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ARTICLE TYPE

Miniaturised silicon biosensor for the detection of Triglyceride in blood serum [†]

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This paper reports on the design and fabrication of Electrolyte Insulator Semiconductor Capacitor (EISCAP) devices to detect triglycerides in the form of microreactors fabricated by bulk micromachining of silicon. We have developed a complete triglyceride biochip wherein the enzyme for hydrolysis and the counter electrode for signal transduction are integrated with the miniaturised EISCAP sensor. A compact readout system that measures the triglyceride concentration in blood serum under test has been developed and is implemented in a Programmable System on Chip (PSoC®). The miniaturised EISCAP devices are tested using blood serum samples to estimate the triglyceride concentration within the clinical range of 50 to 150 mg/dL and the time taken by the readout system to calibrate the sensor and to measure the triglyceride is within 5 minutes.

1 Introduction

Silicon is a well known material that is being used for a wide variety of application-specific sensor systems[1]. A biosensor based on silicon can be of low cost when mass produced. Silicon biosensors can be miniaturised and used with sample volumes as low as few microliters. Our motivation in this work is to design a silicon biochip that allows fast and direct determination of triglycerides within the clinical measurement range. Triglycerides (TGs), which are present in the form of fats in food as well as in the human body are important to monitor as high blood TG levels are indicative of high risk for cardiovascular problems. Presently, TG levels are estimated using spectrophotometric methods wherein the instrumentation is complex and expensive, making it essential to develop simple and inexpensive biosensors. Various types of TG biosensors such as electrochemical,[2] amperometric[3−5] and potentiometric,[2] those using conducting polymer[2] and nano particles[2], have been reported recently. Many silicon biosensors work on the principle of impedance or change in the surface charge when it comes in contact with electrolytes and thereby develops a potential across the semiconductor-liquid interface and are classified as potentiometric biosensors.[6−10] Ion Sensitive Field Effect Transistor (ISFET) and Electrolyte Insulator Semiconductor Capacitor (EISCAP)[11,12] are such type of silicon based sensors used in the determination of bio-analytes like TG and urea through an enzymatic reaction. ISFET and EISCAP devices resemble the structure of Metal Oxide Semiconductor Field Effect Transistor (MOSFET) and Metal Oxide Semiconductor Capacitor (MOSCAP) respectively. The advantage of ISFET sensors is the relative simplicity of design of readout electronics, since the ISFET drain current which is a function of pH can be readily converted into a voltage. On the other hand, EISCAP has the advantage of ease in fabrication and being more robust. The capacitance-voltage (C-V) characteristic of EISCAP gets modulated depending upon the pH of the electrolyte. In the presence enzyme lipase, the triglyceride (bioanalyte) is hydrolysed and there is a change in the pH of the electrolyte that shows up as a shift in the C-V plot.[13,14] Thus, from the shift in the C-V plot, the amount of the TG being hydrolysed can be determined. We have earlier reported on the working of large EISCAP sensors made on silicon to detect triglyceride and urea.[15−18] Miniaturised EISCAPs have been fabricated using bulk micromachining of silicon and have been used to sense tributyrin (a short-chained triglyceride).[17] Direct readout circuits to calibrate the sensor against process variations and to measure the pH under test have been developed for a large sized EISCAP and a miniaturised EISCAP.[19,20] For the EISCAP sensors we have used a stacked gate dielectric layer of silicon oxide and silicon nitride. While the oxide ensures a better interface with silicon, the silicon nitride layer has better pH sensitivity and is more resistant to electrolyte spoiling. In this paper, we discuss the design and implementation details of the miniaturised EISCAP along with the readout circuit as a fully functional triglyceride sensor in clinical application.

2 Materials and Methods

Triglyceride biosensors are fabricated using 400 μm thick 3" p-type 1-10 Ω-cm single side polished crystalline silicon (100) wafers and 500 μm thick 4" boro-float glass wafers. The wafer cleaning and processing chemicals such as Sulphuric

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Analytical Methods Accepted Manuscript

(a) Large area EISCAP used in earlier work[18]

