

Penicillin Detection by Means of Silicon-Based Field-Effect Structures

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(Received July 24, 2000; accepted April 12, 2001)

Key words: penicillin, capacitive EIS sensor, cross-sensitivity, EnFET, low detection limit

Two types of semiconductor-based field-effect biosensors, i.e., a capacitive electrolyte-isolator-semiconductor (EIS) sensor and an enzyme field-effect transistor (EnFET) with different pH-sensitive transducer materials (Si_3N_4 and Ta_2O_5) and with different enzyme immobilisation methods (heterobifunctional cross-linking and physical adsorption) are investigated for penicillin detection. Capacitive EIS sensors with diffusion barriers are suitable for the detection of different kinds of penicillin: Penicillin G, ampicillin and amoxicillin. Compared to the conventional methods of measurement in penicillin solutions, the introduction of a diffusion barrier in combination with a suitable working buffer improves the detectable amount of penicillin to about $0.05 \mu\text{g}$. Here, only $10 \mu\text{l}$ of the analyte is needed for a single penicillin measurement. For a penicillin FET with adsorptively immobilised penicillinase, excellent working characteristics, namely, high penicillin sensitivity ($120 \pm 10 \text{ mV/mM}$ in the linear concentration range of $0.05\text{--}1 \text{ mM}$), low hysteresis ($< 4 \text{ mV}$), low detection limit of $10 \mu\text{M}$ and long lifetime (more than 5 months) were achieved by choosing Ta_2O_5 as a pH-sensitive transducer material, and a low capacity polymix buffer.

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1. Introduction

Biosensors based on the principle of the field effect in semiconductor structures have been extensively studied in recent years.⁽¹⁻⁸⁾ They use a common detection principle: enzyme molecules which are immobilised on the surface of the semiconductor structure convert the respective substrate to output a charged product. This product is detected by the ion-sensitive surface layer of the sensor device, and the resulting surface charge modulates the space charge region at the insulator-semiconductor interface.

The combination of a biologically sensitive material with silicon technology is very attractive due to the possibility of the miniaturisation and integration of multisensor systems and signal processing devices. The enzyme field-effect transistor (EnFET), introduced for the first time by Caras and Janata in 1980,⁽⁹⁾ and electrolyte-insulator-semiconductor (EIS) devices^(5-7,10) are two typical examples of microelectronic potentiometric biosensors. While both sensor types use the same detection principle and are thus similar with respect to the sensitivity characteristics, capacitive EIS sensors are cheaper and easier to prepare.^(5,7) However, since the sensitive area of an EIS sensor is generally larger than the gate region of an EnFET, more enzymes for immobilization and more analyte for measurement are needed. A very important feature of the EIS sensor is its complete flatness and the simplicity of its layout. The sensitive surface of this sensor is free of any metal contacts, i.e., no corrosion-resistant encapsulation of electrical connections against the surrounding liquid is necessary. The same does not apply to EnFETs. Usually the source and drain contacts and bonding wires are on the top surface next to the sensitive gate region, therefore resulting in sensor encapsulation problems. Up to now, there has been no established method of encapsulation of ion-selective FETs (ISFETs) and EnFETs. To achieve "geometrically selective" encapsulation, partially successful attempts have been made at using structures such as Si-on-sapphire⁽¹¹⁾ and Si-SiO₂-Si.⁽¹²⁾ Nevertheless, most encapsulations are currently still made by hand. Such preparation steps are time-consuming and thus, noneconomical and unsuitable for batch fabrication processes.

On the other hand, the capacitive EIS biosensors are less sensitive, from an electrical point of view, than EnFETs and therefore, less suitable for miniaturisation (the measured capacitance values on miniaturised sensors often become too small for accurate measurements). In contrast, the EnFET is an active device and has a greatly reduced need for primary signal processing circuits, which can be more easily integrated on the same chip containing the biosensor. In addition, by using a dual sensor chip with the differential measurement method disturbing variables, such as the influence of pH of the measuring solution, thermal effects and light sensitivity, can be eliminated.^(12,13)

At present, many potentiometric enzyme biosensors have been developed for the detection of penicillin G, mainly for the analysis of fermentation broths.^(3,5,13-15) However, for many fields of application (drug control, clinical laboratories, food control, *etc.*) a rapid and inexpensive analytical method and (bio)chemical microsensors for the detection of small amounts of penicillin are needed. Such sensors should be highly stable over the long term as well as have a low detection limit.

The characteristics of penicillin biosensors based on pH-sensitive semiconductor field-effect structures strongly depend on the type of pH-sensitive material, on the pH and

capacity of the buffer solution, on the thickness of the enzyme membrane and on the immobilisation method. One possible method of improving the detection limit has been suggested by Thust *et al.*⁽¹⁶⁾ By introducing a diffusion barrier, which prevents the analyte from diffusing away from the sensor surface, both the amount of analyte and the detection limit of this capacitive sensor for penicillin G were significantly reduced. Another method is the optimisation of the sensor preparation and measurement conditions through the selection of a transducer material with a high pH sensitivity and stability, as well as an appropriate enzyme immobilisation method and working buffer.

In this work, the results of investigations on two types of penicillin biosensors – a capacitive EIS sensor with a diffusion barrier and a penicillin FET (PenFET) – with different pH-sensitive transducer materials (Si_3N_4 and Ta_2O_5) and with different enzyme immobilisation methods (heterobifunctional cross-linking and adsorptive) are presented. The cross-sensitivity of the EIS sensor to different kinds of penicillin, namely, penicillin G, ampicillin, amoxicillin and cloxacillin, is also presented.

