dissolved NH_3 gas and the NH_4^+ ions:

$$NH_{3\,(diss)} + H_2O \rightleftharpoons NH_4^+ + OH^-$$

By using these reactions it is possible to construct a urea biosensor by combining the enzymatic reaction with an NH_3 gas sensor.

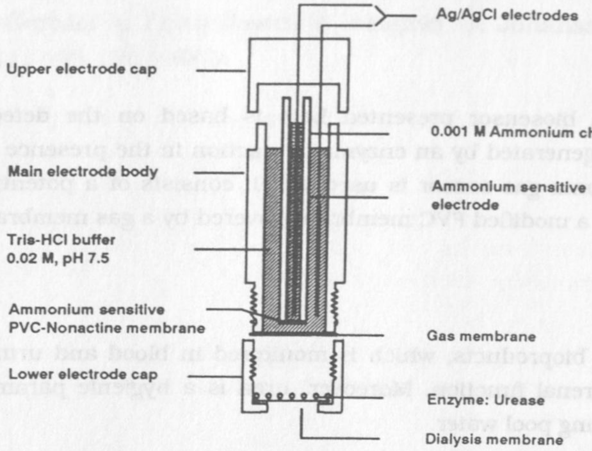

Fig. 1: Construction of the urea biosensor

Our sensor consists of a modified NH_4^+-sensitive PVC/nonactine membrane electrode, a special thin gas permeable membrane, a dialysis membrane and physically entrapped urease (Fig.1).

Caused by a pH gradient (pH 8 for the analyte and pH 7.5 for the inner buffer) the produced NH_3 is transfered over the gas membrane and protonated to NH_4^+ which is detected by the ion sensitive electrode.

Results and discussion

- The combination of the urease reaction with aspecial NH_3 gas probe leads to a reversible urea biosensor, which shows only very low interferences.
- The sensor works in physiological samples as well as in aqueous solution.
- The reproducibility is very high (Fig 2). The standard deviations of the blood measurements were always lower than 4%.

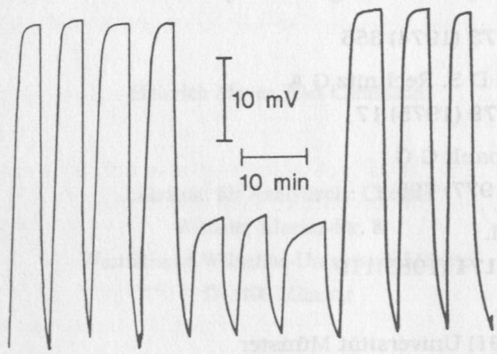

Fig. 2: Response of the sensor (0.02 Tris-HCl, pH 8); 1-4: urea standard solution $2.5 \cdot 10^{-5}$ mol/l, 5-7: swimming pool water, 8-10 blood plasma (sheep, diluted 1:200)

- A linear calibration curve is obtained in the range from $1 \cdot 10^{-6}$ to $4 \cdot 10^{-4}$ mol/l with a typical slope of 50 mV/decade (Fig. 3).
- The response time of the sensor is in the 2 minute range.

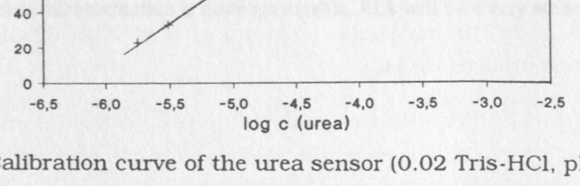

Fig. 3: Calibration curve of the urea sensor (0.02 Tris-HCl, pH 8)

- The observed life time was in the order of 4 weeks.
- The best membrane composition was: 33% PVC, 65.5% dibutylsebacate, 1% nonactine and 0.5% tetraheptylammonium tetraphenylborate.
- With this membrane no faradaic shielding is required.

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