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A (Bio-)Chemical Field-Effect Sensor with Macroporous Si as Substrate Material and a SiO_2 / LPCVD-Si_3N_4 Double Layer as pH Transducer

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Abstract: Macroporous silicon has been etched from n-type Si, using a vertical etching cell where no rear side contact on the silicon wafer is necessary. The resulting macropores have been characterised by means of Scanning Electron Microscopy (SEM). After etching, SiO_2 was thermally grown on the top of the porous silicon as an insulating layer and Si_3N_4 was deposited by means of Low Pressure Chemical Vapour Deposition (LPCVD) as transducer material to fabricate a capacitive pH sensor. In order to prepare porous biosensors, the enzyme penicillinase has been additionally immobilised inside the porous structure. Electrochemical measurements of the pH sensor and the biosensor with an Electrolyte/Insulator/Semiconductor (EIS) structure have been performed in the Capacitance/Voltage (C/V) and Constant capacitance (ConCap) mode.

Keywords: Macroporous silicon, pH sensor, Penicillin biosensor, Thermal oxidation, LPCVD-Si_3N_4, long-term stability.

Introduction

Porous silicon is well-suited as an alternative material for chemical sensors and biosensors [1-3]. The use of porous instead of planar silicon as substrate material provides many advantages. Due to the large surface area of porous silicon [4], a simple miniaturisation of capacitive EIS (Electrolyte/Insulator/Semiconductor) sensors down to the µm scale is possible since the measuring signal (capacitance value) raises with increasing the effective sensor area [5]. Inside the pores sensing components, like biomolecules (e.g., enzymes) can be stable fixated to protect the sensor from a fast leaching out of the molecules that results in a higher mechanical stability. The fabrication of porous Si by means of an anodic etching process is fully compatible to the semiconductor processing in sensor fabrication [6,7]. Moreover, in contrast to ISFETs (ion-sensitive field-effect transistors [8]), no photolithographic process steps and no additional passivation layers are necessary. Thus, the sensor can be prepared in an easy and cheap way and no life-time limiting detachment of the passivation layer will be expected.

Nevertheless, the chemical sensors and biosensors with macroporous silicon as substrate material, developed so far, have one decisive drawback: because of the metallic rear side contact on the silicon substrate that is necessary during the etching process, subsequent process steps only allow to apply low-temperature processes such as Plasma-Enhanced Chemical Vapour Deposition (PECVD); by means of these processes, the necessary dielectric and pH-sensitive materials (SiO_2, Si_3N_4) will be deposited inside the pores. However, a comparison of planar sensors, where the dielectric and sensitive materials were deposited using low-temperature processes, with those fabricated by high-temperature processes, shows a clearly improved behaviour in sensitivity, stability and reproducibility in the case of the sensors prepared in a high-temperature regime [9]. Therefore, in this work a vertical etching set-up was designed to etch silicon substrates without a metallic rear side contact, but an aqueous liquid one. After adjusting the proper etching profile, the subsequent thin film layers can be deposited by means of high-temperature processes (thermal oxidation, LPCVD: Low Pressure Chemical Vapour Deposition), yielding porous EIS sensors with an improved stability in the long term.

Experimental

N-doped silicon <100>-wafers (Fa. Wacker-Chemitronic), with a thickness of 330-430 µm and a specific resistance of 12-27 Ωcm were used to fabricate the porous samples. Before processing, the wafer was quartered and the samples were purified by successively immersing in acetone, propanol and de-ionised water. A macroporous silicon layer was formed in an etching cell that is schematically demonstrated in Fig. 1. The etching of the pores was carried out under constant current conditions (High Current Source Measure Unit 238, Fa. Keithley) with a liquid front and rear side contact. Therefore, a 1:1 mixture of 50% HF and pure ethanol was used as electrolyte, connecting an area of 2.5 cm^2 on both sides of the sample. The etching process was performed with different current densities (10 mA/cm^2-300 mA/cm^2) and an anodisation time of 10 minutes, assisted by illumination of the front side with a halogen lamp (Philips Dichroic, 50 W, 12 V). The resulting microporous layer on top of the macroporous layer was removed with 1 M NaOH. The samples were rinsed in de-ionised water and dried in nitrogen gas atmosphere.

To fabricate the porous pH sensor, 50 nm SiO_2 was thermally grown at 1000°C for one hour on top of the macroporous silicon substrate (oxidation oven, Fa. Tempress). The oxidation process occurred in dry atmosphere of oxygen ($Si + O_2 \rightarrow SiO_2$). As pH-sensitive layer, 70 nm Si_3N_4 was deposited on the SiO_2 by means of LPCVD technique at a temperature of 770°C and a pressure of 750 mTorr

(Centronic E1200HT, Fa. Centrotherm). The Si_3N_4 layer was built from silan and ammoniac ($3\ SiH_2Cl_2 + 4\ NH_3 \rightarrow Si_3N_4 + 6\ HCl + 6\ H_2$) and deposited with a deposition rate of about 3 nm/min.