2. Experimental

2.1 Sensor structure and preparation

2.1.1 EIS sensor

The structure and the operation principle of the capacitive EIS biosensor is shown in Fig.1. The penicillin biosensor consists of a pH-sensitive EIS structure with immobilised β -lactamase (penicillinase). The operation principle of the penicillin biosensor based on the pH-sensitive field-effect structure is as follows. The pH-sensitive transducer detects variations in the H^+ -ion concentration resulting from the catalysed hydrolysis of penicillin by the penicillinase. A resulting local pH decrease near the pH-sensitive layer leads to a change in the capacitance of the EIS structure (or a change of the drain current in the case of the PenFET).

The pH-sensitive EIS structures adopted, consisting of $\text{Al/Si/SiO}_2/\text{Si}_3\text{N}_4$, were fabricated from p-type silicon wafers (18–24 Ωcm , (100) orientation, Wacker Chemitronic) with a thermally grown SiO_2 and a plasma-enhanced chemical vapour deposition (PECVD) Si_3N_4 layer with thicknesses of 30 nm and 50 nm, respectively. As an ohmic contact, 200 nm of Al was evaporated on the back of the wafers. Finally, the wafers were cut into single sensor chips of $10 \times 10 \text{ mm}^2$.

The basic characteristics of the pH-sensitive EIS sensors with a PECVD- Si_3N_4 layer were recently reported.⁽¹⁷⁾ A typical pH response is linear in the range from pH 3 to pH 10 with an average slope of 54 mV/pH. The sensors show a long-term stability of more than 5 months and relatively small drift values of the sensor output signal of less than 3 mV/day.

2.1.2 PenFET

The PenFET developed is composed of a H^+ -sensitive Ta_2O_5 -gate ISFET and an immobilised enzyme layer that covers the gate region of the ISFET. The pH ISFETs were n-channel devices fabricated from a p-type boron-doped silicon wafer with (100) orientation and 20 Ωcm specific resistivity using standard n-channel technology. The channel was approximately 1 mm wide and 30 μm long, and was made by ion implantation of

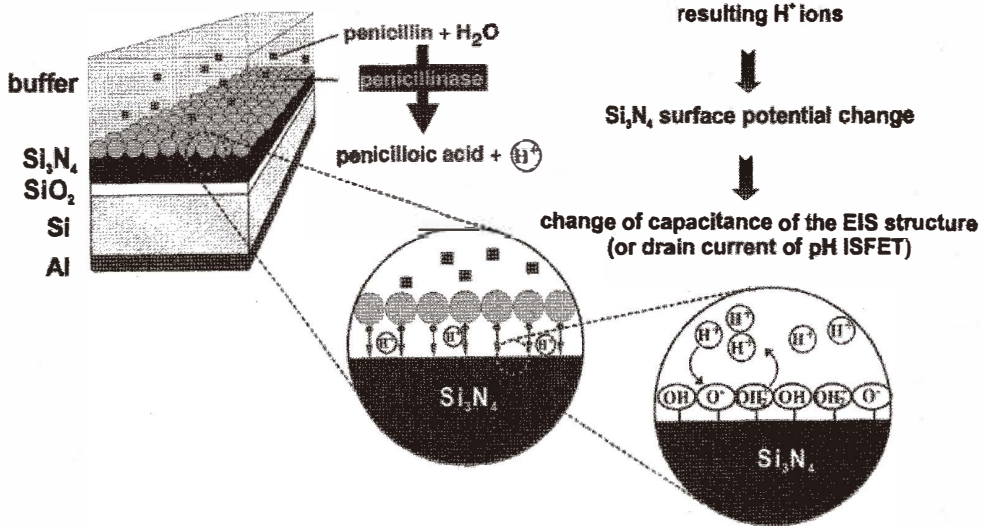


Fig. 1. Structure and operation principle of the capacitive EIS penicillin biosensor.

phosphorus. The source was connected to the bulk. The gate composite insulator consists of an approximately 80-nm-thick thermally grown silicon dioxide layer, prepared in dry oxygen atmosphere at 1,150°C, covered by a Ta_2O_5 film (also 80 nm thick). The Ta_2O_5 layer also served as a passivation layer. The preparation of the pH-sensitive Ta_2O_5 films was optimized with regard to a high pH sensitivity, low hysteresis and small drift. They were prepared by thermal oxidation of sputtered Ta in an oxygen atmosphere at 510–530°C for about 2 h. Aluminium was evaporated on the contact pads, providing contacts to the source and drain of ISFET. For easy encapsulation of the device, the pH-sensitive gate was positioned as far from the bonding pads as possible.⁽¹⁸⁾ After scribing, sensor chips having dimensions of 1.6 mm × 2.67 mm were obtained. For the ISFET parameter analysis, some chips contained a control metal-oxide-semiconductor FET (MOSFET) with an Al gate. Figure 2 shows the scanning electron microscopy (SEM) image of the chip layout with an Al-gate MOSFET.

The sensor chips were bonded and glued onto a printed circuit board. After wiring, the FETs were encapsulated using an epoxy compound (Epo-Tek 77, Epoxy Technology), so that only the ISFET gate was exposed to the environment, while all electrical contacts were insulated by the epoxy encapsulant. The contact area of the ISFET gate with the solution was about 1 mm². Some sensors were prepared in the form of a catheter chip.

The basic characteristics of the developed pH-sensitive Ta_2O_5 gate ISFETs have been reported elsewhere.⁽¹⁸⁾ A typical pH response was linear between pH 2 and 12 with a slope of 55–58 mV/pH. The drift of the output voltage with time was less than 0.5 mV/h, and the hysteresis was about 0.02 pH. The pH response of the PenFETs did not change due to the immobilisation of the penicillinase on the Ta_2O_5 surface.

