

Integration of a Capacitive EIS Sensor into a FIA System for pH and Penicillin Determination

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Abstract: A field-effect based capacitive EIS (electrolyte-insulator-semiconductor) sensor with a p-Si-SiO₂-Ta₂O₅ structure has been successfully integrated into a commercial FIA (flow-injection analysis) system and system performances have been proven and optimised for pH and penicillin detection. A flow-through cell was designed taking into account the requirement of a variable internal volume (from 12 µl up to 48 µl) as well as an easy replacement of the EIS sensor. FIA parameters (sample volume, flow rate, distance between the injection valve and the EIS sensor) have been optimised in terms of high sensitivity and reproducibility as well as a minimum dispersion of the injected sample zone. An acceptable compromise between different FIA parameters has been found. For the cell design used in this study, best results have been achieved with a flow rate of 1.4 ml/min, distance between the injection valve and the EIS sensor of 6.5 cm, probe volume of 0.75 ml, cell internal volume of 12 µl. A sample throughput of at least 15 samples/h was typically obtained.

Keywords: FIA system, capacitive EIS sensor, flow-through cell, pH, penicillin.

Introduction

A flow-injection analysis (FIA) system is a very useful analytical tool with wide possible application fields, like environmental monitoring, biotechnology, drug and food industry, etc. FIA methodology affords the continuous washing of the detector, small sample volumes, possibility of system automation as well as a high sample throughput, which allows the inexpensive, simple and reliable monitoring of chemical parameters in near-real time (i.e., at very short time intervals) [1,2]. Essentially, any sensor capable of flow-through detection can be interfaced to a FIA system as a detector. Therefore, different kinds of electrochemical sensors such as amperometric, conductometric, potentiometric, etc., have been proven as a detector in FIA systems [1-4]. In FIA systems, there is a general need for small and rapidly responding sensors resulting in a small sample volume use, a low sample dispersion, which improves the detection limit, the possibility of a high sampling rate and a simple experimental set-up. In this context, the implementation of an ISFET (ion-sensitive field-effect transistor) as a detector in FIA has been proven to be very useful thanks to its small ion-sensitive gate-surface area, fast response time, the favourable signal-to-noise ratio and the possibility to miniaturise the detector cell [5-13]. The ability to sense multiple species using an ISFET array is a further advantage of the ISFET/FIA system. In addition, the effectiveness of ISFET-based sensors is improved if they are used in continuous flow systems in particular, in FIA systems. The use of a dynamic measurement system minimises or eliminates the well-known disadvantages of ISFETs such as inherent drift and hysteresis effects, which are potential problems in static measurements. However, there are still serious difficulties in adapting ISFET sensors in flow cells because of well-known encapsulation problems, which, in addition, can also hinder the formation of a good stagnant layer [5]. Therefore, different complicated and expensive technologies were developed using anodic glass bonding [14] and backside-contacted ISFETs [15] that could in principle, facilitate the construction of compact flow-through ISFET systems and their integration with FIA systems.

On the other hand, due to the complete flatness of the structure, simplicity of the layout and absence of an encapsulation procedure and thus, easier and cost-effective fabrication [16,17] as well as the possibility of an easy integration in a flow-through FIA system, a second type of semiconductor field-effect-based (bio-)chemical sensors – a so-called capacitive EIS (electrolyte-insulator-semiconductor) sensor – could also be very attractive as detector for FIA systems. Moreover, EIS sensors combine some advantages of ISFETs such as the fast response time, possibility of a multi-analyte detection using an array of EIS sensors, etc. In addition, it can be expected that EIS sensors should have a comparable response behaviour as ISFETs due to their common detection principle and common transducer material [17]. In contrast to ISFETs, the sensitive area of an EIS sensor, in general, is larger than the gate region of an ISFET, and therefore, it is less suitable for a miniaturisation.

