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Short Communication

THE pH-STATIC ENZYME SENSOR
An ISFET-based Enzyme Sensor, Insensitive to the Buffer Capacity of the Sample

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Summary. An ISFET-based urea sensor is combined with a noble-metal electrode which provides continuous coulometric titration of the products of the enzymatic reaction. The sensor thus becomes independent of the buffer capacity of the sample; and because the enzyme is operating at a constant pH, the linear response range is expanded.

Enzyme sensors based on the measurement of pH consist basically of a pH-sensitive electrode to which an enzyme-loaded membrane is attached. The conversion of substrate in the immobilized enzyme membrane results in a local change in pH which can then be measured. The pH-sensitive electrode used is usually a glass membrane electrode or an ISFET. In 1980, Caras and Janata [1] described the first ISFET-based enzyme sensor sensitive to penicillin. The name ENFET was introduced for this sensor.

The response of enzyme sensors based on the measurement of pH is sensitive to the buffering capacity of the sample. Furthermore, these electrodes have a non-linear response for two reasons. First, the buffering capacity of the sample solution is pH-dependent; this implies that pH changes in the immobilized enzyme membrane depend on the initial pH of the sample solution. A second, and perhaps even more important reason for the non-linear response is the pH-dependent enzyme kinetics. It is well known that each enzyme has its own optimal pH value at which its activity is greatest. Thus, at high substrate concentrations, the pH changes in the enzyme membrane may become so large that the enzyme is actually inhibiting its own performance. The complex response characteristics of these enzyme electrodes have been the subject of a number of papers (see, e.g. [2, 3]) and the general conclusion is that the practical applicability is limited.

Recently, ISFET-based sensors have been developed for the performance of coulometric acid/base titrations on a very small scale. This is achieved through the application of a noble-metal electrode that is arranged closely around the pH-sensitive gate of the ISFET. Through the electrolysis of water, H^+ or OH^- ions are formed, depending on the direction of the current from this electrode to a distantly located counter electrode. In this way, a

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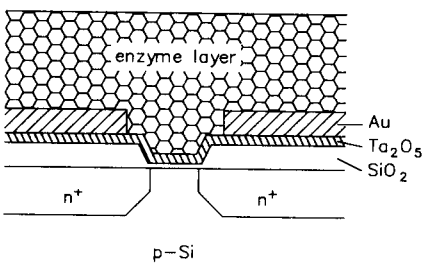
simple pH-actuator is added to the ISFET and local pH changes can be generated. The practical value of this technique has been shown in a system for titrations in microliter samples [4] and in a new type of carbon dioxide sensor with excellent long-term stability [5]. In this communication, the first results of the application of such a system in an enzyme sensor are presented. Through the use of a feedback system, the products of the reaction in the immobilized enzyme membrane are continuously titrated. In this way, the pH in the membrane is kept equal to that of the sample solution and thus the enzyme is operating at a constant pH. Therefore, it seems appropriate to call this sensor a pH-static enzyme sensor. The amount of current that is needed for the coulometric neutralization of the reaction products is proportional to the substrate concentration. The application of this technique for the improvement of the response of enzyme electrodes has also been proposed by Chandler and Eddowes [6].

Experimental

A cross-section of the pH-static enzyme sensor is shown in Fig. 1. The membrane consists of a cross-linked mixture of albumin and urease. The Ta_2O_5 gate oxide of the ISFET is silanized with 3-aminopropyltriethoxysilane. Bovine albumin (25 mg; Sigma A-7030) and 25 mg of urease (EC 3.5.1.5, Sigma Type IX, U-4002) were dissolved in 0.5 ml of 25 mM phosphate buffer, pH 7. To this solution, 0.2 ml of glutaraldehyde (2% in water) was added and a drop of this mixture was deposited over the surface of the sensor/actuator chip that was encapsulated with an epoxy resin. After some hours, the cross-linking reaction was complete and the sensors were ready for use.

The sensor that is thus created can of course also be operated like a "normal" ENFET, i.e., without the use of the generating electrode. In that



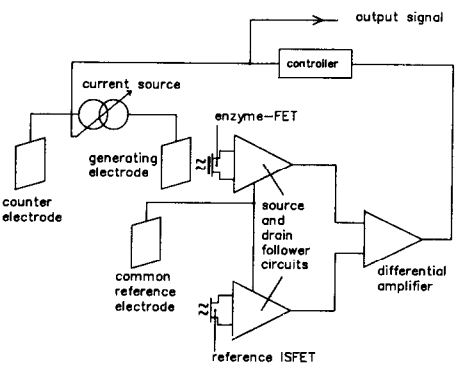


Fig. 1. Schematic cross-section of the pH-static enzyme sensor. The enzyme membrane is deposited over the entire structure.

Fig. 2. Coulometric control system in which the pH in the enzyme membrane is kept equal to that in the sample solution.