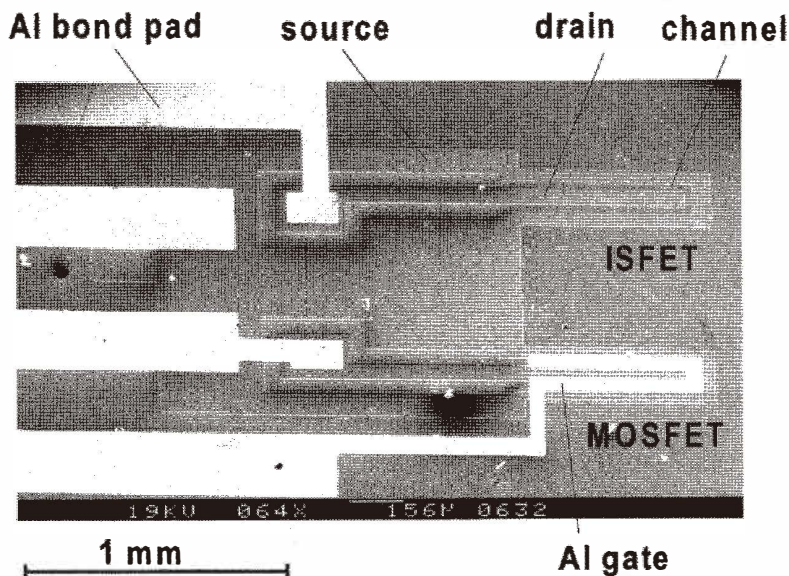


Fig. 2. SEM image of the sensor chip layout with a Ta₂O₅-gate pH ISFET and a control Al-gate MOSFET.

2.2 Enzyme immobilization

2.2.1 Heterobifunctional cross-linking (EIS sensor)

The capacitive EIS biosensor was realised by immobilising the enzyme penicillinase (EC 3.5.2.6., *Bacillus cereus* from Sigma, with a reported specific activity of 1650 units/mg protein) by means of heterobifunctional cross-linking on top of the pH-sensitive Si₃N₄ films. The choice of Si₃N₄ as a pH-sensitive material is predetermined by the presence of amine groups on the Si₃N₄ surface, which are necessary for linking the cross-linker molecules. As a heterobifunctional cross-linking reagent, N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS, from Pierce) was used. The immobilisation of penicillinase was performed in two distinct steps. In the first step, the amine-specific functional groups of the cross-linker molecules were directly linked to the Si₃N₄ surface. For this purpose, the Si₃N₄ surface of the sensor chip was incubated with 5 mM ANB-NOS in 200 mM triethanolamine (TEA) buffer, pH 8, for 30 min to allow the succinimide ester group of the cross-linker to react with the surface amine groups of Si₃N₄. Thereafter, the chip was rinsed with TEA buffer to remove surplus cross-linker molecules which were not bound to the transducer surface. The immobilisation with the cross-linker ANB-NOS had to be carried out in the dark since the cross-linker molecule possesses a phenyl azide group that can be photoactivated by UV light.

In the second step, the second reactive groups of the fixed cross-linker molecules were made to bind with the enzyme molecules. For this purpose, the enzyme solution consisting

of 2 mg penicillinase per ml TEA buffer, pH 8, was incubated for 5 min on the sensor surface. The activation of the phenyl azide ring, and hence, the binding of the enzyme to the cross-linker molecules, was achieved by UV radiation.

Since no enzyme is present in the first step, the polymerisation of the enzyme molecules, which often occurs when homobifunctional cross-linkers are used, is prevented. Thus, the enzyme molecules are stably bound to the surface instead of being cross-linked to each other. Finally, the sensors were rinsed with deionised water and dried for at least one hour at room temperature. Before the first use, they were incubated in the working buffer for at least 12 h to equilibrate the enzyme membrane. When not in use, the penicillin sensors were stored in 0.2 mM polymix buffer, pH 8, at 4°C. Details of the immobilisation procedure are given in ref. 19.

2.2.2 Adsorptive immobilisation (PenFET)

Many kinds of EnFETs have been developed so far. In general, enzymes were immobilised to the gate surface of ISFETs by the use of cross-linking or enzyme entrapment methods. We have examined the performance of PenFETs with penicillinase adsorptively immobilised onto the Ta₂O₅ gate of a pHISFET. The main advantages of the adsorptive immobilisation are the simplicity and low cost of this method without any loss of enzyme activity and with the possibility of a subsequent enzyme regeneration.

The enzyme solution was prepared by dissolving the enzyme penicillinase in 200 mM TEA buffer, pH 8. Ten μ l enzyme solution per sensor was pipetted onto the samples and incubated at room temperature for about 1–2 h. Then, the sensors were rinsed for 1 min in deionised water and dried at room temperature for 3 h.

2.3 Measurement setup

2.3.1 Constant capacitance method (EIS sensors)

The characterisation of the EIS biosensors was performed with an impedance analyser (Zahner Elektrik) by means of the constant capacitance (ConCap) method. The EIS structures form a plate capacitor with a variable capacitance depending on the pH value of the contacting solution. Figure 3 shows results of measurements in both the C/V and ConCap modes. The C/V curves of the EIS structure are shifted along the voltage axis as the penicillin concentration (and consequently, the pH value) of the solution is changed (Fig. 3, top, left). In the ConCap mode, by setting the capacitance at a fixed value (usually ~60% of the maximum capacitance), the voltage shift which results from the increase of H⁺-ion concentration at the sensor surface due to the enzymatic reaction can be directly recorded (Fig. 3, top right). The ConCap measuring mode allows a direct investigation of the dynamic behaviour of the sensor signal.⁽²⁰⁾ To obtain the calibration curve of the sensor, the measured voltage values are plotted against the corresponding penicillin concentration (Fig. 3, bottom).