Miniaturisation

(b) Enzyme immobilised mini-EISCAP used in this work

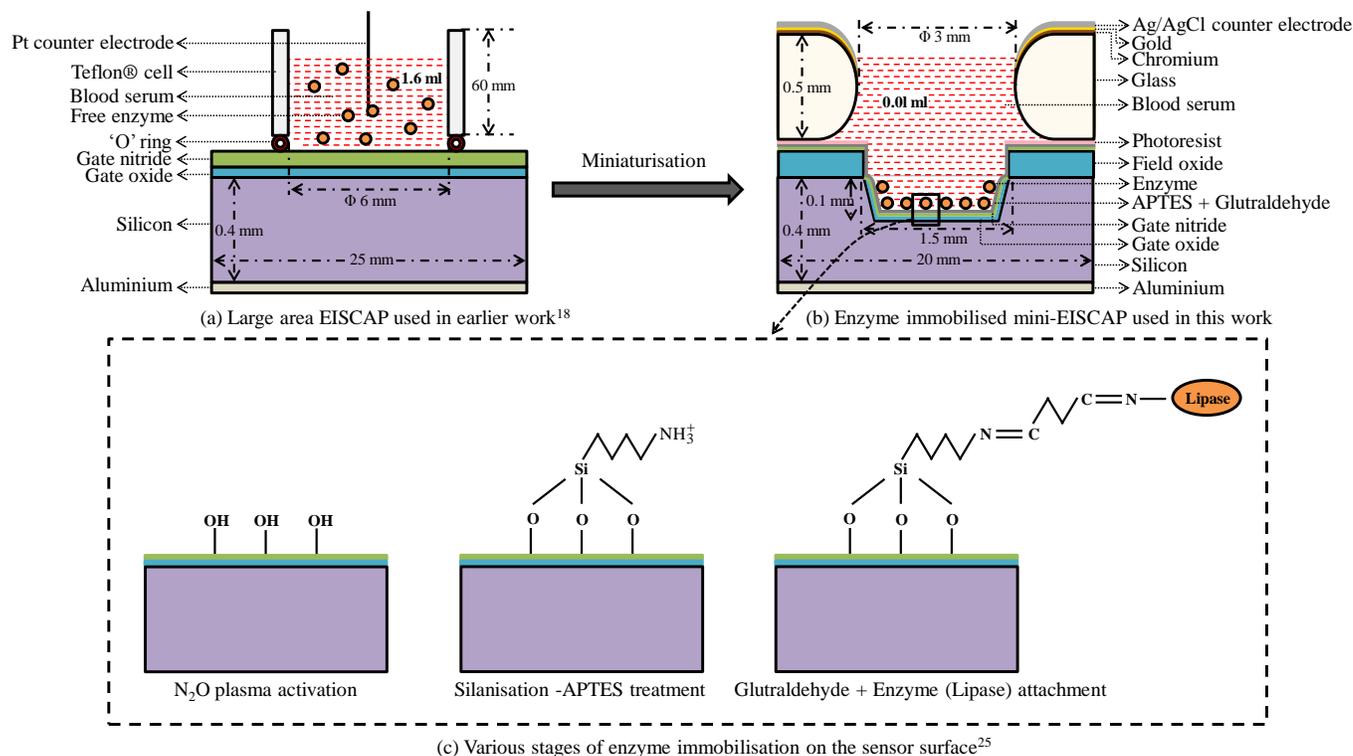(c) Various stages of enzyme immobilisation on the sensor surface[25]

Fig. 1 Schematic of miniaturised EISCAP TG biosensor (not drawn to scale)

acid, Hydrogen peroxide, Ammonium hydroxide, Hydrocholric acid, Hydrofluoric acid, Potassium iodide, Iodine, Ammonium cerric nitrate, Acetic acid, Ethanol, Aminopropyltriethoxysilane (APTES), Toulene, Glutaraldehyde, Tributyrin, Tris-HCl buffer, Phosphate buffer, Acetonitrile, *p*-Nitro Phenol Butyrate (*p*NPB) are purchased from Sigma Aldrich, India. Lipase from *Pseudomonas cepacia* is purchased from Amano, Japan. Buffer and other solutions are prepared using DI water of 18.2 MΩ-cm resistivity.

2.1   Sensor fabrication

The sensors are fabricated using processes similar to those reported earlier by us.[20,21] Briefly, the silicon wafer after cleaning using standard RCA cleaning procedure are bulk micromachined to a depth of 100 μm within masking silicon dioxide windows of dimensions 1.5 mm × 1.5 mm. The miniaturised microreactors are electrically isolated from each other by a field oxide. In each microreactor, a 10 nm thin layer of gate oxide is grown in a rapid thermal oxidation (RTO) system (AnnealSys, France) and a 30 nm of gate nitride is deposited in a plasma enhanced chemical vapor deposition (PECVD) system (Oxford Plasma Technology, UK). Aluminium is deposited in the rear side of the sensor for back contact after removing any residual oxide and nitride insulations. Detailed explanation of

the sensor fabrication is in the ESI[†].

