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# Field-effect sensors with charged macromolecules: Characterisation by capacitance–voltage, constant-capacitance, impedance spectroscopy and atomic-force microscopy methods

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## Abstract

Field-effect-based capacitive electrolyte–insulator–semiconductor (EIS) sensors have been utilised for the deoxyribonucleic acid (DNA) immobilisation and hybridisation detection as well as for monitoring the layer-by-layer adsorption of polyelectrolytes (anionic poly(sodium 4-styrene sulfonate) (PSS) and cationic poly(allylamine hydrochloride) (PAH)). The EIS sensors with charged macromolecules have been systematically characterised by capacitance–voltage, constant-capacitance, impedance spectroscopy and atomic-force microscopy methods. The effect of the number and polarity of the polyelectrolyte layers on the shift of the capacitance–voltage curves has been investigated. Alternating potential shifts of about 30–90 mV have been observed after the adsorption of each polyanion and polycation layer, respectively. The DNA immobilisation and hybridisation signals were 35–55 and 24–33 mV, respectively. The possible mechanisms for the sensor responses are discussed.

*Keywords:* Field-effect sensor; DNA; Polyelectrolytes; Layer-by-layer adsorption

## 1. Introduction

Semiconductor field-effect devices (FED) based on an electrolyte–insulator–semiconductor (EIS) system are currently the basic structural elements of chemical and biological microsensors. These devices have been shown to be versatile tools for detecting pH, ion concentrations, enzymatic reactions, cellular metabolism and action potentials of living cells, etc. (see e.g., recent reviews by Schöning and Poghossian, 2002; Schöning, 2005; Bergveld, 2003; Offenhäusser and Knoll, 2001; Poghossian and Schöning, 2006).

The detection of molecular interactions at the solid–liquid interfaces is of great interest for a wide variety of applications, ranging from coatings for lenses, biomedical implants and drug-carrier systems up to biosensors such as deoxyribonucleic acid (DNA) arrays and protein chips. In the techniques actually employed for DNA-hybridisation detection, the readout of the DNA-hybridisation event requires the application of various labels (radiochemical, enzymatic, fluorescent, redox, etc.) and thus, have been proven to be time-consuming, expensive and complex to implement (e.g. De-los-Santos-Alvarez et al., 2004). Label-free detection is thus highly desirable. Therefore, recently considerable research effort was devoted to a label-free electronic detection of charged biomolecules, such as DNA and proteins, by their intrinsic molecular charge using FEDs (e.g. Fritz et al., 2002; Uslu et al., 2004; Pouthas et al., 2004; Han et al., 2006a; Uno et al., 2004; Sakata et al., 2005; Kim et al., 2004; Shin et al., 2004; Estrela et al., 2005; Lud et al., 2006). These devices offer the advantage of their compatibility with advanced micro- and nano-fabrication technology and thus, they are attractive for a fast, simple, inexpensive, and real-time analysis of nucleic acid samples with a direct electrical readout. It was suggested that during the binding event of the charged macromolecules, the intrinsic molecular charge effectively changes

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surface potential at the gate, which adds to the applied gate voltage, and thus, could measurable alter the operating characteristics of the FED. However, non-ideality of the molecular layer as well as so-called counter-ion screening effect are major obstacles for the direct electrostatic detection of the intrinsic charges of biomolecules. Although FEDs are principally able to measure the charge of adsorbed macromolecules or the charge change due to a hybridisation event, however, the counter-ion condensation effect will mask or reduce the expected signal, especially in high ionic-strength solutions (e.g., in physiological solutions). This subject has been discussed in Bergveld (1991), Poghossian et al. (2005a), Lud et al. (2006), Landheer et al. (2005). Recently, an alternative mechanism based on the detection upon the DNA-hybridisation induced redistribution of the ion concentration within the intermolecular spaces or in molecular layers was proposed (Poghossian et al., 2005a).

Today there are still insufficient correct models for a clear understanding of the mechanism of molecular detection by means of FEDs. Theoretical basis of the sometimes experimentally observed large sensor signals (see e.g. Kim et al., 2004; Shin et al., 2004; Dashiell et al., 2002) still remains unclear. In this context, polyelectrolyte (PE) multilayers could be very useful as a model system for direct monitoring of the various interfacial effects induced in field-effect sensors by the adsorption and binding of charged macromolecules. Recent experiments with the ISFETs (Uslu et al., 2004; Ingebrandt et al., 2005; Poghossian et al., 2006), capacitive EIS sensors (Kassab et al., 2004; Poghossian et al., 2005b, 2006) and silicon-on-insulator based thin-film resistors (Neff et al., 2006) as transducers, have demonstrated the possibility of application of semiconductor field-effect platform for the detection of layer-by-layer adsorbed polyelectrolytes.