The ConCap measurements were carried out at a frequency of 120 Hz with an ac voltage amplitude of 20 mV.⁽¹⁹⁾ For this purpose, the sensor was mounted in a measuring cell, as shown in Fig. 4. The front of the sensor was in contact with the working buffer and a Ag/AgCl reference electrode and the back, with a gold-plated pin. Recently performed investigations of penicillin G biosensors with a diffusion barrier in Tris and polymix

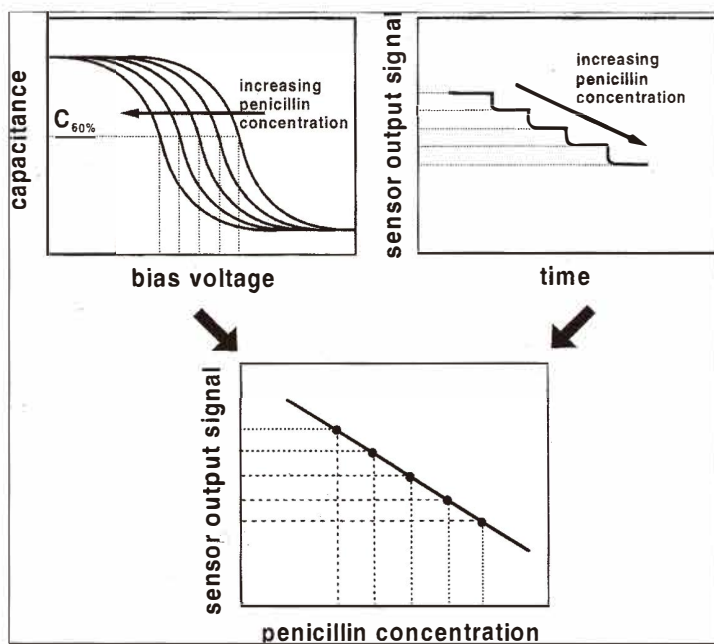


Fig. 3. Evaluation of capacitance-voltage (C/V) and constant-capacitance (ConCap) measurement modes.

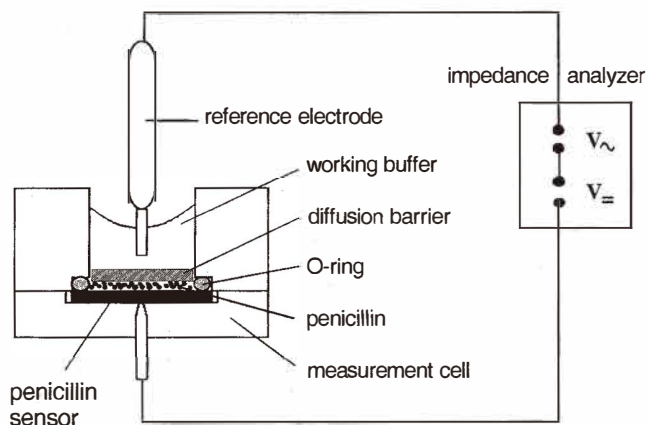


Fig. 4. ConCap measurement setup consisting of the sensor with a diffusion barrier, the reference electrode and the impedance analyzer.

buffers with different buffer capacities have shown⁽¹⁶⁾ that a low capacity in the case of the polymix buffer (pH 8) is optimal with regard to a lower detection limit. Therefore, in the present work, a 0.2 mM polymix multicomponent buffer solution containing 100 mM KCl to adjust the ionic strength was used as a working buffer. The pH of the polymix buffer was adjusted to pH 8 by titration with NaOH solution. This buffer type has a constant buffer capacity over a wide range of pH (pH 5 to pH 9). A detailed description of the preparation of the polymix buffer is given in refs. 21 and 22.

For sensor measurements, discs of PVC plastic foil (Xerox) were used as a diffusion barrier. The diffusion barrier prevents the analyte from diffusing away from the sensor surface. Ten μl of a solution containing 0.05 to 5 μg penicillin G, ampicillin or amoxicillin, or 0.3 to 30 μg cloxacillin were dried on the discs (all analytes were purchased from Sigma). The diameter of the diffusion barrier was slightly smaller than the inner diameter of the O-ring, in order to retain the electrical contact with the conventional Ag/AgCl reference electrode. The contact area of the sensor with the solution was about 0.4 cm^2 .

The measurement cycle starts with a ConCap measurement in the working buffer. The measurement cell was filled with 1 ml of the working buffer and the voltage was recorded for 2.5 min. Subsequently, the disc was placed with the penicillin-covered side on the sensor surface and the voltage was recorded for each amount of analyte for 2.5 min. Then the respective sensor signal was taken as the measured value. After each concentration step, the cell was rinsed with working buffer. The voltage difference $\Delta V = V_{\text{analyte}} - V_{\text{buffer}}$ was taken as the sensor signal, where V_{analyte} and V_{buffer} are the output signals of the penicillin sensor with the analyte-covered diffusion barrier and in the buffer solution without analyte, respectively. The measurements were performed in a dark Faraday cage at room temperature. All chemicals used were of analytical-reagent grade. Bidistilled or deionised water were used for the preparation of the penicillin and buffer solutions.