2.2   TG sensor fabrication

In the above sub-section, fabrication of a pH sensor is discussed where the gate nitride is a pH sensitive layer. This sensor becomes a TG sensor only after making it specific to detecting TG by covalently immobilising the bioreceptor lipase to the surface of the sensor. The enzyme (lipase) with amine (-NH$_2$) terminations can be covalently attached to a molecular layer that has same amine (-NH$_2$) terminations using a linker molecule (glutaraldehyde). A molecular layer with amine terminations (-NH$_2$) is formed on the sensor surface by a process referred as silanisation and it is carried out using APTES. The linker molecule (glutaraldehyde), in principle, can covalently bond with the amine terminations forming imine (C=N) linkages. Enzymes are immobilised first by silanising the sensor surface followed by treating the sensor surface with activated glutaraldehyde and lipase.[22] The process is initiated by activating the nitride surface with a N$_2$O plasma[23] in a PECVD system. This results in the formation of -OH bonds on the surface of the nitride. The N$_2$O plasma treatment helps to promote uniform covalent adhesion of APTES and other subsequent functional layers to the nitride surface of the sensor.[24] The sensors are treated in N$_2$O
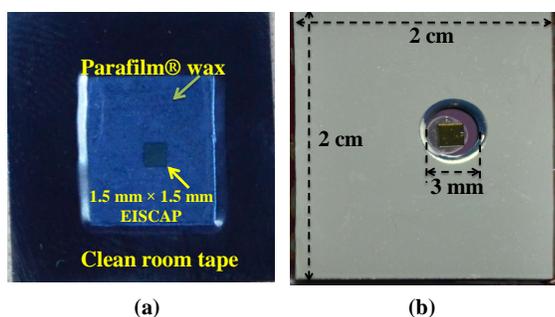
Fig. 2 Photographs of (a) diced sensor covered with parafilm® and clean room adhesive tape (b) singulated bonded and enzyme immobilised EISCAP
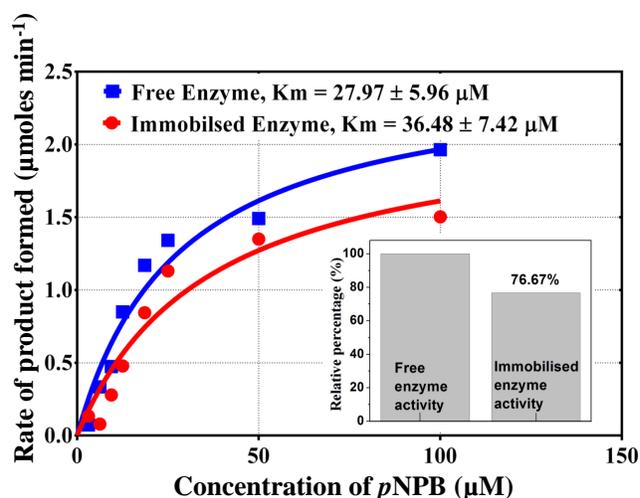

Fig. 3 Free and immobilised enzyme activity plots. Inset shows the percentage of specific activity of immobilised enzyme relative to that of free enzyme

plasma for 5 minutes in the PECVD system at 250 °C. Plasma enhanced sensors are immediately transferred to 1% APTES in anhydrous toluene (v/v) and allowed to react at 70 °C for 1 hour. The wafer after the APTES treatment are rinsed gently thrice in toluene, ethanol and DI water respectively to remove any adsorbed molecules on the surface. Subsequently, the samples after curing at 120°C for 10 minutes are treated with 0.5% glutaraldehyde in DI water (v/v) for 2 hours at room temperature and again cured at 120 °C for 10 minutes. Batch processing is carried out till this step to get maximum process matching among the sensors. After this step, individual sensors are diced out using an Ultra slice dicing machine (Ultra Tech Manufacturing, USA) to singulate the sensors. Active device area (microreactors) of the sensor are protected using parafilm® wax and clean room tape while dicing the wafer. The sensor surface is treated with the enzyme (lipase) after integrating the counter electrode to the sensor.

Procedure of counter electrode integration to the sensor has been reported in our earlier work.[20,21] Briefly, through holes of 3 mm diameter are etched in the boro-float glass wafers with 49% electronic grade HF acid. Thick photoresist and Cr-Au acts as masking layers. After removing any residue, the glass wafers are diced out before depositing the counter electrode. A thin film Ag-AgCl electrode is formed on the through hole etched glass wafer by depositing Ag with Cr-Au as pre-layers in a vacuum e-beam metallisation (HindHivac, India) system. AgCl is electrochemically deposited on the Ag layer. The singulated glass and the silicon wafers are bonded together using photoresist as an adhesive material. After bonding the glass and silicon wafers, the device active area (microreactors) are activated in 3 μl of lipase (1 mg/ml) in Tris-HCl buffer (200 mM, pH7.2) for 24 hours. The samples are finally washed thoroughly with DI water to remove any adsorbed enzyme. Fig. 1(a) shows with dimensions, the structure of the large ares EISCAP used in our earlier work[18] where the electrolyte (blood serum sample and free enzyme) was held in a Teflon®

cell and a platinum (Pt) wire dipped in the electrolyte was used as a counter electrode. The schematic of the miniaturised EISCAP TG biosensor with dimensions marked are shown in Fig. 1(b). The stages of immoblising the enzyme (lipase) to the nitride surface are shown in Fig. 1(c). Fig. 2(a) shows the photograph of the singulated sensor covered with parafilm wax® and clean room tape. Fig. 2(b) shows the photograph of the the final sensor with dimensions marked.