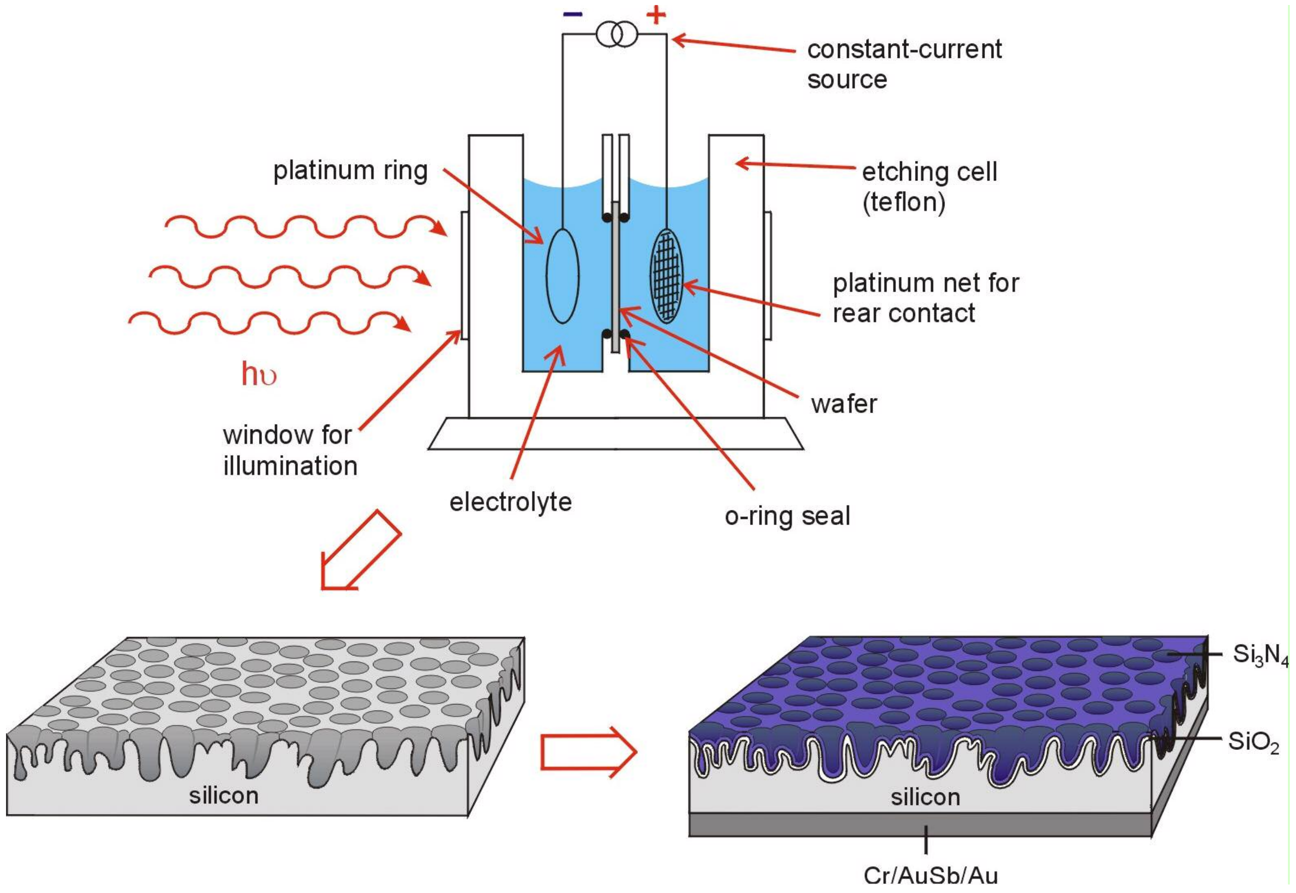

Figure 1. Schematic picture of the set-up, used for etching the silicon substrates (top) and a cross section of a resulting macroporous sample (bottom, left). After etching, 50 nm of SiO_2 was thermally grown on top of the macroporous substrate and 70 nm of Si_3N_4 was deposited by means of LPCVD technique to fabricate the pH sensor.

To contact the porous pH sensor for electrochemical investigations, a layer sequence of 5 nm Cr, 150 nm AuSb and 20 nm Au as an ohmic contact was deposited by means of electron beam evaporation (L 560, Fa. Leybold AG). Fig. 1 also gives a schematic view of the capacitive layer structure of the porous pH sensor.

To realise a porous biosensor, penicillinase (β-lactamase from *Bacillus cereus*, P0389, Fa. Sigma) as an enzyme sensitive to penicillin with a specific activity of 2660 units per mg protein was adsorptively immobilised into the pores. A detailed description of the procedure and the immobilisation conditions are given in [10].

The electrochemical characterisation of the pH sensor and the biosensor occurred with a computer-controlled impedance measuring system (IM5d, Fa. Zahner Elektrik). Measurements were made in a two-electrode configuration. To examine the potentiometric sensor response, the porous EIS sensor was mounted in a o-ring sealed measuring cell and contacted on its front side by the electrolyte and a Ag/AgCl reference electrode. The rear side was contacted by a gold-plated pin. For all pH measurements, technical buffer solutions in the range of pH 4 to pH 9 (Titrisol, Fa. Merck) were utilised as electrolyte. Penicillin measurements in the range of 0.01 mM to 10 mM were performed using Penicillin G (benzylpenicillin, sodium salt) dissolved in 0.2 mM Polymix buffer, pH 8, containing 140 mM NaCl. The preparation of the Polymix buffer is described in detail in [11]. The measurements were carried out in a dark Faraday cage at room temperature. When not in use, the pH sensor was stored in de-ionised water at room temperature, whereas the penicillin sensor was stored in Polymix buffer, pH 8, at a temperature of 4°C.