2.3.2 Constant charge method (PenFETs)

The characteristics of the PenFETs have been measured in the constant-charge mode with a grounded reference electrode using a 4-channel ISFET/BioFET-meter fabricated in our laboratory (Research Centre Jülich). The measurement setup is presented in Fig. 5. For the PenFETs, the source voltage is measured while the drain current I_D and the drain-source voltage V_{DS} are kept constant. In the constant-charge mode, by setting the drain current at a fixed value, the voltage shift ΔV which results from the increased H^+ -ion concentration at the transducer surface due to the enzymatic reaction can be directly recorded (the sensor output signal V_{output} is proportional to the voltage shift ΔV). The constant-charge mode with the grounded reference electrode allows a simultaneous measurement of the characteristics of multisensors to be carried out using only one reference electrode. The measurement system is controlled by a personal computer, using appropriate data-acquisition software that allows the user to carry out the measurement easily.

The PenFET chip was immersed in the measurement cell filled with about 3 ml of working buffer or respective penicillin solution, and the sensor output signal was recorded for about 3 min. The measurement cycle starts with a constant-charge-mode measurement in the working buffer. To achieve a low detection limit, a low-capacity working buffer of 0.2 mM polymix buffer, pH 8, was used. The penicillin solutions were prepared by

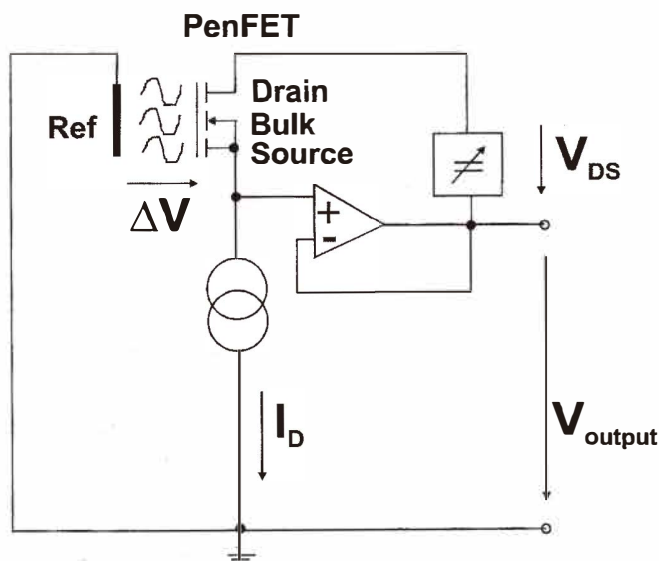


Fig. 5. Constant-charge-mode measurement setup for the PenFET. Ref.: reference electrode; V_{DS} : drain-source voltage; I_D : drain current; V_{output} : sensor output signal; ΔV : voltage shift due to the enzymatic reaction.

dissolving penicillin G (benzylpenicillin, 1,695 units/mg, Sigma) in the working buffer. After each concentration step, the PenFET gate was rinsed with distilled water. The storage, equilibration and measurement conditions (temperature, reference electrode, etc.) of the PenFETs were the same as those described for the EIS sensors.

3. Results and Discussion

3.1 Penicillin measurements by means of an EIS biosensor with a diffusion barrier

As an example, Fig. 6 shows the profile of a typical ConCap measurement of the EIS biosensor with a diffusion barrier. The amount of penicillin G was changed at the times marked by arrows. The sensor output signal was recorded for each amount of penicillin G and in the buffer solution over a time period of 2.5 min. Before each measurement with the diffusion barrier, the sensor output signal was recorded in the buffer solution to reproduce the starting conditions. When the amount of penicillin G on the diffusion barrier decreases from 50 to 0.5 μg , the concentration of the H^+ ions resulting from the penicillin hydrolysis is decreased. As a result, the voltage that is necessary to adjust the constant capacitance value decreases. Thus, the recorded voltage signal correlates directly with the respective amount of penicillin G on the diffusion barrier.

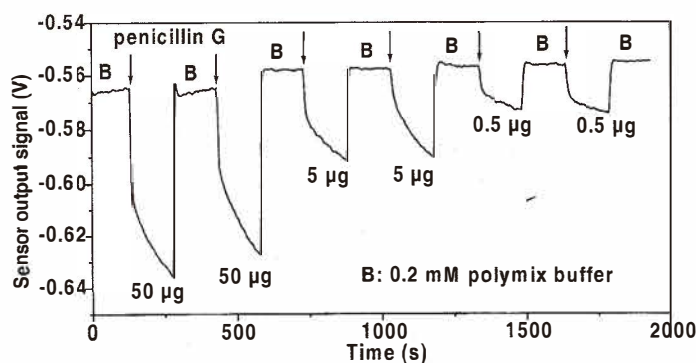


Fig. 6. Typical results of ConCap measurement of an EIS biosensor with a diffusion barrier. The amount of penicillin G was changed at the times indicated by arrows. B: 0.2 mM polymix buffer, pH 8.

The time-dependent behaviour of the biosensor signal towards penicillin G and ampicillin over a measurement period of 2 months is presented in Fig. 7. The respective sensor signal was evaluated at a concentration of $0.5 \mu\text{g}$. Within this investigation period, the sensor shows a reproducible output signal for both penicillin G and ampicillin, and relatively small deviations of the sensitivity.

Figure 8 shows the calibration curves obtained from ConCap measurements of the EIS biosensor with a diffusion barrier for the various kinds of penicillin. The sensor signal is plotted vs the logarithm of the amount of analyte. Additionally, in this figure the calibration curve of the same sensor without a diffusion barrier in amoxicillin solutions is presented. For visual comparison, the amoxicillin concentration in the solution is presented in units of $\mu\text{g}/\text{ml}$. The results for penicillin G, ampicillin and amoxicillin are nearly equal. Reproducible sensor signals of about -2 mV were measured for $0.05 \mu\text{g}$ penicillin and about -11 mV and -23 mV for 0.5 and $5 \mu\text{g}$ penicillin, respectively. For cloxacillin, the sensor signal changes from -3 to -23 mV for analyte amounts between 0.3 and $30 \mu\text{g}$. As can be seen from Fig. 8, compared to the usual measurements in amoxicillin solutions, the introduction of a diffusion barrier in combination with a suitable working buffer improves the detection limit of amoxicillin (as well as of penicillin G and ampicillin) to about $0.05 \mu\text{g}$. Since the analyte is concentrated near the sensor surface, the sensor signals are comparable to those obtained with penicillin solutions though the amounts of penicillin detected using a diffusion barrier are two orders of magnitude lower. All data presented here are averages of at least 15 measurements.