2.3   Biochemical characterisation and calibration

The activity of the enzyme *P.cepacia* lipase after purification is determined by *p*NPB assay as reported.[25,26] The Michaelis constants K_m (shown in Fig. 3) for free and immobilised enzyme kinetics measured at half of the maximum enzyme velocity at the maximum substrate concentration ($V_{max}/2$) are found to be 27.97 μM and 36.48 μM. The specific activities of the free and immobilised enzyme kinetics are found to be 37.8 U/mg and 28.95 U/mg (one enzyme unit 'U' is defined as the amount of enzyme that converts 1 μM of *p*NPB into product per minute). The immobilised enzyme retained 76.58% of its native activity. Fig. 3 shows the rate of the enzymatic reactions of free and immobilised enzyme.The percentage of specific activity of immobilized enzyme relative to that of free enzyme varies from 70% to 80% between different chips that are batch processed. The Fourier transform infrared (FTIR) spectra measured as reported earlier[25] confirms the formation of hydroxyl, amine and imine bonds after each of the immobilisation process steps. The details of the enzyme assay and FTIR are mentioned in the ESI†.

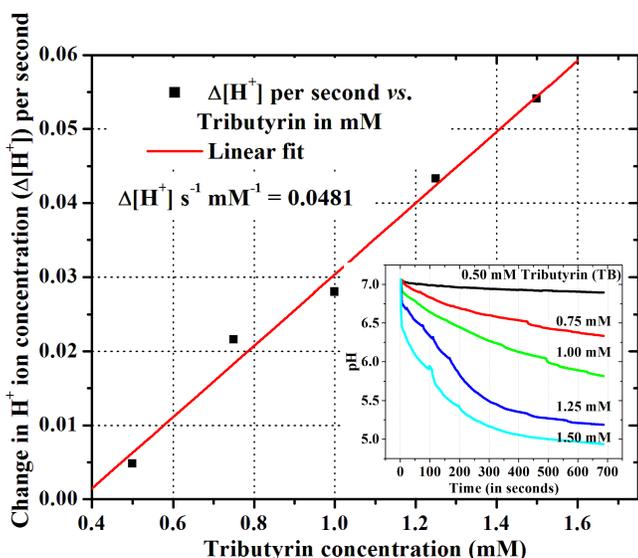The TG concentration in blood serum is measured based on

Fig. 4 Tributyrin standard plot-Average rate of hydrolysis measured at different time instances between 1 and 2 minutes of the enzymatic reaction[21] is found to be 0.0481 [H$^+$] s^{-1} mM^{-1}. Inset shows the pH change with time due to hydrolysis of tributyrin of different cocentrations with lipase

Fig. 5 Calibration and measurment modes using C-V curves

the information on rate of TG hydrolysis (calibration point). Tributyrin, a standard TG is used in this experiment. Tributyrin concentrations varying from 0.5 mM to 1.5 mM in steps of 0.25 mM are prepared using Tris-HCl buffer (pH 7.2) and acetonitrile as a co-solvent. The co-solvent plays a major role in mixing the tributyrin homogeneously in the buffer. A buffer is necessary to provide suitable environment for optimal enzyme activity. In our earlier work,[18] we have optimised the buffer concentration as 0.25 mM phosphate buffer, pH 6. However, with this buffer, it was not possible to determine the final pH at lower substrate concentration such as 1 mM tributyrin that is critical in clinical applications. Hence, in the improved version, we adopted Tris-HCl buffer system. The rate of tributyrin hydrolysis is measured using a pH meter. The inset in Fig. 4 shows the measured pH change with time for different tributyrin concentrations. A standard plot (Fig. 4) of average rate of tributyrin concentration *vs.* [H$^+$] ion concentration measured between 1 and 2 minutes of hydrolysis is obtained from the pH *vs.* time graph measured using a pH meter. The interval of 1 to 2 minutes is chosen to confine the measurement to the linear region of the enzyme kinetics. The biochemical sensitivity (S$_{std}$) from the standard plot for the tributyrin is measured to be 0.0481 [H$^+$] s^{-1} mM^{-1} or 0.00481 pH s^{-1}mM^{-1}.