While the ISFET/FIA systems are intensively studied today, there are, however, only a few works concerning the application of EIS (bio-)chemical sensors in FIA systems. In references [18-20], EIS biosensors consisting of a Si/SiO₂/Si₃N₄/LaF₃ structure with different immobilised enzyme layers have been developed and integrated into a FIA system for the measurement of several analytes. Here, the sensor detects the changes in the fluoride-ion concentration in the sample solution resulting from the

enzymatic reaction. However, most of enzyme sensors known are based on the detection of a pH change as a result of the enzymatic reaction.

In this work, EIS sensors with Ta₂O₅ as a pH-sensitive layer have been successfully integrated into a home-made flow-through cell and combined with a commercial FIA system. System performances have been proven and optimised for the pH and penicillin detection taking into account the best compromise between different FIA parameters. The Ta₂O₅ layer combines both a high pH sensitivity and high corrosion-resistant properties in a wide pH range.

Experimental

FIA system

The EIS sensors have been characterised in both static and FIA mode using a measurement system presented in Fig. 1. It consists of a commercial single-line flow-injection analyzer FIA-Lab-2500 (FIA-Lab-Instruments), a home-made flow-through cell with integrated EIS sensor and a miniature Ag/AgCl reference electrode, an impedance analyzer (Zahner Elektrik) and a personal computer. The carrier stream was continuously pumped by a peristaltic pump, while the samples to be measured (standard buffer of pH 3-11 or penicillin solution) were injected into the carrier stream manually using an injection valve. The tubing distance between the injection valve and the detector was 6.5 cm. The carrier buffer was propelled through Tygon tubings (1.0 mm in diameter). The carrier stream used for pH and penicillin measurements in the FIA mode was a standard buffer of pH 7 and 0.5 mM polymix buffer, pH 8, 100 mM KCl, respectively. The penicillin solutions with different concentrations from 0.1 to 5 mM were prepared by dissolving penicillin G (benzylpenicillin, 1695 units/mg, Sigma) in the polymix working buffer. All chemicals used were of analytical reagent grade. The measurements were performed in a dark Faraday cage at room temperature.

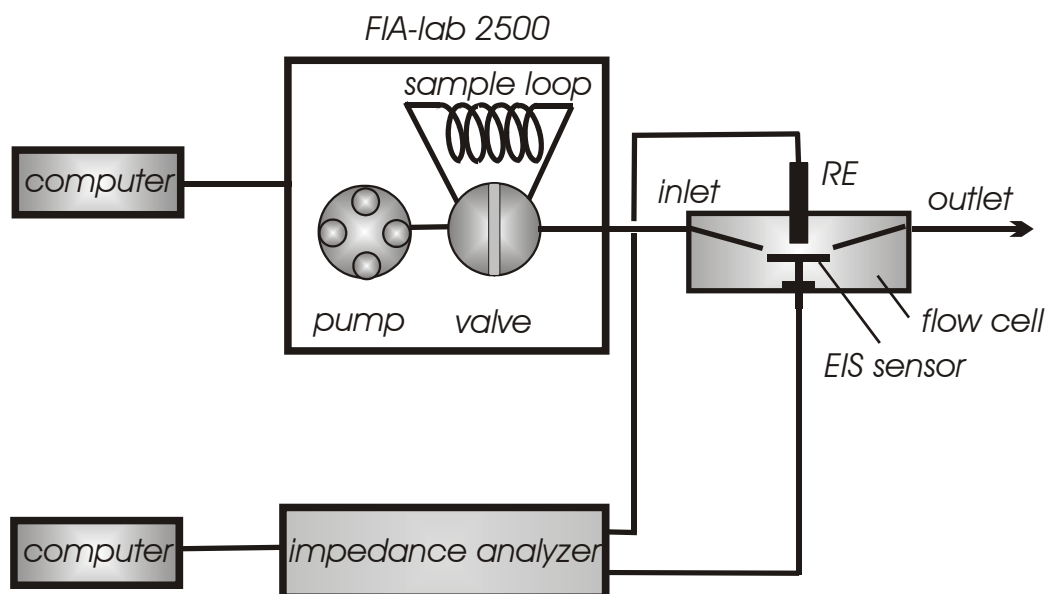


Figure 1. FIA measuring system (schematically); RE: reference electrode.

