# Potentiometric Biosensors Based on Silicon and Porous Silicon

Indranil Basu*, R.Venkata Subramanian**, Arun Mathew*,
Anju Chadha** and Enakshi Bhattacharya*

*Department of Electrical Engineering , Indian Institute of Technology Madras, Chennai 600036, India, enakshi@ee.iitm.ernet.in
**Department of Chemistry, Indian Institute of Technology Madras, Chennai 600036, anjuc@iitm.ac.in

## ABSTRACT

We report fabrication of potentiometric biosensors with silicon for the estimation of triglycerides and urea based on enzymatic reactions. The sensor is an Electrolyte–Insulator-Semiconductor capacitor (EISCAP) that shows a shift in the measured CV with changes in the pH of the electrolyte. Enzyme mediated biological reactions involve changes in the pH of the electrolyte and an EISCAP can be effectively used for detection of biological compounds. Optimization of the conditions for the enzymatic reaction and calibration of the sensor are included. Effect of replacing silicon with porous silicon is discussed.

***Keywords***: biosensor, potentiometric, EISCAP, pH, enzymatic

## 1 INTRODUCTION

Detection and estimation of Triglycerides and Urea are clinically important, the first being correlated to heart disease while urea is an important analyte for the diagnosis of diseases such as renal malfunction. There is immense scope for improving sensitivity and ease of operation of commercial sensors presently used for estimating triglycerides and urea. We report fabrication of silicon and porous silicon based potentiometric biosensors for the estimation of tributyrin, a short chain triglyceride, and urea. The sensor is an Electrolyte–Insulator-Semiconductor capacitor (EISCAP) that shows a shift in the measured CV with changes in the pH of the electrolyte [1]. Many biological reactions, especially enzyme mediated ones, involve changes in the pH of the electrolyte. Hence an EISCAP can be effectively used for the detection of biological compounds through enzymatic reactions and can function as a biosensor.

## 2 PRINCIPLES

We can consider the basic EISCAP structure with silicon as the semiconductor, the oxide $SiO_2$ as the insulator and the electrolyte as the sample to be tested. When in contact with an aqueous solution, the $SiO_2$ surface is hydrolyzed to form silanol surface groups. These groups are positively charged, negatively charged, or neutral depending on the pH of the electrolyte[1]. The presence of this surface charge at the interface of $SiO_2$ and electrolyte affects the depletion layer within the silicon at the Silicon and $SiO_2$ interface, thereby producing a change in the flat band voltage of the electrolyte-insulator-semiconductor (EIS) system. Hence for solutions with different pH, C-V curves of the EIS system shift along the voltage axis with essentially no change in their shape.

The situation is similar if the insulator is silicon nitride[2,3]. Silicon nitride ($Si_3N_4$) has replaced $SiO_2$ as the sensing material in EISCAPs due to its superior sensing properties as compared to $SiO_2$. It not only offers a better pH sensitivity but is also stable in basic solutions in contrast to $SiO_2$, which dissolves in basic solutions [4]. Hence with $Si_3N_4$ based EISCAP, the detection can be extended to the basic range.

The pH sensitivity is determined by calculating the parallel shift in C-V curves in the depletion region along the voltage axis at a point of constant capacitance (usually at the midpoint of the C-V curve). The voltage value at that point is termed as $U_{bias}$. The EISCAPs, thus, can function as pH sensors and can be utilized for biosensing in case of enzyme mediated biological reactions resulting in the release of acid/base [5].

The amount of acid/base produced as a result of the enzymatic reaction is proportional to the amount of bioanalyte consumed. The pH of the electrolyte changes due to the production of acid/base that can be detected by the EISCAP. By correlating the change in pH of the electrolyte solution to the concentration of the bioanalyte, which produces this change, a pH sensing EISCAP can be used for detection of biological compounds.

When tributyrin, a short chain triglyceride, is hydrolysed by the enzyme lipase it results in the production of butyric acid as a product.

$$\text{Tributyrin} + \text{Water} \xrightarrow{\text{LIPASE}} \text{Glycerol} + \text{Butyric acid}$$

The pH of the solution changes with the butyric acid produced and the change is proportional to the concentration of tributyrin in the solution.

In the case of urea, the reaction is:

$$\text{Urea} + \text{Water} \xrightarrow{\text{UREASE}} \text{Ammonia} + \text{Carbon dioxide}$$

Ammonia and carbon dioxide liberated as a result of the enzymatic reaction dissolve in water thereby producing ammonium hydroxide and carbonic acid respectively. Ammonium hydroxide is a strong base while carbonic acid is a weak acid. Hence pH of the electrolyte solution after the enzymatic reaction shifts towards the basic range.