2.4 TG measurement protocol

The principle of readout of TG in blood serum is fundamentally similar to the pH readout system reported earlier.[20] In our earlier work, we used pH 4 and pH 8 as reference electrolytes to calibrate the sensor against process variations. In this work, the TG measurement is done on an enzyme immobilised sensor and hence, the standard pH reference solution of pH 4 cannot be used as the enzyme activity could be deteriorated at this pH. Hence, the reference pH solutions for the TG readout system are chosen to be (pH 6 and pH 8.5) and they are used in calibrating the sensor against process variations using (1). The readout circuit principle and the details of the calibration and measurement protocols are discussed in the ESI[†]. The calibration and measurement modes are shown in Fig. 5 using the EISCAP C-V characteristics.

$$\Delta V_{ref} = 59.2\alpha(pH8.5 - pH6) \qquad (1)$$

The TG under test is measured as follows-

- The sensor is calibrated by biasing it in the middle of the C-V curve (where, EISCAP produces f = f$_{mid}$ clock frequency) and by measuring the pH sensitivity (ΔV$_{ref}$/2.5)
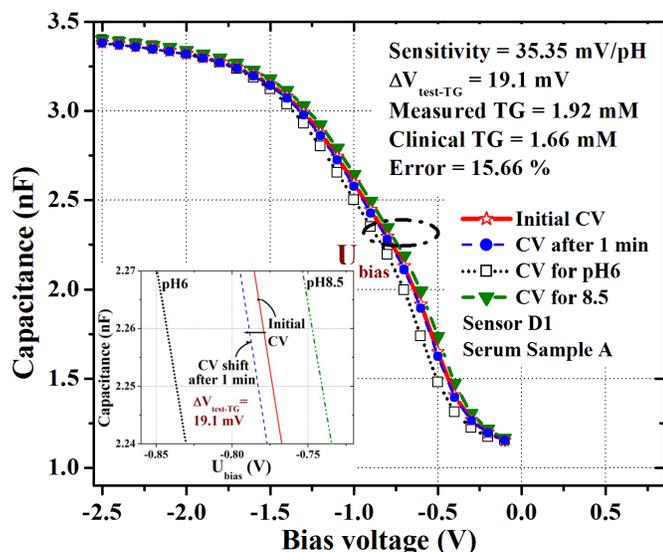
Fig. 6 C-V plots showing the device sensitivity and the TG hydrolysis in sensor D1 with blood serum A

Fig. 7 C-V plots showing the device sensitivity and the TG hydrolysis in sensor D2 with blood serum B after diluting with Tris-HCl buffer

using pH 6 and pH 8.5 electrolytes to account for the variations in the flat band voltage and the device sensitivity. The voltage shift (in mV) corresponding to a change in the pH from 6 to 8.5 at 25 °C (in mV) is given as (rewriting (1))

$$\Delta V_{ref} = 59.2\alpha(pH8.5 - pH6) \quad (2)$$

- The biochemical sensitivity from the standard plot discussed in subsection 2.3 (S_{std} after 1 minute duration) is given as

$$S_{std-1min} = 0.28 \; pH/mM \quad (3)$$

- pH sensitivity (S, in mV/ΔpH) of the device is calculated from (2)

$$S = \Delta V_{ref}/2.5 \quad (4)$$

- The blood serum sample, whose TG is to be determined, is placed in the sensor. The initial voltage and the voltage after 1 minute to bring back the frequency of oscillation to f_{mid} are measured. The voltage shift ($\Delta V_{test-TG} = \Delta V_{after-1min} - \Delta V_{intial}$) corresponds to the rate of hydrolysis depending on the concentration of the TG present in the blood serum in one minute interval.

$$\Delta V_{test-TG} = 59.2\alpha(pH_{intial} - pH_{after-1min}) \quad (5)$$

- From (3-5), $S_{std-1min}$, S, $\Delta V_{test-TG}$, the unknown concentration of TG in the blood serum is estimated to be

$$TG(inmM) = \Delta V_{test-TG}/(S \times S_{std-1min}) \quad (6)$$

- The unknown TG in blood serum is now determined using (6). The result of the test TG is displayed on the LCD screen.
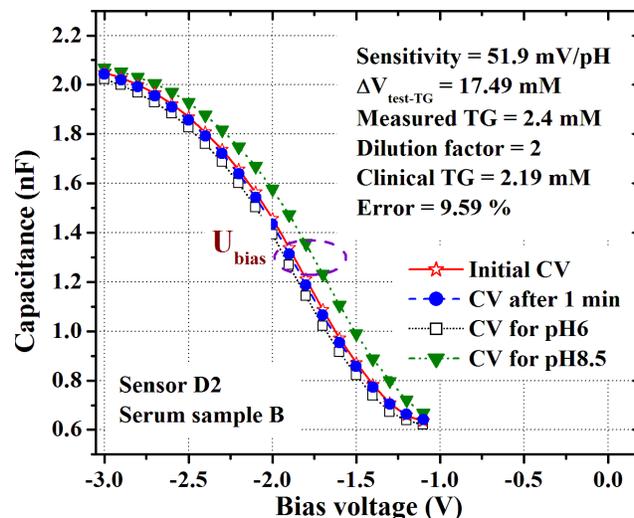
3 Results and Discussion

3.1 Blood serum TG measurements

The miniaturised EISCAP TG sensors are tested using blood serum sample to estimate the TG content. Blood samples were taken from volunteers of both sexes in the age group from 20 to 35 years. The samples were collected at the IIT Madras institute hospital after obtaining ethical clearance. One part of the collected sample was tested for TG in the clinical laboratory and the other part was used in this study to validate the miniaturised EISCAP sensors. The serum samples of 10 μl are delivered to the device using a micropipette.