The EIS sensor has been characterised by constant capacitance (ConCap) method using an impedance analyzer. In this mode, the capacitance of the EIS structure is kept constant using a feedback control circuit and the voltage change is measured as a function of the pH value or analyte concentration. An AC voltage with an amplitude of 20 mV and a frequency of 180 Hz has been used for all types of measurements.

Flow-through cell

The flow-through cell, which is the key element for a successful operation of the FIA system, was designed and constructed considering the requirements of a variable internal volume, an easy integration of the EIS sensor and reference electrode within the cell as well as a simple replacement of the exchangeable sensors. A photograph of the developed flow-through cell with the size of $2 \times 3 \times 2 \text{ cm}^3$ is presented in Fig. 2.

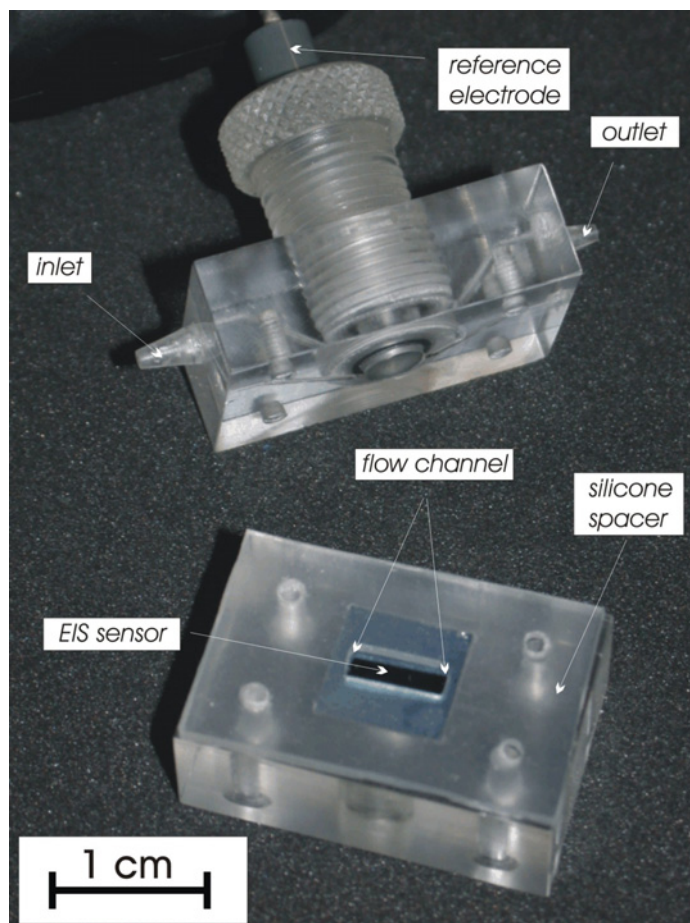


Figure 2. Photo of the developed flow-through cell.

The cell was made from two separate blocks of transparent polymer (plexiglass), the “channel” block and the “sensor” block. The transparency of plexiglass was very useful for controlling the air-bubble distribution and flow properties inside the channel. The EIS sensor was fixed in the cavity in the “sensor” block and contacted on its rear side by a gold-plated pin. A miniature Ag/AgCl reference electrode (Fa. BAS, type MF-2021) with ~5 mm in diameter was placed in the “channel” block. In

order to press the “channel” and the “sensor” block firmly together, a 0.5 or 1 mm thick silicone spacer was placed between them. A rectangular opening of $3 \times 8 \text{ mm}^2$ in the silicone spacer defines the active sensor area (24 mm^2) that is contacted with the solution. A variable internal cell volume (12, 24, 36 and $48 \text{ }\mu\text{l}$) has been obtained by varying the numbers of silicone spacer. The cell was assembled and then, connected with the commercial FIA-Lab-2500 system.