The change in pH is detected by CV measurements on the EISCAP and co-related to the concentration of tributyrin or urea[6].

## 3 EXPERIMENTAL DETAILS

Fig 1 shows the basic structure of the fabricated EISCAP. In order to obtain a good interface between the insulator layer and semiconductor in an EISCAP, $Si_3N_4$ is deposited on thermally oxidized silicon sample since the interface state density is negligible at the Si-$SiO_2$ interface. p-type(100) silicon wafers with a resistivity of 4 to 11 Ωcm were oxidized at 1000°C in dry oxygen for 2 hours. $Si_3N_4$ was then deposited by PECVD at 300°C for 7 minutes followed by a 10 min annealing at 800°C. The annealing step is essential because untreated $Si_3N_4$ when kept exposed to ambient conditions results in the oxidation of $Si_3N_4$ surface and degradation of pH sensitivity of $Si_3N_4$ [1,7].

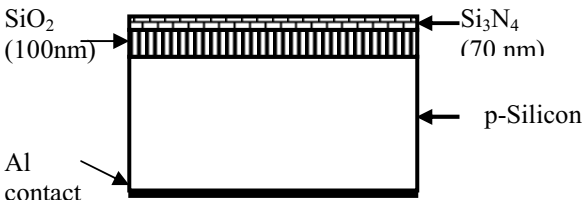


Fig 1: Cross-section of EISCAP

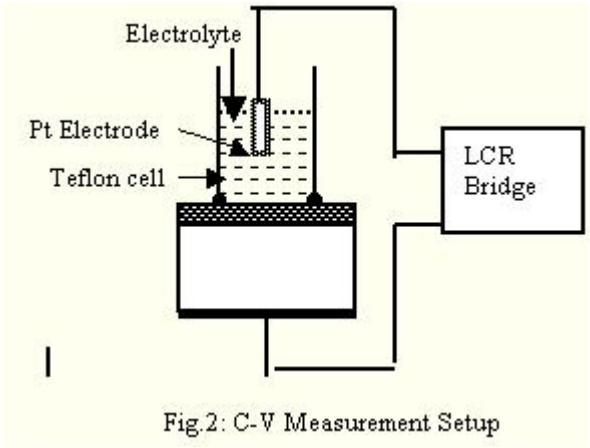


Fig.2: C-V Measurement Setup

The experimental setup used for characterizing the EISCAP is as shown in Fig. 2. The electrolyte is held with the help of a teflon cell and O-ring and the contact is taken from a platinum-wire dipped in the electrolyte. The semiconductor contact was taken from the back aluminium. C-V measurements were done at room temperature with an HP 4275A LCR meter at 10kHz with 15mV signal amplitude by sweeping the dc bias from -10 to -3 V in steps of 100mV. Solutions of different pH were prepared by adding HCl and NaOH to 0.1M phosphate buffer with 0.5M KCl added as ionic strength adjuster for C-V measurements. After measurements, the device was thoroughly rinsed in deionised water. When the device is not in use it is stored in deionised water at $4^0$C.

## 4 RESULTS AND DISCUSSIONS

The CV curves were normalized with respect to the accumulation capacitance value. With increasing pH of the electrolyte the C-V curves were found to shift towards right along the voltage axis as indicated in Fig. 3. $U_{bias}$ is defined as the voltage where the capacitance falls to 70% of its maximum value. A plot of $U_{bias}$ vs. pH shows a maximum slope of 55mV per unit change in pH, Fig. 4.