Fig. 6 - 9 shows typical C-V plots measured on miniaturised EISCAP sensors using an Agilent E4980A LCR meter. The TG measurement protocol discussed in the sub-section 2.4 is used to estimate the blood serum TG concentration. The measurment procedure is discussed taking examples of a sensor D1 with blood serum sample A and a sensor D2 with blood serum sample B. The pH sensitivity of the sensors D1 is measured by calibrating it using two reference pH solutions (pH6 and pH8.5) and it is found to be 35.35 mV/pH. After placing blood serum sample A in D1, the shift $\Delta V_{test-TG}$ (= $\Delta V_{after-1min} - \Delta V_{initial}$, indicated in Fig. 5) in the C-V from the moment the sample is placed to the end of 1 minute interval is measured to be 19.1 mV. The inset in Fig. 6 clearly shows a shift in C-V after 1 minute to the left of the initial C-V curve confirming the hydrolysis of the TG in blood serum. From $S_{std-1min}$, S, and the shift $\Delta V_{test-TG}$ (+19.1 mV), the concentration of TG in serum sample A is estimated as 1.92

Table 1 Comparison of Triglyceride concentrations in blood samples measured using EISCAP and CV meter with clinical data

Sensor	Serum Sample	Blood serum dilution with TrisHCl buffer pH7.4	Device Sensitivity(S= $\Delta V/pH$)	ΔV_{test_TG} (mV)	Measured TG (mM)	Clinical TG (mM)	% Error
D1	A	No dilution	35.35	+19.10	1.92	1.66	15.66
D2	B	No dilution	51.90	-20.00	—	2.19	–
D2	B	1:1	51.90	+17.49	2.40	2.19	09.59
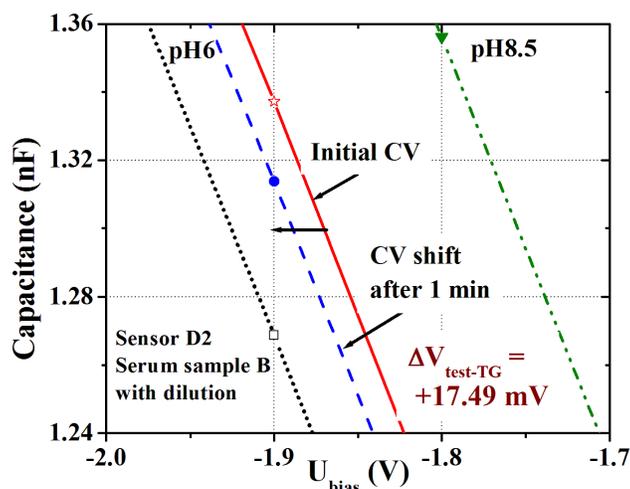
Fig. 8 Enlarged plots of C-V measurements (at the mid point of the C-V) on sensor D2 with no dilution of buffer with blood serum B
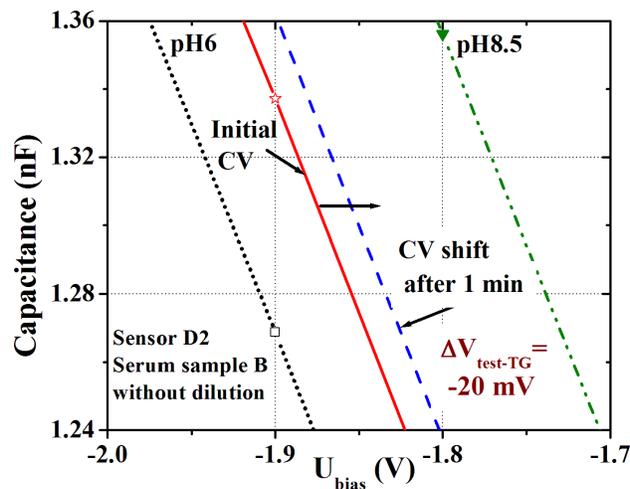
Fig. 9 Enlarged plots of C-V measurements (at the mid point of the C-V) on sensor D2 with dilution of buffer with blood serum B

mM. Clinical lab measurement was 1.66 mM for the same serum sample A.