In the present work, two types of capacitive EIS sensors consisting of Si-$SiO_2$ and Si-$SiO_2$-$Ta_2O_5$ structures with self-assembled PE multilayers of cationic poly(allylamine hydrochloride) (PAH)) and anionic poly(sodium 4-styrene sulfonate) (PSS) have been systematicaly investigated by means of capacitance–voltage (*C–V*), constant-capacitance (ConCap), and impedance-spectroscopy (IS) methods. In addition, the surface morphology and roughness of the PE mono- and multilayers has been characterised by atomic-force microscopy (AFM). The effect of the number and polarity of the polyelectrolyte layers on the shift of the capacitance–voltage curves along the capacitance and voltage axis has been studied. The experimental results of label-free detection of DNA immobilisation and hybridisation with EIS sensors are presented, too. The possible mechanisms of signal generation are discussed.

## 2. Materials and methods

### 2.1. Preparation of EIS sensors

Two types of capacitive EIS sensors consisting of an n-Si-$SiO_2$ structure with different thickness of $SiO_2$ (native oxide of about 1–2 nm, thermally grown oxide of 15 and 30 nm, respectively) and p-Si-$SiO_2$-$Ta_2O_5$ (30 nm $SiO_2$, 60 nm $Ta_2O_5$) were fabricated. The $Ta_2O_5$ layer has been prepared by means of thermal oxidation of sputtered Ta in an oxygen atmosphere at 515 °C for about 2 h. As contact layer, a 5 nm Cr/150 nm Au-Sb/20 nm Au film or a 300 nm Al film was deposited on the rear-side of the n- or p-type wafer, respectively. The wafer was then cut into single chips with a square surface of about 10 mm × 10 mm. The contact area of the EIS sensor with the solution was $0.5\ \mathrm{cm}^2$.

### 2.2. Measurement set-up

The EIS sensors with charged macromolecules have been characterised by means of *C–V*, ConCap and IS methods before and after each step of gate-surface functionalisation with the charged macromolecules using an impedance analyser (Zahner Elektrik). The *C–V* and ConCap measurements have been performed at a frequency of 1 kHz. An ac voltage with an amplitude of 20 mV has been used for all types of measurements. In the ConCap mode, the capacitance of the EIS sensor is kept constant by using a feedback-control circuit that allows direct monitoring of potential changes during multilayer build-up. The impedance measurements were carried out in both accumulation and depletion regions in a frequency range varying from 1 Hz up to 1 MHz. For comparison, the *C–V*, ConCap and IS curves of the bare EIS structures (as prepared or cleaned) were also recorded. A conventional liquid junction Ag/AgCl electrode (Metrohm) was used as a reference electrode. The measurements have been performed in a dark Faraday cage at room temperature.

Before the PE adsorption, the pH sensitivity of $SiO_2$ and $Ta_2O_5$ layers has been investigated in standard buffer solutions of pH 4–9 by the *C–V* and ConCap methods. The EIS sensors with $SiO_2$ and $Ta_2O_5$ gate-insulator showed a pH sensitivity of about 39–42 and 56 mV/pH, respectively, that are in good accordance to values typically reported for a $SiO_2$ and $Ta_2O_5$ layer (see e.g., Cane et al., 1997; Poghossian and Schöning, 2004).

### 2.3. Layer-by-layer build-up of polyelectrolytes

The PE multilayers were obtained using the layer-by-layer assembly technique (e.g. Decher et al., 1998; Hammond, 2000; Schönhoff, 2003) by a consecutive adsorption of PAH and PSS (MW ∼ 70,000, Sigma–Aldrich) from the PE solution (maximum number of PE layers was 10). The weak polyelectrolyte PAH is approximately fully charged at neutral and acidic solution, and is neutral at a pH > 10 (Smith et al., 2004). The PSS is a strong polyelectrolyte that totally dissociates in aqueous solutions and therefore, is fully negatively charged in a wide pH range as it liberates $Na^+$ ions (Smith et al., 2004).

The layer-by-layer adsorption technique is very simple. During the experiments, EIS sensors were consecutively exposed to the respective PE solution (50 µM PSS or PAH, unbuffered 0.1 M NaCl, pH 5 (pH 6)) for a time necessary for the adsorption of a single monolayer as well as for obtaining a stable sensor signal (usually 3–20 min), followed by rinsing with a solution of the same salt concentration and pH value as the respective PE solution or with a ultrapure water (Milli-Q, Millipore, Billerica, MA, USA) without any drying steps. These procedures were repeated until the desired number of layers was adsorbed