For the C/V (Capacitance/Voltage) measurements, the d.c. voltage was swept from –2.0 V to 2.0 V in steps of 100 mV and an a.c. voltage was superposed with a frequency of 1 kHz and a signal amplitude of 20 mV. Depending on the change of the pH value, the position of the C/V curve shifts along the voltage axis. In the ConCap (Constant capacitance) mode, by setting the capacitance at a fixed value (e.g., 60% of the maximum capacitance), the voltage shift, which results from changing the pH of the electrolyte, can be directly recorded (decreased voltage value by increased H^+-ion concentration). The ConCap measurements were carried out at the same frequency and a.c. voltage amplitude as for the C/V measurements. ConCap measurements, carried out with the pH sensor, were started at pH 9 and the voltage was recorded 5 minutes for each pH value. The solution was changed stepwise from pH 9 to pH 4 and backwards to pH 9. Before each concentration step, the pH sensor was rinsed with de-ionised water. To obtain the calibration curve of the pH sensor, the measured voltage values after 300 seconds were plotted *versus* the corresponding pH values.

In the case of the biosensor, the immobilised enzyme catalyses the reaction of penicillin in aqueous solution to penicilloic acid and H^+ ions, which results in a pH change at the surface of the sensor, depending on the concentration of penicillin in the solution [12]. Detailed information of the principle of the penicillin sensor is given elsewhere [13]. ConCap measurements of the biosensor were started with pure Polymix (without penicillin); the solution was changed after each 150 seconds. If the sensor signal in Polymix buffer was constant, the measurement was continued with a concentration of 0.01 mM penicillin G. Penicillin solution and penicillin-free Polymix buffer were alternately measured each for 150 seconds, increasing the penicillin concentration from 0.01 mM to 10 mM subsequently. To obtain the calibration curve, the sensor signal was evaluated as the difference of potential of the respective penicillin concentration after 150 seconds and the previous penicillin-free buffer solution after 150 seconds. Thus, the sensor signal can be plotted *versus* the penicillin concentration.

Results and Discussion

Physical characterisation of the porous silicon substrates

Macroporous silicon substrates, etched with different current densities in the range between 10 mA/cm^2 and 300 mA/cm^2 were characterised by means of Scanning Electron Microscopy (SEM) to evaluate the influence of the current density on the pore formation. Fig. 2 depicts typical SEM cross sections of four samples etched under identical conditions, only varying the current density. These pictures are taken with an angle of 60°. Using low current densities (10 mA/cm^2-50 mA/cm^2), a homogeneous distribution of the pore depth can be observed with an averaged depth between 5 µm

(10 mA/cm^2) and 8 µm (50 mA/cm^2). Increasing the constant current density (>100 mA/cm^2) effects the formation of thinner pore walls. Additionally, the generation of crater-like tubes with a maximal deepness of 40 µm can be recognised.