Some blood serum samples exhibit negative ΔV_{test_TG} values and have to be diluted with Tris-HCl buffer (pH 7.4) to bring it within the clinical range 0.56 - 1.68 mM (50 - 150 mg/dL, unit conversion: 1 mM $\sim$ 89 mg/dL). The dilution is necessary to reduce the effect of the buffering action of blood[28] which is more rapid when the amount of TG in blood is higher than the normal clinical range. A negative value of ΔV_{test_TG} indicates that the C-V after 1 minute of TG hydrolysis shifts to the right of the initial C-V, due to the rapid buffering action of blood buffer within a minute time interval. An example of higher TG measurement (Device D2, serum sample B, Fig. 7) details are discussed as follows. The pH sensitivity of the sensors D2 is measured by calibrating it using two reference pH solutions (pH6 and pH8.5) and it is found to be 51.9 mV/pH. After placing blood serum sample B in D2, the shift (ΔV_{test_TG}) in the C-V (Fig. 8) from the moment the sample is placed to the end of 1 minute interval results in negative

ΔV_{test_TG} value (–20 mV) confirming the rapid blood buffering action. Fig. 9 shows the hydrolysis of the same sample after diluting it with Tris-HCl buffer (pH 7.4) solution. The shift in the C-V after 1 minute of TG hydrolysis is positive ΔV_{test_TG} (+17.49 mV), confirming that the rapid buffering action of the blood initiated by the high TG concentration has been mitigated. Table 1 shows the comparison of TG concentration in blood serum (A and B) measured using our sensors (D1 and D2) with the pathology laboratory data.

Fig. 10 shows photograph of the TG readout system connected with the mini-EISCAP. The nominal frequency of the EISCAP relaxation oscillator is set as 4 kHz and the PSoC® system clock frequency is set as 10 MHz. The mini-EISCAP is kept in a black box that prevents the light interfearing with sensor measurments and has a provision to connect the top and bottom contacts of the sensor to the readout system as shown in Fig. 10. Table 2 compares the readout measured TG against the clinically measured TG and it gives data on device sensivity and C-V shift (ΔV_{test_TG}) after 1 minute of hydrolysis.

Table 2 Comparison of TG concentrations in blood serum samples measured using the readout system with clinical data

Sample/ Sensor No.	Age group	Blood serum dilution with TrisHCl buffer pH7.4	Device Sensitivity(S= $\Delta V/pH$)	ΔV_{test_TG} (mV)	Measured TG (mM)	Clinical TG (mM)	% Error
1	20-25	No dilution	57.0	34.6	2.17	1.86	16.66
2	20-25	No dilution	45.3	10.5	0.83	0.77	07.79
3	20-25	No dilution	53.3	11.5	0.77	0.71	08.45
4	20-25	1:1	49.4	18.7	2.70	2.42	11.57
5	26-30	No dilution	48.2	20.9	1.55	1.43	08.39
6	26-30	No dilution	47.0	25.3	1.92	1.66	15.66
7	26-30	1:1	48.9	16.6	2.42	2.24	08.03
8	31-35	No dilution	39.6	19.2	1.73	1.50	15.33
9	31-35	No dilution	40.2	17.5	1.55	1.39	11.51
10	31-35	1:1	50.1	21.9	3.12	2.86	09.09
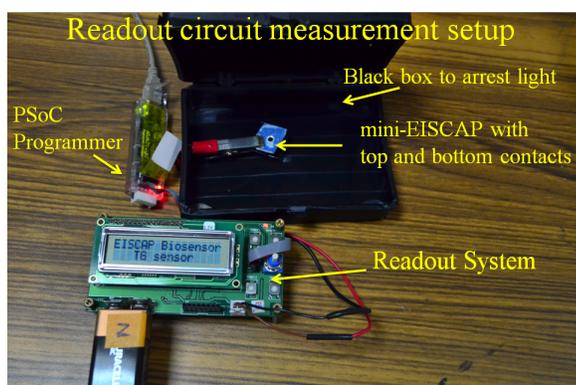
Fig. 10 Photograph of readout measurement setup with packaged miniaturised EISCAP sensor

If the measured ΔV_{test_TG} is negative then the readout system indicates the need for dilution of the blood serum with TrisHCl buffer (pH 7.4) in 1:1 ratio. A multiplication factor of 2 is automatically used to calculate the TG value when the diluted sample is used for testing. The error obtained from the TG readout measurements (Table 2) varies from 7.79 to 16.66%. The EISCAP device sensitivity and the enzyme activity play a major role in appropriate measurement of the TG concentration. The reduction in either of the sensitivities could be the reason for the measured error. The device sensitivity depends on the $-NH_2$ bonds on the surface of the nitride layer. But they get replaced by $-OH$ bonds due to the surface hydration by the electrolyte. Enzyme activity could be affected by non-uniform surface functionalisation. The enzyme activity depends on the active sites orientation on the functionalised surface and this could vary from device to device.