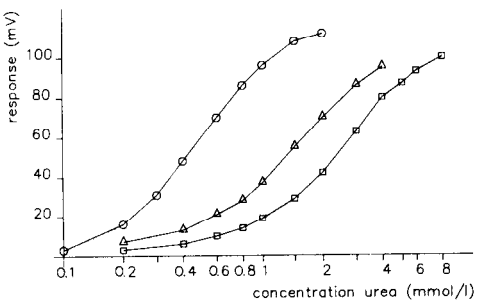
case, pH changes are measured as a function of substrate concentration. Measurements were made in phosphate buffers of various concentrations at pH 7. The buffer solutions were contained in an ordinary 100-ml glass beaker and were not stirred. The substrate concentration was changed through the addition of small aliquots of a concentrated urea solution.

If the sensor is to be operated in the pH-static mode, the control system outlined in Fig. 2 is used. In this system, the pH in the enzyme membrane is continuously compared to that of the sample solution as it is measured with the reference ISFET. If urea is added to the solution, the pH in the enzyme membrane will rise and the controller will then activate the current source that is connected to the generating electrode. The operation of the system is such that the alkaline products of the enzymatic reaction, i.e., ammonia, are constantly neutralized.

Results

Figure 3 shows that the response of the ENFET is dependent on the buffer capacity of the sample solution. The pH-sensitivity of the ISFET's used for these experiments was approximately 50 mV/pH. It can be seen that the output values level off when the response is about 100 mV. This change in output voltage corresponds to a pH value of ca. 9 in the immobilized enzyme membrane.

When the pH-static mode is used (Fig. 2), the amount of current required to provide continuous neutralization is linearly related to the substrate concentration (Fig. 4). The response is almost independent of the buffer capacity of the sample. A second important advantage of this method is that the linear range can be expanded. Without continuous neutralization, the response levels off at high substrate concentrations (Fig. 3). In the case of the pH-static sensor, however, the enzyme operates at constant pH and its activity is not inhibited by its own products. It must be noted that in the



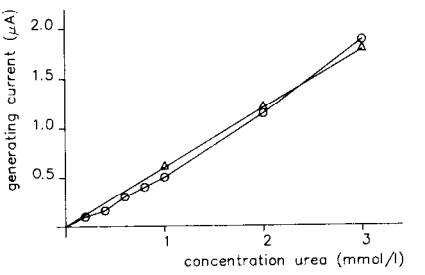


Fig. 3. The sensitivity and dynamic range of the urea-ENFET depend on the buffer capacity of the sample. Buffer concentration (mmol l^{-1}): (○) 1; (△) 5; (□) 10.

Fig. 4. The output of the pH-static enzyme sensor is independent of the sample buffer capacity and the dynamic range is expanded. Buffer concentration (mmol l^{-1}): (○) 1; (△) 5.

case of the normal ENFET, the response to added urea is logarithmic so that Fig. 3 is plotted on a semilogarithmic scale in the conventional manner. In contrast, the coulometric response with the pH-static sensor is linearly related to urea concentration (Fig. 4). Comparison of these two figures show that the linear response ranges for the 1 and 5 mmol l^{-1} buffers are clearly expanded with the latter method of measurement.

Conclusion

The first results obtained with the pH-static enzyme sensor show that this method of controlling the internal membrane pH is a practical solution for the problem of buffer dependence. However, various technological problems have yet to be solved. The results are fairly reproducible but it was found that albumin is not the most suitable membrane material. Because the pH-sensitive gate area of the ISFET and the generating electrode cannot be physically located in the same point, there is necessarily a small distance between the two. Therefore, during the application of the coulometric compensation current, a pH gradient is formed in the membrane. The pH directly at the surface of the generating electrode is considerably lower than the pH at the ISFET gate where it is kept equal to the bulk pH of the solution. Through these lateral pH differences, deformations occur in the protein layer and the response time of the sensor is increased. The construction of the sensor must therefore be optimized by choosing a different geometry or other membrane materials.

The integration of an enzymatic membrane with a sensor/actuator system offers the possibility of controlling the internal membrane pH. Of course, the method is not limited to keeping the membrane pH equal to that of the sample solution. It should also be possible to control the membrane pH in such a way that an enzyme is always operating at its optimal pH value.

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REFERENCES

1 S. Caras and J. Janata, Anal. Chem., 52 (1980) 1935.
2 S. D. Caras, J. Janata, D. Saupe and K. Schmidt, Anal. Chem., 57 (1985) 1917.
3 M. J. Eddowes, Sensors and Actuators, 7 (1985) 97.
4 B. H. van der Schoot and P. Bergveld, Sensors and Actuators, 8 (1985) 11.
5 B. H. van der Schoot and P. Bergveld, Proc. 2nd International Meeting on Chemical Sensors, Bordeaux, France, July, 1986, Université Bordeaux 1, ISBN 2-906257-00-1, 1986, pp. 665—668.
6 G. K. Chandler and M. J. Eddowes, Proc. 2nd International Meeting on Chemical Sensors, Bordeaux, France, July, 1986, Université Bordeaux 1, ISBN 2-906257-00-1, 1986, pp. 531—533.