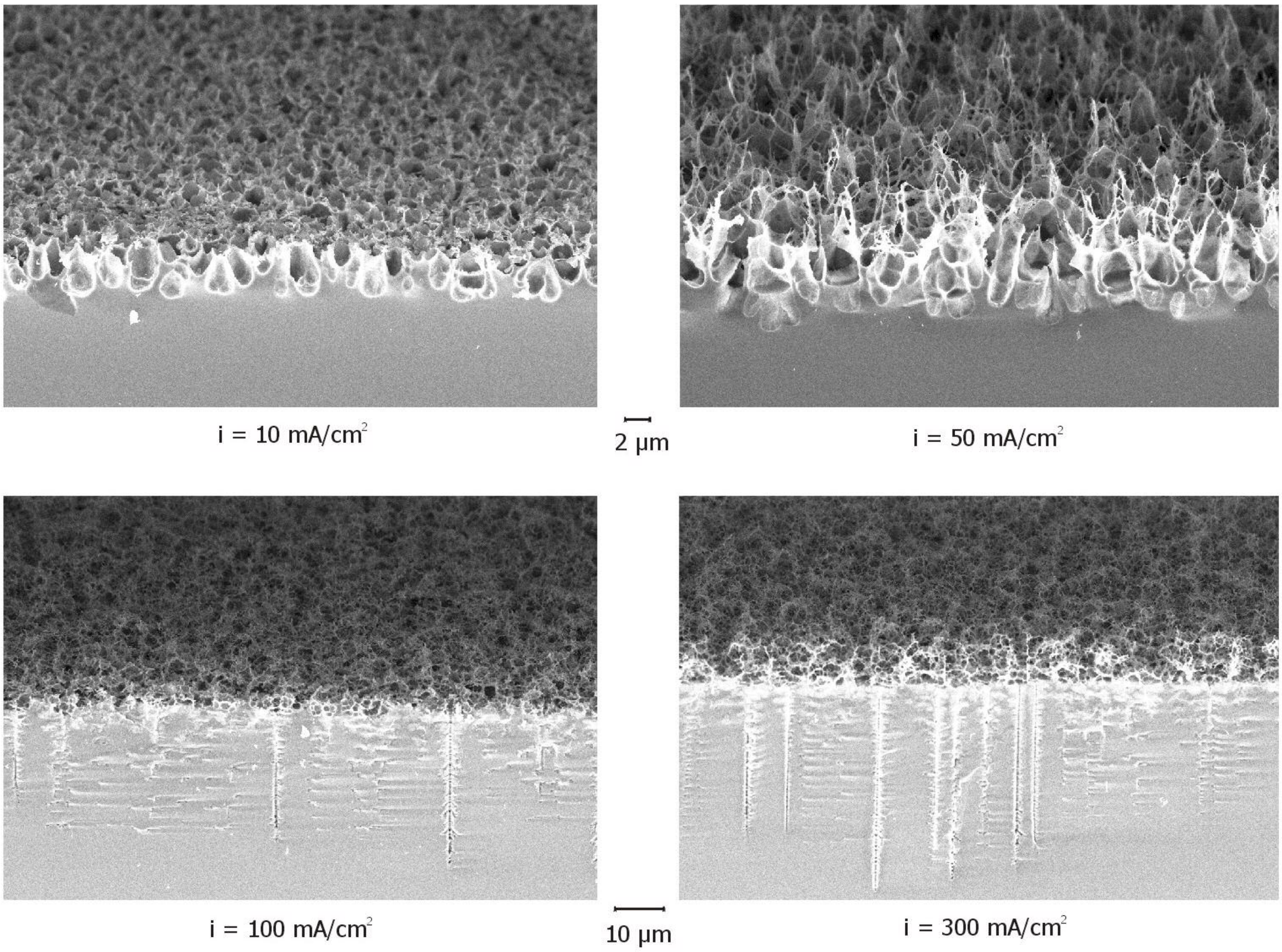

Figure 2. SEM cross section of macroporous silicon samples, etched with current densities between 10 mA/cm^2 and 300 mA/cm^2. When using low current densities (10 mA/cm^2-50 mA/cm^2), a homogenous distribution of the pore depth (5 µm-8 µm) can be observed. Utilising high current densities (100 mA/cm^2-300 mA/cm^2) effects the formation of crater-like tubes.

In Fig. 3, the corresponding SEM top views of the four samples are presented. The dark areas in the top view show the bottom of the etched pores whereas the pore walls are shining in bright colour. All samples are completely covered with pores, with an uniform distribution of circular pores for low current densities in the range between 10 mA/cm^2 and 50 mA/cm^2. An increased pore diameter can be recognised with increasing the current density. When using current densities of 10 mA/cm^2 to 30 mA/cm^2, the pore diameter is in the order of about 2 µm (10 mA/cm^2-20 mA/cm^2) and 3 µm (25 mA/cm^2-30 mA/cm^2). A further raise in the current density of greater than 50 mA/cm^2 leads to the formation of many small pores inside a main pore (not visible in this figures) with a crater-like tube in the centre (Fig. 2). The main pore's diameter amounts to about 7 µm for a current density of

300 mA/cm^2. In addition, the increase of the pore diameter is accompanied by a decrease of the thickness of the pore walls. In the experiments, 10 porous substrates have been characterised, which have been etched in the for the sensor application interesting range between 10 mA/cm^2 and 50 mA/cm^2. In all cases, the porous silicon layer could be fabricated with high reproducibility.