Sensor preparation

The pH-sensitive EIS sensors, consisting of a Al-Si-SiO₂-Ta₂O₅ structure, have been fabricated from a p-Si wafer with specific resistivity of 1-10 Ωcm . A double layer insulator consists of 35 nm thermally grown SiO₂ and 67 nm Ta₂O₅, which has been prepared by means of thermal oxidation of sputtered Ta in an oxygen atmosphere at 510°C for about 2 h 15 min. A 300 nm Al film was deposited on the rear-side of the wafer as contact layer. The wafer was then, cut into chips with a square surface of about $10 \times 10 \text{ mm}^2$.

The penicillin sensor was realised by adsorptive immobilisation of the enzyme penicillinase (*Bacillus cereus* from Sigma, specific activity: 1650 U/mg) directly onto the Ta₂O₅ surface. For the immobilisation, the enzyme solution was prepared by dissolving the enzyme penicillinase in a 200 mM triethanolamine (TEA) buffer, pH 8. 80 μl enzyme solution per sensor was pipetted onto the chips and incubated at room temperature for about 1-2 h. After rinsing and drying at room temperature, the sensors were stored in a 0.2 mM multi-component polymix buffer (pH 8, 100 mM KCl) at 4°C.

Results and discussion

The FIA method is based on the injection of a liquid sample into a carrier stream. The injected sample forms a zone in the carrier stream, which is diluted as it is transported to a detector (here, the EIS sensor). Because of the dispersion of the sample zone within the carrier stream, pH values measured by the flow-injection method will always be a result of some interchanges between the two solutions (carrier stream – sample solution). However, conditions can be found under which the potential and thus, the pH measured at peak maximum, will truly reflect the pH of the sample prior to the injection. To perform reliable and accuracy measurements, the dispersion coefficient, which is defined as the ratio of the original sample concentration to the concentration of the dispersed sample solution as measured by the sensor [1], should be as close as possible to one. The low dispersion generates high peaks and thus, a high sensor signal. There are several ways (or better their combination, if possible) to achieve a low dispersion-coefficient value: by injecting a large sample volume, reducing the distance between the injection valve and the sensor or by reducing the volume of the flow channel, etc. [1,2]. However, a large sample volume can result in a large sample consumption and low sample frequency. On the other hand, an achievement of minimum distance between the injection valve and the sensor or a minimum volume of the flow channel is often limited by the cell design and sensor size. Therefore, FIA parameters should be optimised for the specific requirement and an acceptable compromise between different FIA parameters should be found in order to achieve the desired results. In this study, the FIA parameters have been optimised in terms of a minimum

dispersion of the injected sample zone, a high sensitivity and reproducibility, and a reasonable fluid consumption and sample throughput.

pH sensor

Before FIA measurements, the pH sensitivity of the developed EIS sensors has been previously evaluated under static conditions. The results of this study show that the EIS sensors exhibit a nearly-Nernstian response of 58–59.7 mV/pH in the measured range from pH 3 to pH 10. Then, the EIS sensors were characterised in the FIA mode by determining the influence of the injected sample volume (from 0.5 to 1 ml) and flow rate (from 0.6 to 1.6 ml/min) on the peak height of the sensor's output signal and thus, on the pH sensitivity. The distance between the injection valve and the sensor was as short as possible (6.5 cm). The results of these investigations are summarised in Fig. 3 in the form of a 3D-diagram.