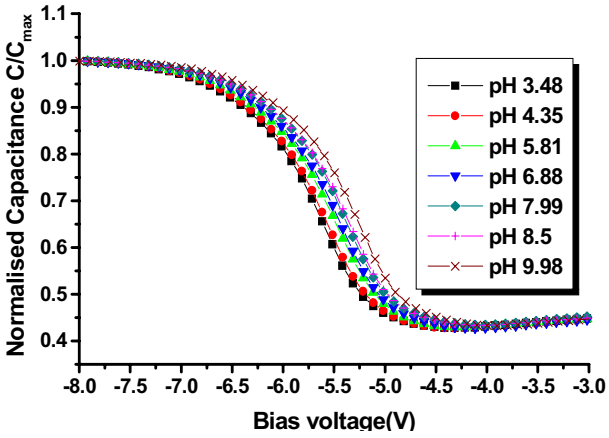


Fig.3: Normalized C-V curves for solutions of different pH

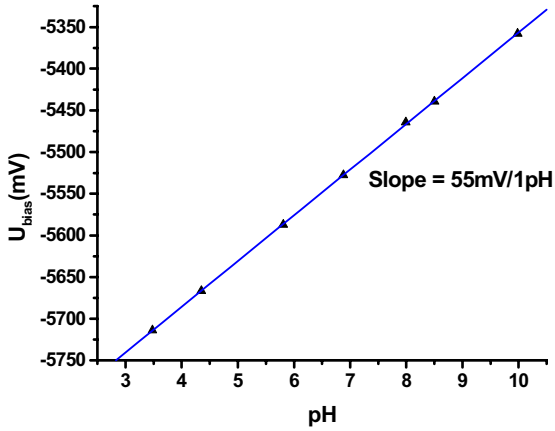


Fig.4: $U_{bias}$ vs. pH

The rate of an enzymatic reaction is a function of the sample concentration and will be linear until the catalytic sites of the enzyme are saturated with the sample. At saturation, the rate of the reaction is independent of the

sample concentration. It is important therefore to optimize various parameters of an enzyme catalyzed reaction in the linear region where the specificity of the enzyme to the sample is maximum. The parameters optimized for this study include enzyme concentration, sample concentration, time of the reaction, buffer concentration and its pH. For the detection of tributyrin, the amount of the enzyme, Lipase - with an activity of 40 units/mg - and the time of reaction were optimized to 12 mg for 30 ml of buffer solution and 30 min respectively, for the enzyme reaction to be in the linear region[8]. The buffer used was phosphate buffer, 0.01M, pH 7 and all reactions were done at room temperature.

The activity of the enzyme Urease (Jack beans, Fluka) was determined by the spectrophotometric method[3] before carrying out the optimisation studies and the activity was found to be 30 U mole/mg/min[9]. For the estimation of urea, the optimized values for a sample concentration of 150mM were 0.16mg of Urease in 0.5M phosphate buffer for 30 minutes.

The pH of the electrolyte changes after the enzymatic hydrolysis and is detected by CV measurements on the EISCAP. The final calibration plot of the sensor is a plot of $U_{bias}$ vs. sample (tributyrin/urea) concentration. This is constructed by combining the two curves, namely: (1) sample concentration vs. pH which is obtained by carrying out the enzymatic reaction on known concentrations of the sample, monitoring the change in pH using a digital pH meter and noting down the pH value after the optimised interval of time (i.e. 30 min). (2) $U_{bias}$ vs. pH which is obtained by carrying out the C-V measurements using known concentrations of the samples, after subjecting them to enzymatic reaction for the optimised interval of time. This calibration curve can be used to determine unknown concentrations of the sample. Figs 5 and 6 give the calibration plots for tributyrin and urea.

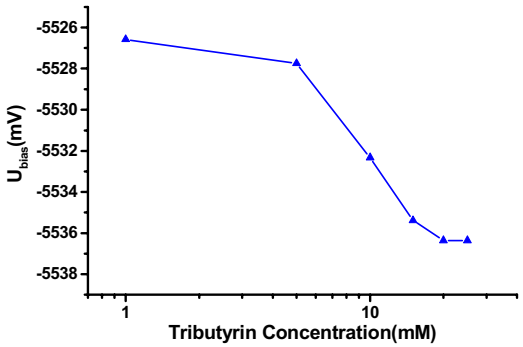


Fig.5: Calibration plot for sensing Tributyrin

Measurements were done with unknown concentrations of tributyrin and urea and the calibration plots, Figs 5 and 6, were used to determine the concentrations. For this, unknown concentrations of the sample to be estimated, tributyrin and urea, were prepared and the optimized value of the enzyme, lipase and urease , were added. After

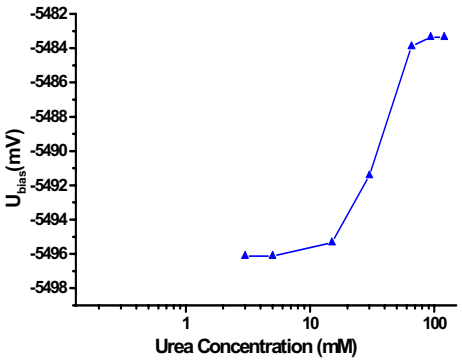


Fig.6: Calibration plot for sensing Urea

carrying out the enzymatic reaction for the optimized time, C-V measurements were taken and the corresponding values of $U_{bias}$ were determined. From the calibration curve the tributyrin/urea concentration corresponding to the measured $U_{bias}$ value was obtained and was compared with the actual value. The measured and actual values are given in Tables 1 and 2 and show good agreement.