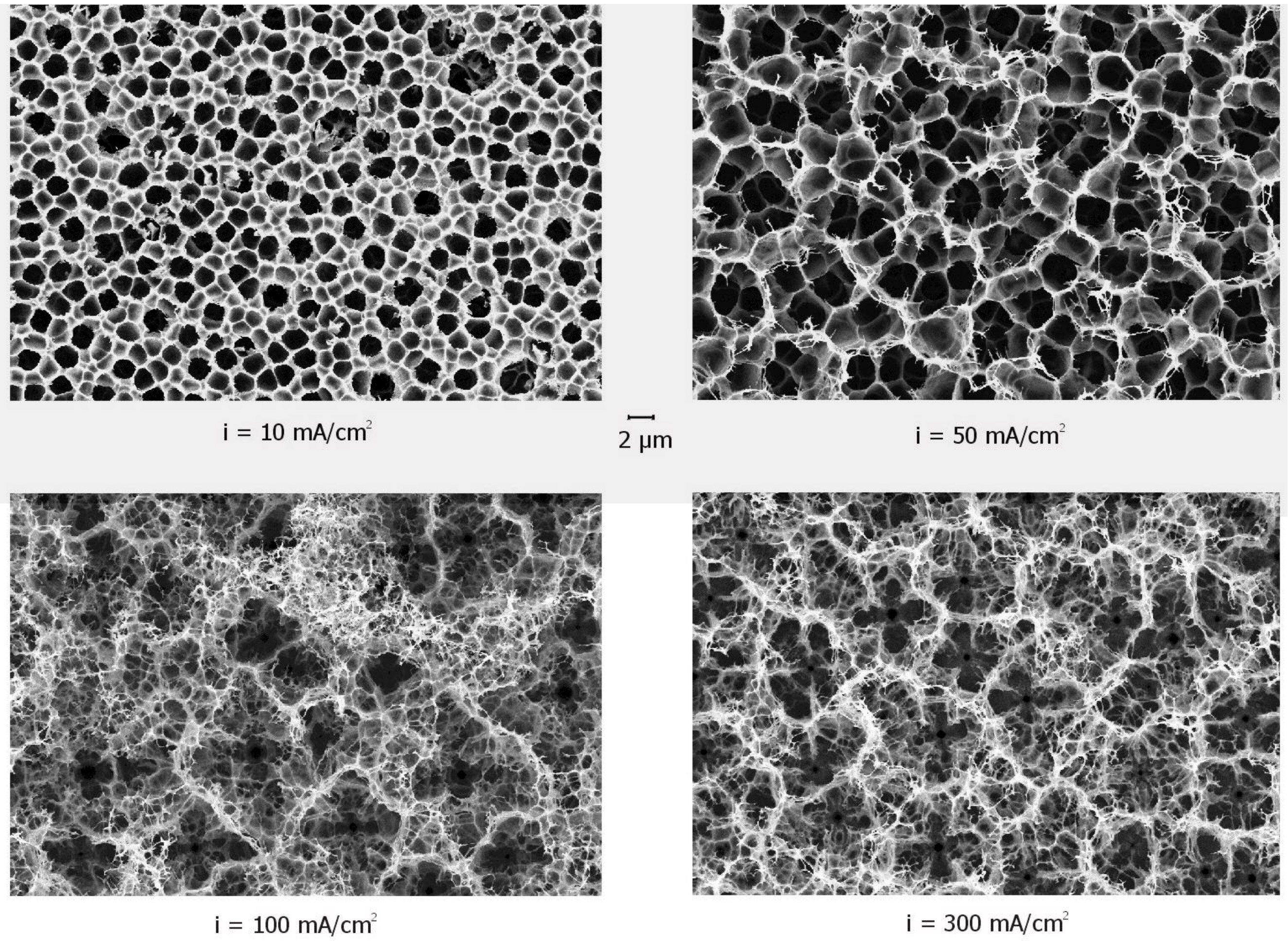

Figure 3. SEM top view of macroporous silicon samples, etched with current densities between 10 mA/cm^2 and 300 mA/cm^2. When using low current densities (10 mA/cm^2-50 mA/cm^2), an uniform distribution of circular pores is observed. An increased pore diameter can be achieved with higher current densities.

Electrochemical characterisation of the porous EIS sensors

As could be demonstrated by the SEM pictures, only such porous substrates are favourable for sensor applications, which were etched with current densities between 10 mA/cm^2 and 50 mA/cm^2. Porous substrates, etched with current densities lower than 10 mA/cm^2 show inhomogeneities in the pore distribution, visible with the pure eye or a simple light microscope. In this current-density range, the effect of generated air bubbles due to the etching process is not negligible. Therefore, porous substrates, etched under constant current conditions of 20 mA/cm^2 to 30 mA/cm^2, were used to fabricate the pH sensors and biosensors.

In order to investigate the electrochemical properties of the porous pH sensors, C/V and ConCap measurements have been carried out. To evaluate the influence of the measurement frequency, C/V measurements with frequencies of 120 Hz, 600 Hz, 1 kHz and 5 kHz have been performed. In Fig. 4, exemplary C/V measurements of a pH sensor (S1) in pH 7 buffer, etched with a current density of 30 mA/cm^2 are presented. As can be seen, there is a C/V-curve behaviour of the sensor according to the theory of semiconductor field-effect structures with the regions accumulation (1), depletion (2) and inversion (3). For a frequency of 120 Hz a slight deviation from the ideal C/V curve was found. This is probably due to additional surface states at the Si/SiO$_2$ or SiO$_2$/Si$_3$N$_4$ interface. The maximal capacitance amounts about 150 nF at a frequency of 120 Hz and decreases to about 135 nF at a frequency of 5 kHz. On the basis of these results, for all further investigations in the C/V and ConCap mode a frequency of 1 kHz was applied, to get an ideal curve behaviour combined with a high accumulation capacitance. Due to the enlargement of the sensor surface, the maximal capacitance of the porous pH sensors is about a factor six higher compared with that of planar pH sensors.

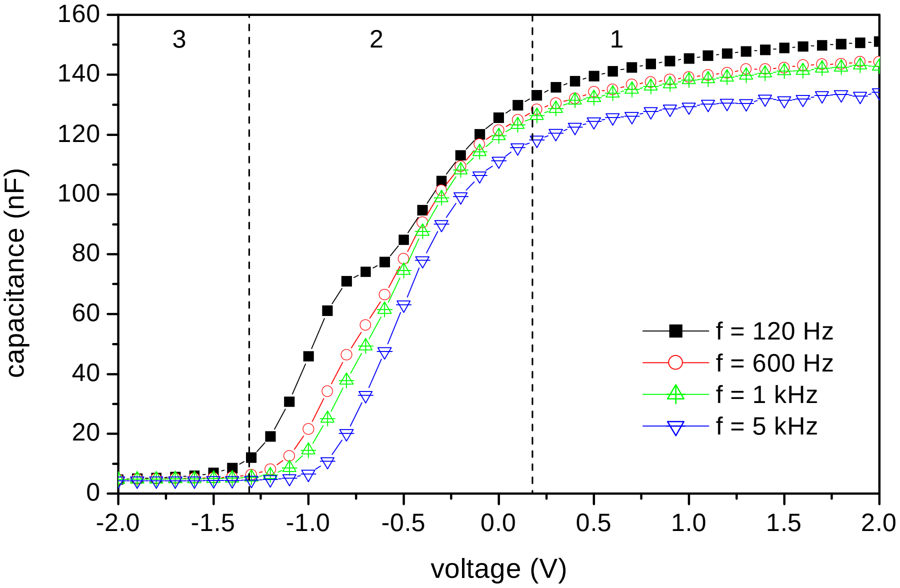

Figure 4. Characteristic C/V measurement of a pH sensor (S1), etched with a current density of 30 mA/cm^2 in the frequency range between 120 Hz and 5 kHz. A frequency of 1 kHz has been found as optimal for C/V and ConCap measurements.