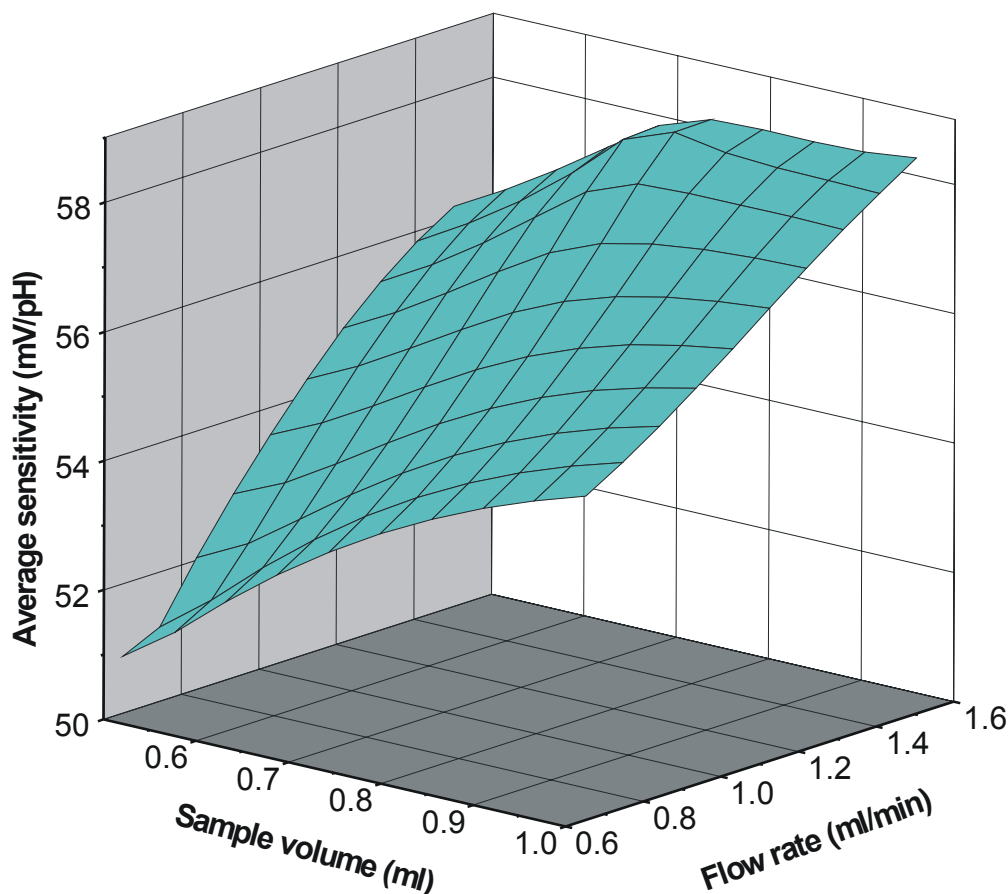


Figure 3. pH sensitivity of the EIS sensor in the FIA mode as a function of the injected sample volume and flow rate.

A raising sample volume leads to a reduction in the mixing of the sample with the carrier buffer. Thus, the degree of dispersion decreases. As a result, as it can be seen from Fig. 3, the pH sensitivity of the EIS sensor increases with increasing the sample volume and it trends to a constant value for sample volumes higher than 0.75 ml. A further well-known behaviour of FIA systems is the dependency on the flow rate. With increasing the flow rate, the effect of diffusion processes is

decreased, too, thereby increasing the sensitivity of the sensor. For the cell design used in this study, best operational conditions have been found to be: a flow rate of 1.4 ml/min, a distance between the injection valve and the EIS sensor of 6.5 cm, a sample volume of 0.75 ml and a cell internal volume of 12 μ l.

A typical FIA response of the developed pH-sensitive Ta₂O₅-gate EIS sensor, recorded under optimal conditions, is presented in Fig. 4. The sensor shows well-reproducible signal peaks in a wide pH range from pH 3 to pH 11, a high sensitivity and a minimal dispersion of the injected sample zone. The surface of the sensor is continuously rinsed by the carrier solution of pH 7, thus re-establishing the baseline after each sample injection. The recorded peak shape is non-symmetrical. This means that the sample is only slightly dispersed into the carrier solution, when propelled from the injection valve to the sensor. The calibration curve evaluated by using the height of the peaks was linear with a nearly-Nernstian slope of 58.6 mV/pH. The time elapsed between the sample injection and the maximum peak height was about 30 s and the baseline-to-baseline time was found to be 4 min. Thus, an acceptable sample throughput of at least 15 samples/h was typically achieved.