Table 1: Unknown concentrations of Tributyrin as determined by the sensor

| No. | $U_{bias}$ (mV) | Concentration (mM) | | % Error |
|---|---|---|---|---|
| | | Measured | Actual | |
| 1. | -5530.8 | 8.08 | 8.29 | -2.53 |
| 2. | -5534.2 | 12.97 | 12.89 | 0.62 |

Table 2: Unknown concentrations of Urea as determined by the sensor

| No. | $U_{bias}$ (mV) | Concentration (mM) | | % Error |
|---|---|---|---|---|
| | | Measured | Actual | |
| 1. | -5486.2 | 51.63 | 50 | 3.26 |
| 2. | -5488.3 | 41.72 | 40 | 4.3 |

## 4.1 Porous Silicon as the Substrate

Electrochemical dissolution of crystalline silicon in hydrofluoric acid (HF) based electrolyte solutions at constant current gives a sponge-like silicon matrix with pores, which is known as porous silicon [10]. Due to the large specific surface area ( $> 200\ m^2\ cm^{-3}$ ) [11] and its

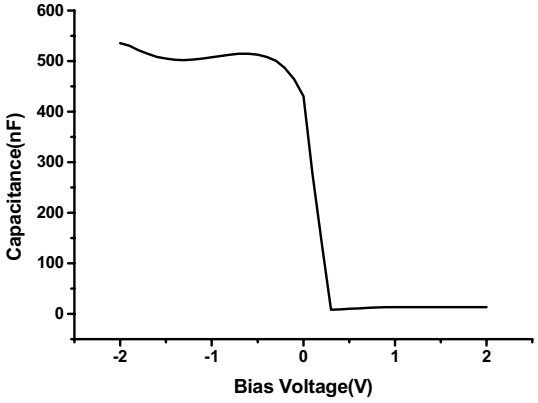


Fig.7: CV with porous silicon substrate

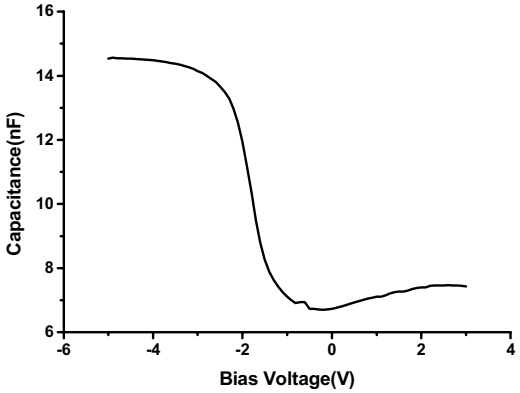


Fig.8 : CV with single crystalline Si substrate

porous structure, PS can adsorb molecules easily from the surroundings and can be used effectively as a sensor material. The pore size can be varied by varying the substrate doping, concentration of the etching solution, the anodisation current density and the time. Porous silicon was prepared under the following conditions:

Substrate – p-Si (ρ=4-11Ω-cm)
Etching solution – 1:2::HF:Iso-propanol
Anodisation current density – 10mA/cm$^2$.
Anodisation time- 10mins

The thickness of the porous silicon layer was found to be 5 μm and the average width of a silicon filament was about 10 μm while the average pore size was 2-3 μm. The porous silicon sample was subjected to dry oxidation at 400$^0$C followed by $N_2$ annealing after which 40nm of $SiO_2$ and 80nm of $Si_3N_4$ were deposited on it by PECVD technique. C-V measurements were then taken on the porous silicon sample using phosphate buffer solution (0.1M $Na_2HPO_4/KH_2PO_4$ in 0.5M KCl) at a signal frequency of 10kHz and is shown in Fig 7. C-V measurements were also performed using the phosphate buffer solution at a frequency of 10kHz on a similarly processed single crystalline silicon sample and is shown in Fig 8. As is observed, the porous silicon sample shows a 34.5 times increase in accumulation capacitance over the single crystalline silicon sample. This is due to the much larger surface area of the porous silicon sample and will be useful in miniaturization of the sensor.

## 5 CONCLUSIONS

A potentiometric sensor has been developed for detection and estimation of tributyrin and urea, which can be extended to detect other biological compounds as well. This sensor is an EISCAP with silicon as the substrate and Silicon Nitride as the sensing dielectric. Sensors using porous silicon as the substrate material have been shown to be more sensitive due to the increase in surface area as compared to the single crystal planar sensors. The sensors show good reproducibility.

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