3.2 Comparison of the present work with eariler works

Miniaturisation of EISCAP has led to one order improvement in the surface area to volume ratio compared to the large EIS-CAP, thereby increasing the device sensitivity. The device dimensions of the large area EISCAP[18] and the miniaturised EISCAP are shown in Fig. 1. The volume of the electrolyte that can be held by the miniaturised EISCAP before packaging is 0.2 μl and after packaging is 10 μl, while that of Teflon® cell held large EISCAP[18] was 1.6 ml and hence there is 160 times reduction in the total volume of the electrolyte. The through hole in the glass wafer not only helps in containing the electrolyte, but also ensures the contact of electrolyte with the thin film electrode in the side walls. Miniaturisation can help in improving the uniformity in enzyme immobilisation among batch processed EISCAP devices. But immobilised enzyme activity depends on the availability of the enzyme active sites for the hydrolysis of TG. A comparative study of our present and earlier work are given in Table 3. Comparisons between some of the reported strategies[29−32] and the present work are given in Table 4. Compared to other strategies this work presents the design of a TG biochip with integrated enzyme and counter electrode along with a compact readout system to measure and display the unknown TG concentration in blood serum within a reasonable time duration of 5 minutes. In this strategy, it is possible to make an array of miniaturised EISCAP biosensors on silicon to monitor various blood compositions on a single chip.

Table 3 Comparison of analytical parameters of the large EISCAP (earlier work)[18] and the miniaturised EISCAP (present work)

Parameters	Large EISCAP - Earlier work[18]	Miniaturised EISCAP - Present work
Blood serum sample volume	80 μl	5 to 10 μl
Buffer and Enzyme	Phophate buffer (pH 6), 0.25 mM; *P.cepacia* lipase - free enzyme	Tris-HCl buffer (pH 7.4), 200 mM; *P.cepacia* lipase - immobilized enzyme
Total solution volume	1.6 ml	10 μl
Device sensitivity (minimum-maximum)	30 - 55 mV/pH	35.35 - 57 mV/pH
Clinical TG reported	1.9 to 2.9 mM	0.7 to 2.86 mM
Surface area to volume ratio (mm^2/μl)	0.0177	0.285
Measurement time	30 minutes + post processing time	Total readout time is within 5 minutes
Handling	Complex since enzyme and counter electrode are not integrated with the sensor. Post processing is required to determine the unknown TG	Simple since enxyme and counter electrode are integrated with the sensor. Readout system determines and displays the unknown TG

Table 4 Comparison of analytical parameters between some of the reported TG biosensors

Type	Immobilisation mechanism	Type of electrode	Detection limit	Linear range	Response time	Shelf life	Reference
Amperometric	Polyvinyl Alcohol - Glutaraldehyde cross linkage	Pt	0.21 mM	0.56 to 2.25 mM	2 s	50% loss in 70 days	[29]
Electrochemical	ZnO nano particles - Covalent cross linkage	Pt	20 mg/dL	50 to 650 mg/dL	6 s	50% loss in 70 days	[30]
Electrochemical	Conducting ploymer (Gold polypyrrole) - Covalent linkage	Au	20 mg/dL	50 to 700 mg/dL	4 s	50% loss in 70 days	[31]
Potentiometric	External immobilisation - enzyme attached to a separate reactor	Ion Sensitive FET	Not reported	5 to 30 mM	<5 min	45% loss in 2 weeks	[10]
Potentiometric	Glutaraldehyde cross linkage - immobilisation on the sensor surface	EISCAP	0.5 mM	0.7 to 2.86 mM	<5 min	30% loss in 2 weeks	Present work

4 Conclusions

Miniaturised EISCAP biosensors that use enzymatic hydrolysis to detect the triglyceride in blood serum are batch processed with optimised process steps to improve the device and the biochemical sensitivity. The miniaturised sensors are fabricated on silicon wafers and are bonded to the through hole etched glass wafers using photoresist as an adhesive at room temperature. Enzyme *P. cepacia* lipase is immobilised on the surface of the sensor and the activity of the enzyme is quantified using biochemical assay. A compact electronic readout system is designed using custom made printed circuit board and PSoC® chip from Cypress semiconductors. The enzyme immobilised miniaturised sensor is embedded in a successive approximation analog to digital conversion algorithm to calibrate the sensor and to measure the triglyceride concentration in blood serum and display the same within 5 minutes. The integrated sensor along with an electronic readout system is used for sensing and estimating the triglyceride concentration in blood serum sample and the results are compared with the clinical lab data. The device sensitivity of the miniaturised devices that we measured varies between 35.35 and 57.00 mV/pH. The error obtained from the TG readout measurements varies between 7.79 and 16.66 % and this could be because of the reduction in the device sensitivity or enzyme activity in the final sensor. The miniaturised EISCAP sensors are designed to detect and estimate the amount of TG present in blood serum within the clinical range of 50 to 150 mg/dL. This type of biosensors sensors are of relevance to the industries like biomedical and food. The sensor can also work for other bio-analytes like urea and glucose with appropriate enzymes and change in the calibration and measurement algorithm to suit the specific application.

5 Acknowledgement

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