It is known that freshly prepared reference standard solutions of different kinds of penicillin usually contain a certain amount of the corresponding penicilloic acid (0.5 – 1% of the penicillin concentration as reported in ref. 23) due to the hydrolysis of penicillin. Consequently, the penicillin biosensor can observe a certain blank pH response, particularly in a working buffer with low buffer capacity. In order to correct the calibration curves

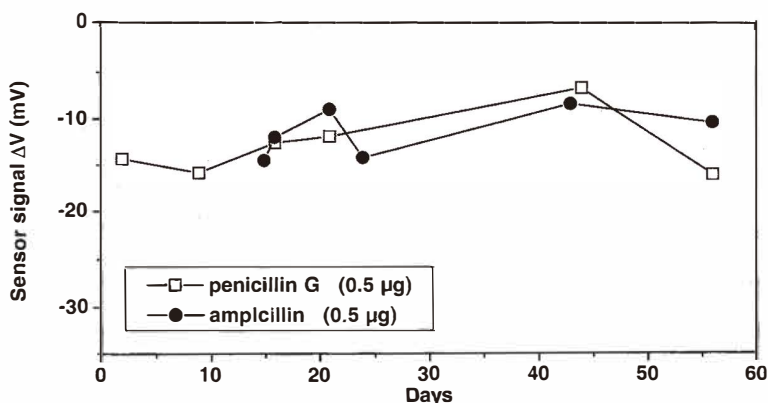


Fig. 7. Time-dependent change of the signal of penicillin G and ampicillin of an EIS biosensor with diffusion barrier over a measurement period of 56 days. The amount of penicillin G and ampicillin was 0.5 μg .

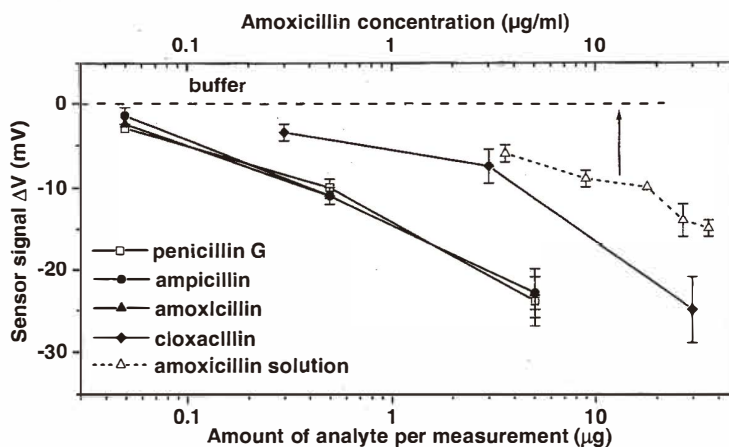


Fig. 8. Calibration curves obtained from the ConCap measurements of the EIS biosensor with a diffusion barrier for various kinds of penicillin, and the calibration curve of the same sensor without a diffusion barrier for an amoxicillin solution (Δ).

for possible pH interferences, the same measurements as described above were performed with a capacitive pH sensor (without the enzyme penicillinase) instead of the biosensor. The net calibration curves corrected for the pH interferences are shown in Fig. 9. Since the results obtained for cloxacillin with the penicillin sensor and the pH sensor are nearly equal, no net sensitivity exists for this kind of penicillin in the investigated concentration range. In contrast, the corrected calibration curves for penicillin G, ampicillin and

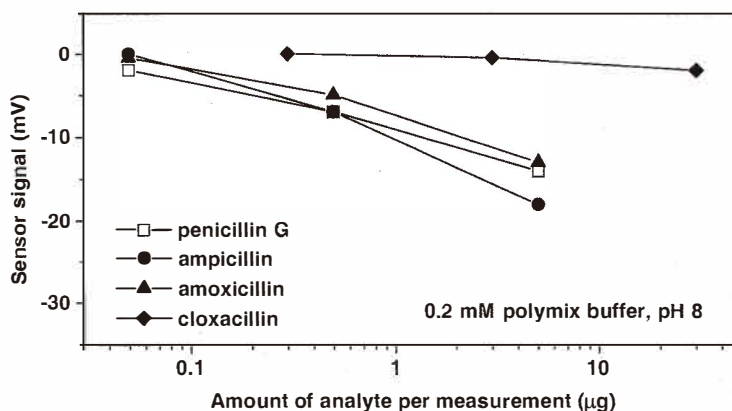


Fig. 9. Net calibration curves corrected for pH interferences.

amoxicillin exhibit very similar shapes of the sensor signals up to about -20 mV for $5 \mu\text{g}$ penicillin. The similar sensitivities of the developed capacitive penicillin sensors (with the enzyme β -lactamase) to penicillin G, ampicillin and amoxicillin can be explained by the fact that all of these different kinds of penicillins have the same lactam ring. In all cases, the lactam ring is hydrolyzed by the enzyme penicillinase, resulting in liberated H^+ ions which can be detected by the pH-sensitive Si_3N_4 layer.