Fig. 5(a) depicts a typical ConCap measurement, carried out with a pH sensor, where the substrate was etched at a current density of 20 mA/cm^2 (S2). At this time, the sensor has been already stored in de-ionised water for 44 days. The measurement shows reproducible potentials for each pH value with short response times of smaller than one minute for both directions of changing the pH.

In Fig. 5(b), the respective calibration curves for both directions of changing pH (pH 9 → pH 4, pH 4 → pH 9) demonstrate linear sensor responses. The sensitivity was calculated by linear fit to 59.6 mV/pH (pH 9 → pH 4) and 59.1 mV/pH (pH 4 → pH 9), respectively. The difference of potential in buffer solution, pH 9, at beginning and at the end of the measurement (hysteresis) was less than 3 mV. ConCap measurements of pH sensors, where the substrates were etched at current densities of 25 mA/cm^2 (S3) and 30 mA/cm^2 (S4) yielded comparable results.

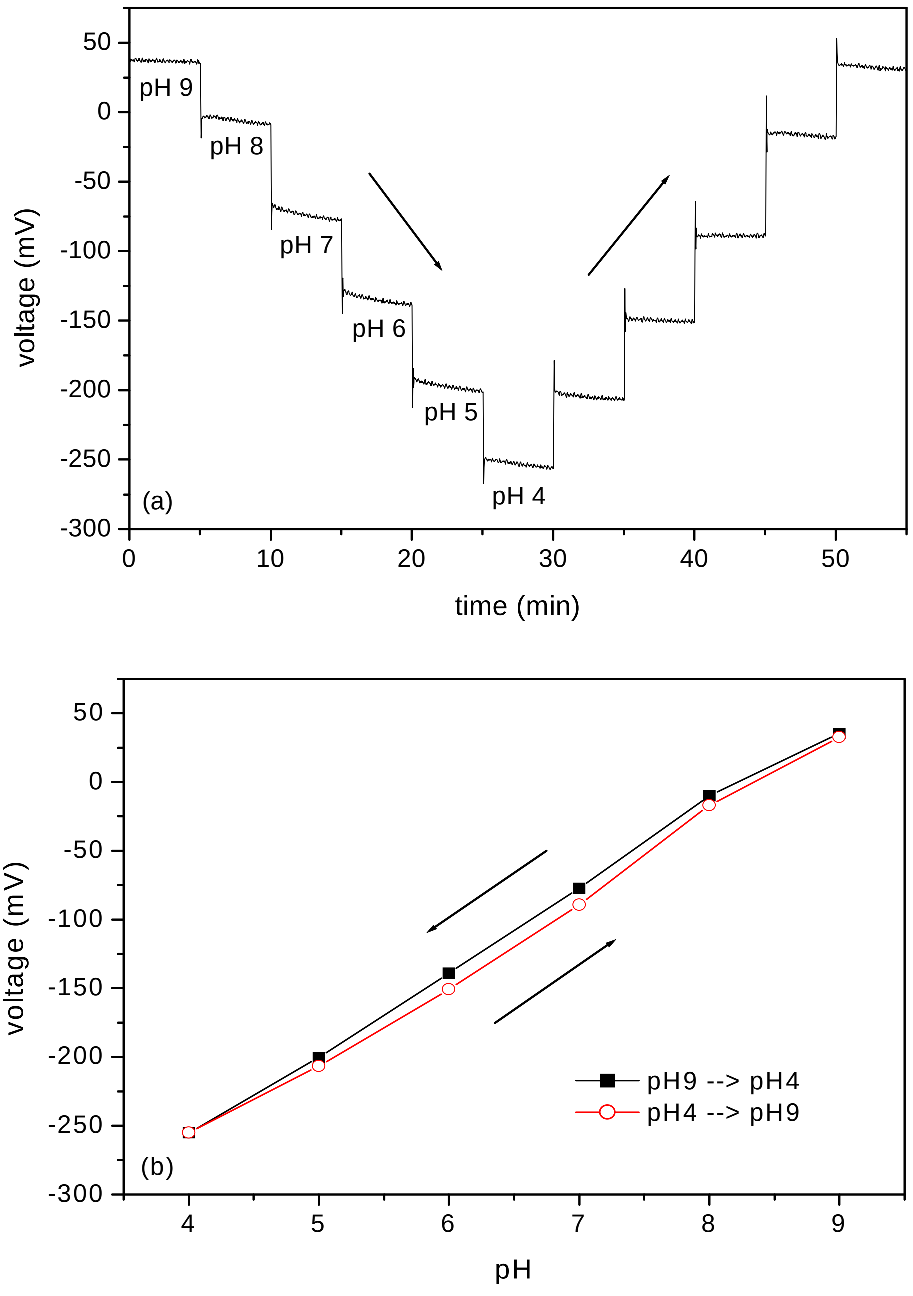

Figure 5. ConCap measurement (a) and corresponding calibration curve (b) of a pH sensor (S2), etched with a current density of 20 mA/cm^2. The sensitivity was calculated to 59.6 mV/pH (pH 9 → pH 4) and 59.1 mV/pH (pH 4 → pH 9) after the sensor has been measured for several times and stored in de-ionised water for 44 days.