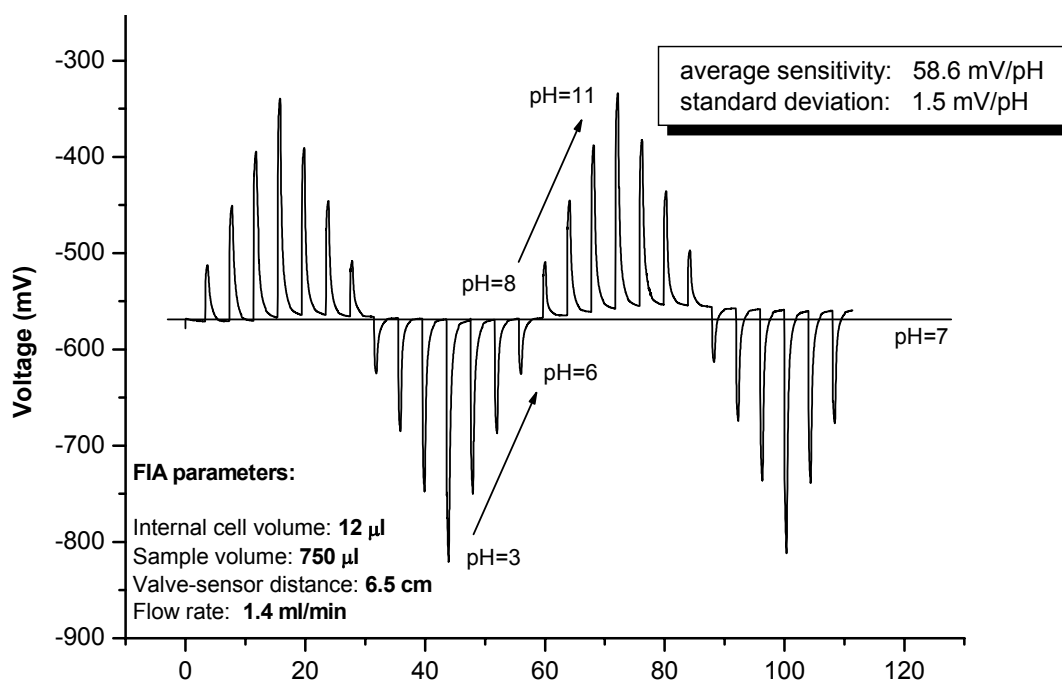


Figure 4. Typical FIA response of an EIS sensor under optimised FIA parameters.

Penicillin measurement in FIA mode with EIS biosensor

In further investigations, the developed EIS/FIA system has been tested for the penicillin detection. For comparison, penicillin measurements have also been performed in static conditions. Fig. 5 shows a typical ConCap response of the EIS penicillin sensor in the static (a) and FIA mode (b) in the concentration range from 0.1 mM to 5 mM penicillin. The FIA parameters were the same as for the pH measurements. The carrier stream used for the penicillin measurements in the FIA mode was a 0.5 mM polymix buffer, pH 8, 100 mM KCl. The EIS penicillin sensor detects variations in the H⁺-ion concentration resulting from the catalysed hydrolysis of penicillin by the enzyme penicillinase. A

resulting local pH decrease near the pH-sensitive layer leads to a change in the capacitance of the EIS structure. When increasing the penicillin content in the sample solution, the H^+ -ion concentration raises, too, yielding a distinct change in the sensor output signal. For instance, at a penicillin concentration of 1 mM, the change of the sensor signal was about 135 mV and 130 mV in static conditions and the FIA mode, respectively. In contrast to static measurements, the FIA measurements show a very reproducible baseline that enables a more accurately determination of the penicillin content in the solution.