The observed negligible sensitivity of the sensor to cloxacillin in the investigated concentration range, and consequently the higher stability of cloxacillin against penicillinase, correlates well with the results recently reported in ref. 24. The fact that cloxacillin is more resistant to β -lactamase than penicillin G, ampicillin and amoxicillin indicates that different types of enzyme-analyte complexes may be formed, depending on the nature of the penicillin side-chain group. For the cloxacillin, this is due to the aminoalicyclic side-chain which prevents penicillinase from attaching to the β -lactam ring.

3.2 Penicillin determination by means of PenFET

For comparison with the EIS biosensor, Fig. 10 shows the typical input characteristics of a PenFET in a working buffer and in penicillin G solutions with concentrations of 0.25, 0.5 and 1 mM. With increasing concentration of penicillin in the solution from 0.25 to 1 mM, the input curves of the PenFET are shifted in the direction of a decreasing threshold voltage V_T , which results from the increase of the H^+ -ion concentration at the sensor surface due to the enzymatic reaction. For the PenFETs used (n-channel), an additional positive charge on the gate surface is expected to lead to a decrease of the threshold voltage. The threshold voltage shift ΔV_T and gate voltage shift ΔV_G are proportional to the change of H^+ -ion concentration (ΔpH) at the Ta_2O_5 surface. At a penicillin concentration of 1 mM, ΔV_T (or ΔV_G) was about 130 mV. Considering the average pH sensitivity of the PenFETs to be 58 mV/pH, this voltage shift corresponds to a pH value at the Ta_2O_5 surface of 5.76 (by the bulk buffer solution of pH 8), i.e., ΔpH of 2.24.

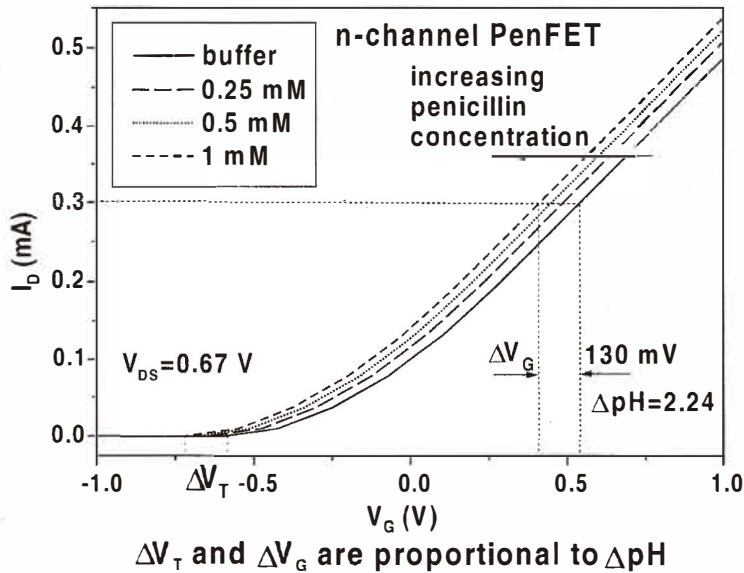


Fig. 10. Input characteristics of a PenFET (n-channel) in a working buffer and in penicillin G solutions with concentrations of 0.25, 0.5 and 1 mM. I_D : drain current; V_{DS} : drain-source voltage; V_G : gate voltage; V_T : threshold voltage.

As in the ConCap mode for the EIS sensors, in the constant-charge mode, the output signal of the PenFET can be directly recorded by adjusting the drain current at a fixed value. As an example, Fig. 11 shows the constant-charge-mode measurement of a PenFET at the 150th day after enzyme immobilisation. During this time period, the sensor characteristics were periodically measured. The sensor output voltage correlates with the respective penicillin concentration in the solution. Moreover, the sensor output signal was almost the same (with a small hysteresis) in the upward and downward series of the measurement within one measurement cycle. The sensor response time was between 0.5 and 3 min, depending on the penicillin concentration.

Figure 12 shows the typical calibration curves of the PenFET for the upward and downward series obtained from the constant-charge-mode measurement (see Fig. 11). Here, the output signal in the buffer solution for upward measurements is taken as zero. The PenFET shows a high average sensitivity of 130 mV/mM in the penicillin concentration range from 0.05 to 1 mM. The upper detection limit is about 20 mM. The lower detection limit is a complex function of the diffusion rate, the buffer capacity and the enzyme activity and amounted to 0.01 mM.

The results for long-term stability are presented in Fig. 13. When stored in 0.2 mM polymix buffer, pH 8, at 4°C, and periodically measured in penicillin solutions at room temperature (as described in section 2.3.2), the PenFETs were stable for at least 5 months;

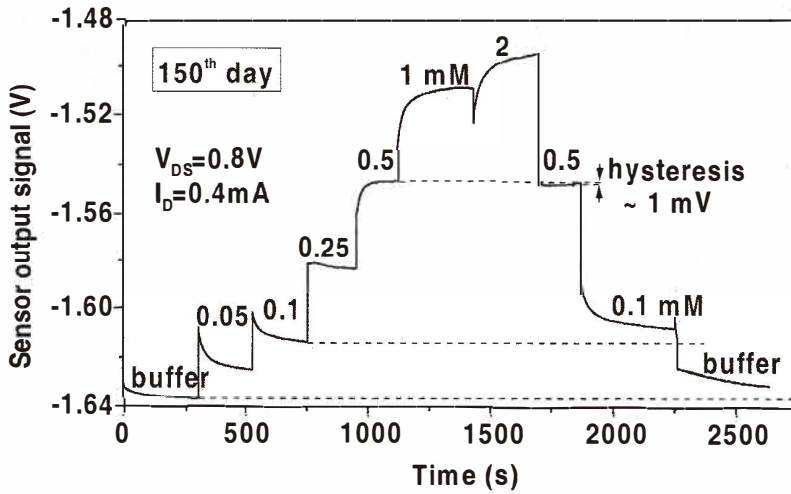


Fig. 11. Constant-charge-mode measurement of a PenFET at the 150th day after enzyme immobilisation. I_D : drain current; V_{DS} : drain-source voltage.