Fig. 6 summarises the averaged pH sensitivities of the three investigated porous sensors during a measurement period of more than six months. In this diagram, the sensitivities for each ConCap measurement were averaged from pH 9 to pH 4 and *vice versa*. The values of all sensors, spanning sensitivities from 58.5 mV/pH (after the first measurement cycle) up to 61 mV/pH, were stable during the complete period of operation. In order to characterise the drift of the porous pH sensors, the shift of the sensor’s flat-band voltage (at pH 9) has been evaluated. Within the measuring period of up to six months, the averaged shift is about 1 mV/day (calculated by means of linear regression with starting after 23 days).

The results of the porous pH sensors are comparable to those of planar pH sensors with Si_3N_4 as sensitive layer [9]. The sensitivity of planar sensors is about 60 mV/pH during a measurement period of 190 days. The hysteresis of planar sensors was calculated to about 3 mV and the drift was less than 3 mV/day.

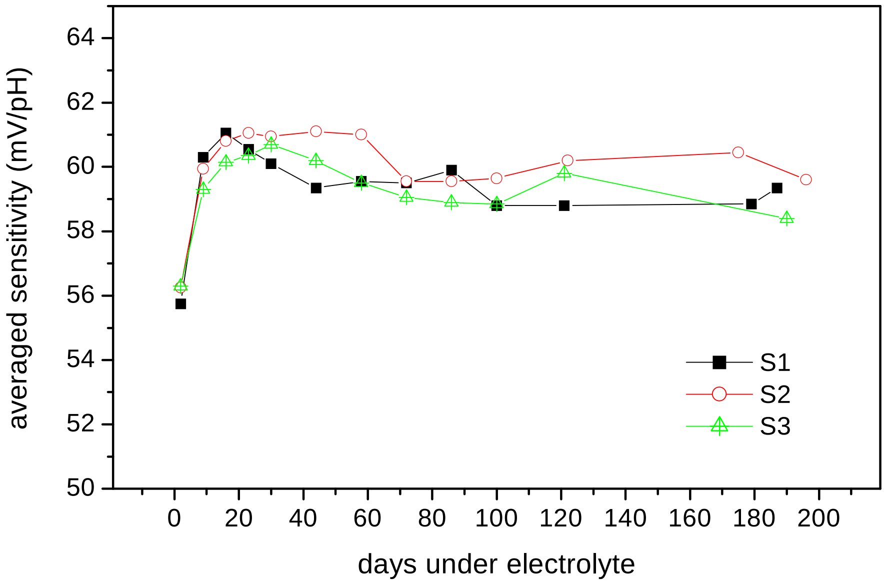

Figure 6. Sensitivities, averaged from pH 9 to pH 4 and from pH 4 to pH 9, of three pH sensors investigated. The substrates of the pH sensors were etched with current densities of 20 mA/cm^2 (S1), 25 mA/cm^2 (S2) and 30 mA/cm^2 (S3), respectively.

First ConCap measurements of a porous biosensor, fabricated with the vertical etching set-up, have been carried out, too. This porous penicillin biosensor was immobilised with an enzyme (penicillinase) concentration of 45 Units/cm^2. In Fig. 7(a) a typical ConCap measurement of such a biosensor, after storage in Polymix buffer for 5 days, is demonstrated. The sensor potential was stable after measuring with penicillin-free Polymix buffer for 3 cycles (after 450 seconds). A clear dependence of the sensor signal on the respective penicillin concentration exists in the concentration range between 0.01 mM and 10 mM penicillin G. The respective calibration curve was evaluated in a linear and a logarithmic scale. In the linear calibration graph, a sensitivity of about 140 mV/mM was obtained in a concentration range between 0.01 mM and 0.5 mM. The logarithmic calibration curve is demonstrated in Fig. 7(b). Here, in the concentration range between 0.1 mM and 1 mM, a sensitivity of about 85 mV/pPen was calculated.