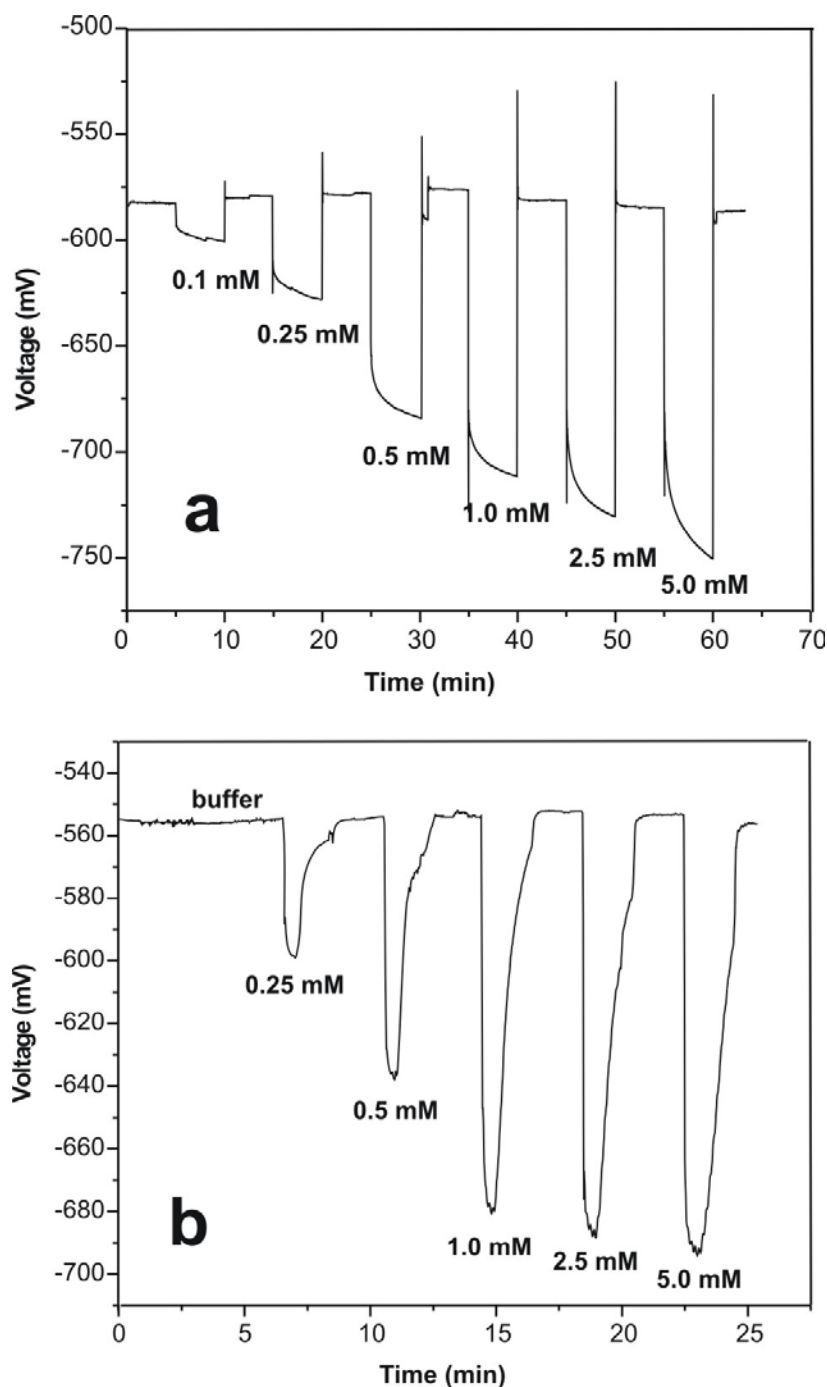


Figure 5. Typical ConCap response of the EIS penicillin sensor in static (a) and FIA (b) mode, respectively.

The respective calibration curves of the EIS biosensor evaluated from static- and FIA-mode measurements are presented in Fig. 6. Both calibration curves are nearly identical. However, a better lower and upper detection limit has been observed in the case of the static measurement. Further investigations are needed in order to optimise the FIA parameters with respect to exactly these detection limits.