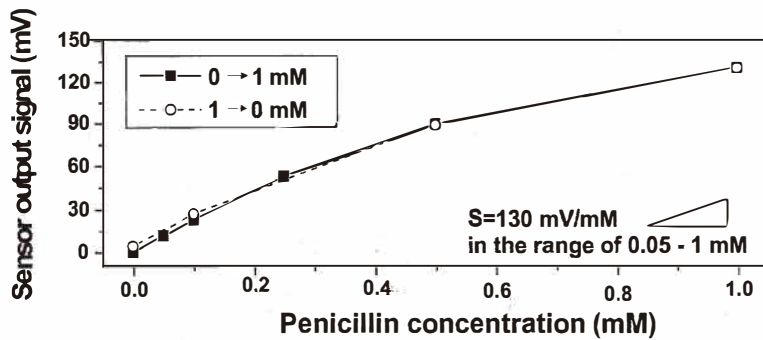


Fig. 12. Calibration curve of the PenFET obtained in the constant-charge mode.

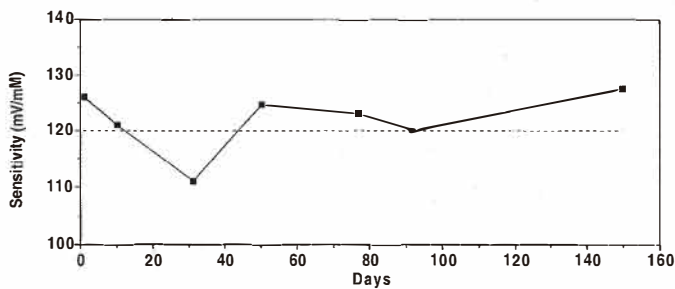


Fig. 13. Long-term stability of the PenFET over a period of 150 days.

sensitivity values fluctuated within ± 10 mV/mM at average sensitivity values of about 120 mV/mM. During this time, the sensors were used for more than 250 measurements.

Figure 14 shows the results of the investigation of the PenFET hysteresis at different penicillin concentrations (from 0.05 to 10 mM) for different time periods (31 days, 50 days, 77 days and 150 days). The hysteresis is defined as the sensor output signal difference between the upward and downward series of measurements (see Fig. 11). The developed PenFETs with the adsorptively immobilised penicillinase possess a small hysteresis that is generally less than 4 mV.

4. Conclusions

Two types of semiconductor-based field-effect biosensors (capacitive EIS sensors with diffusion barrier and PenFETs) were developed and tested for the detection of small amounts of penicillin.

1. The capacitive EIS biosensor with a diffusion barrier is suitable for the detection of different kinds of penicillin: penicillin G, ampicillin and amoxicillin. Due to its resistance against the enzyme β -lactamase, cloxacillin cannot be detected in the investigated concentration range using the sensor adopted in this work. Compared to conventional measurement methods in penicillin solutions, the introduction of a diffusion barrier in combination with a suitable working buffer lowers the detection limit to about $0.05 \mu\text{g}$. Only $10 \mu\text{l}$ of the analyte solution is needed for a single penicillin measurement. Since a shift of the pH value can occur during sample preparation, a correction for pH interferences in the sensor signal or differential mode measurements should be performed. The suggested measurement principle of using a diffusion barrier is not restricted to a certain kind of sensor or analyte.

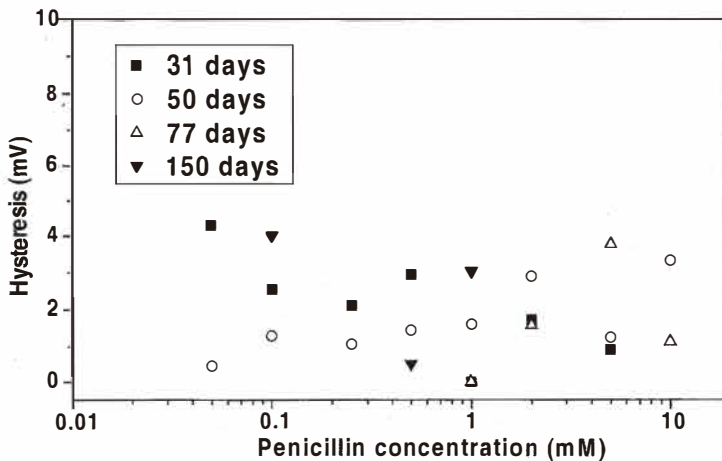


Fig. 14. Hysteresis of the PenFET at different penicillin concentrations.

It can be applied to a variety of chemical sensors and biosensors without any modification of the existing sensor geometry.

2. The results of the PenFET measurement demonstrate that by choosing the optimal pH-sensitive transducer material and low-capacity working buffer, excellent working characteristics, namely, a high penicillin sensitivity (120 ± 10 mV/mM in the linear concentration range from 0.05 to 1 mM), a low hysteresis (< 4 mM), a low detection limit of about $10 \mu\text{M}$ and a long lifetime (more than 5 months) can be achieved for this type of biosensor with adsorptively immobilised penicillinase. The main advantages of the adsorptive immobilisation method are its simplicity and low cost, without any loss of the enzyme activity and with the possibility of enzyme regeneration.

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