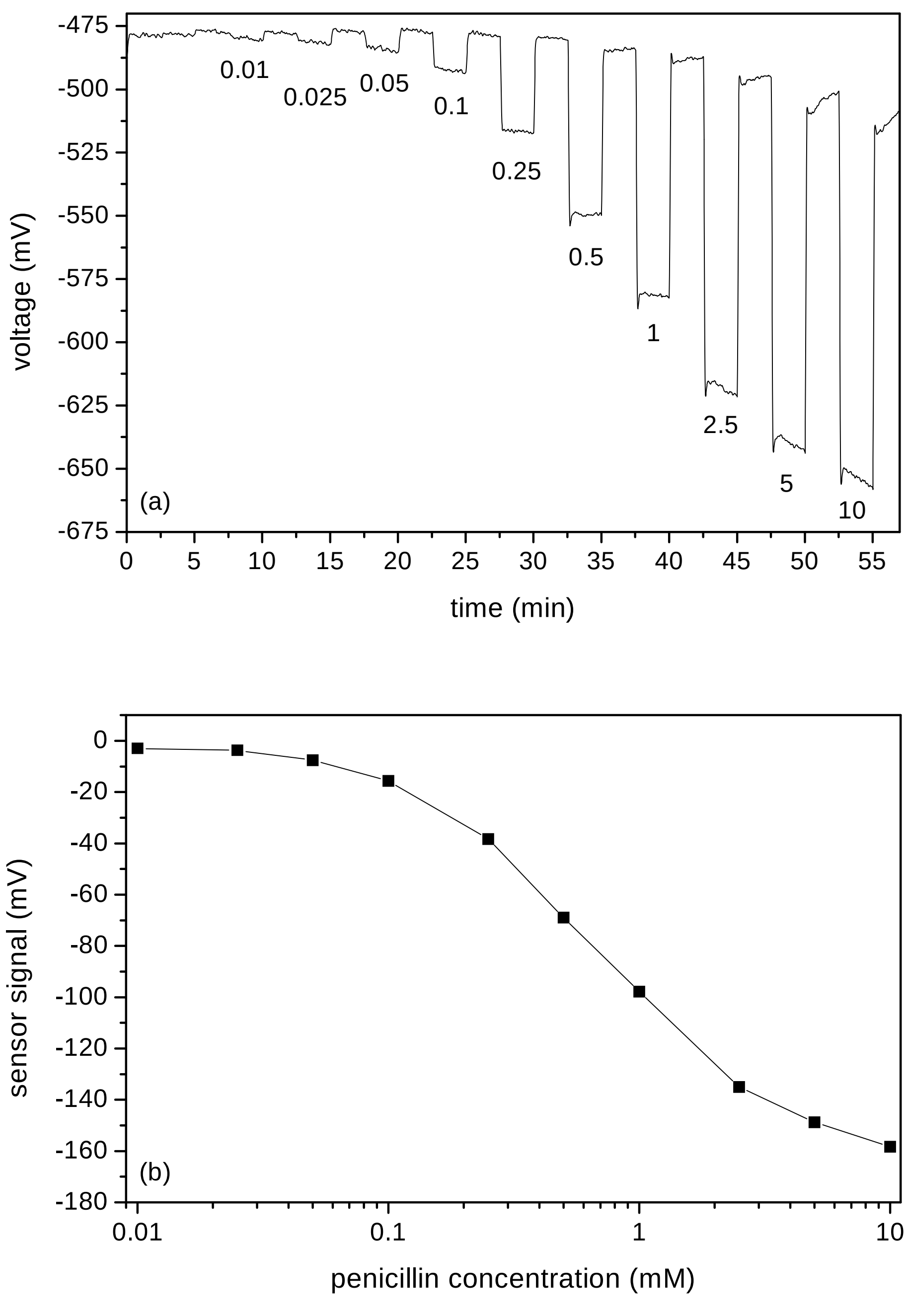

Figure 7. ConCap measurement (a) and corresponding logarithmic calibration curve (b) of a porous penicillin biosensor, etched with a current density of 20 mA/cm^2. The sensitivity was calculated to about 85 mV/pPen in the concentration range between 0.1 mM and 1 mM penicillin G.

Conclusions

pH sensors and biosensors on the basis of macroporous silicon with thermally grown SiO_2 and LPCVD-Si_3N_4 as transducer material have been realised. The macroporous silicon layer has been formed using a vertical etching set-up, where no solid rear side contact on the substrate is necessary. Different samples were etched under constant current conditions with current densities between 10 mA/cm^2 and 300 mA/cm^2, and characterised by means of SEM. As could be demonstrated with the SEM cross section and top view, only such porous substrates are usable for sensor applications, which were etched with current densities between 10 mA/cm^2 and 50 mA/cm^2. A further raise in the current density of greater than 50 mA/cm^2 leads to the formation of many small pores inside a main pore with a crater-like tube in the centre. The pH sensors were fabricated from substrates, which have been etched with current densities of 20 mA/cm^2, 25 mA/cm^2 and 30 mA/cm^2. In order to investigate the pH sensor's properties, C/V and ConCap measurements have been performed. C/V measurements with different frequencies in the range between 120 Hz and 5 kHz showed that a frequency of 1 kHz is favourable. The maximal capacitance of these porous pH sensors was about six times higher than the values of planar sensors with an identical layer sequence of Si | SiO_2 (thermal) | LPCVD-Si_3N_4. Measurements in the ConCap mode resulted in a nearly ideal curve behaviour, including high sensitivities, short response times and low hysteresis. The pH sensitivities, achieved for the complete period of investigation during six months, were close to the theoretical Nernst value. In addition, first ConCap measurements have been performed using a porous penicillin biosensor.

In further experiments, we plan to combine both a porous pH and a porous penicillin sensor onto a single sensor chip. Since first attempts could already successfully prove the realisation of a Ag/AgCl reference electrode – also on the basis of macroporous silicon [5] – , the fabrication of a porous EIS array with an integrated reference might combine the benefits of silicon-compatible process technologies together with the preparation of chemically and/or biologically sensitive membrane materials.

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Sample Availability: Available from the authors.