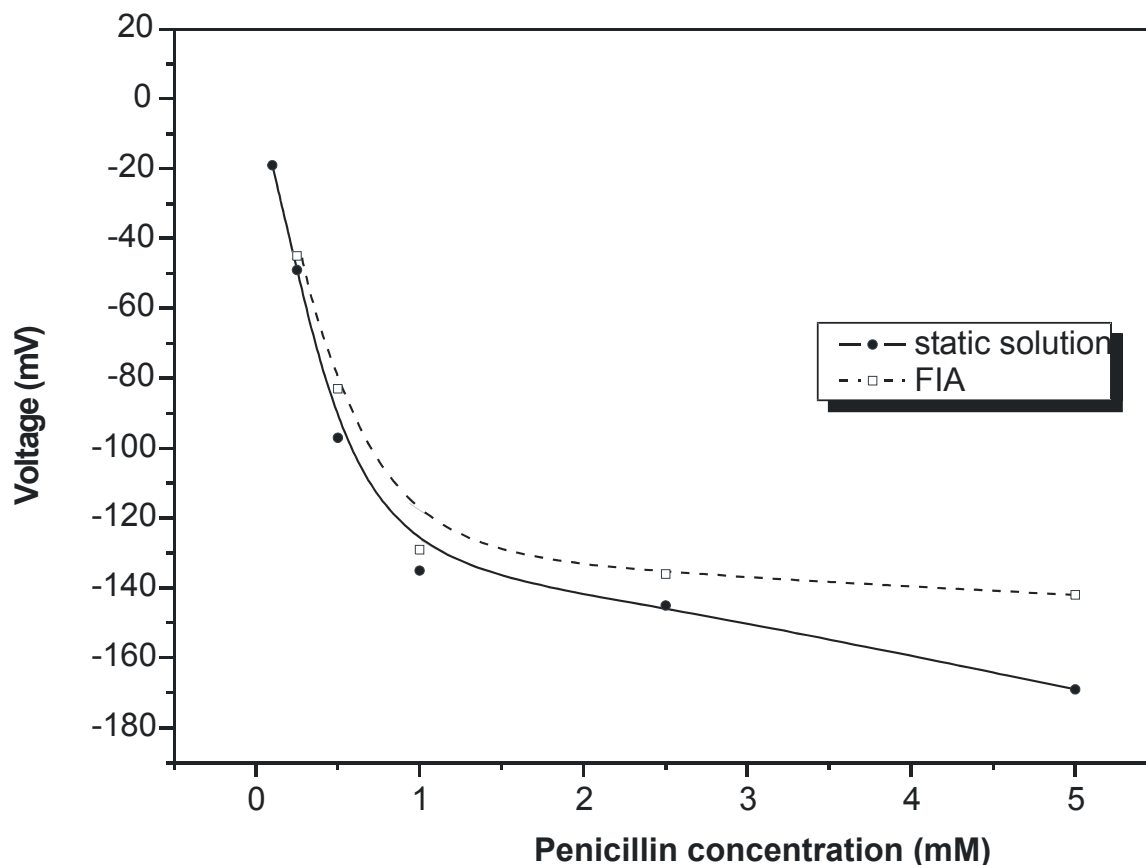


Figure 6. Calibration curves of the EIS penicillin biosensor evaluated from static and FIA measurements, respectively.

Conclusions

A capacitive EIS sensor consisting of a p-Si-SiO₂-Ta₂O₅ structure has been successfully integrated into a commercial FIA system for the pH and penicillin detection. A flow-through cell with a variable internal volume from 12 to 48 μ l was designed taking into account the requirement of an easy integration of the exchangeable EIS sensors and reference electrode within the cell. The FIA parameters have been optimised in terms of a minimum dispersion of the injected sample zone, a high sensitivity and reproducibility. The EIS/FIA system performances have been proven for pH and penicillin detection considering the best compromise between different FIA parameters. For the cell design used in this study, following optimal FIA parameters were chosen for the operation of the EIS sensors in the FIA mode: a flow rate of 1.4 ml/min, a distance between the injection valve and the EIS sensor of 6.5 cm, a probe volume of 0.75 ml and a cell volume of 12 μ l. The obtained results show the suitability of the capacitive EIS sensors as a detector for FIA systems.

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