Research Academy for Young Scientists

The Development of an Organic Electrochemical Transistor for the Detection of Sodium L-lactate

Fanni Välisalo
fanni.valisalo@gmail.com

under the direction of
Yunfan Lin
KTH Royal Institute of Technology
Department of Fibre and Polymer Technology

July 12, 2023

*

Abstract

The detection of sodium L-lactate to determine the status of energy metabolism is vital in several medical applications, including oncology. This study will describe the development of a biosensor, specifically an organic electrochemical transistor, that can detect different concentrations of sodium L-lactate. The functionality, as well as the signal amplification in response to different concentrations of sodium L-lactate solution, of four devices and seven channels of the same size that have undergone identical sample preparation have been tested, with the results showing that the drain current of a majority of them comes close to zero when turning OFF and returns back to the original starting value when it returns to ON mode. The results also display that all of the devices and channels are responsive to changes in sodium L-lactate concentration, with the gate current, drain current, or both signals being amplified. In the future, a promising biosensor can potentially be used in the diagnosis of many diseases, such as cancer.

Acknowledgements

To start, I would like to thank my mentor, Yunfan Lin, whose guidance has been absolutely instrumental in providing a safe space, where no questions are considered dumb, for me to not only complete my experiment, but also learn to explore different possibilities of how a goal can be achieved. I am indebted to my family and friends who supported me through the highs and lows, and gave me their endless support and time throughout the duration of this project. One cannot forget the instrumental support provided by the organizers of the summer research program, namely Victor Sundström, Julia Mårtensson, Linnea Jonröd, and Max Eriksson. Many thanks to AstraZeneca and Beijerstiftelsen for providing the funding necessary for the completion of this research experience. Last, but not least, my gratitude to Rays — for Excellence knows no limits as they have given me an opportunity to further explore my interests in research.

Contents

1 Introduction

Biosensors are devices that produce signals proportional to the concentration of the analyte in a biological or chemical reaction [1]. These instruments are used in several medical applications, including the detection of sodium L-lactate to monitor energy metabolism. This has applications in brain cell and tumor cell research [2]. One type of biosensor, the organic electrochemical transistor (OECT), employs an electrolyte, which injects ions to an organic thin film of a polymer to change its doping state once a gate voltage is applied, consequently impacting the transistor's conductivity [3].

1.1 Structure of an OECT

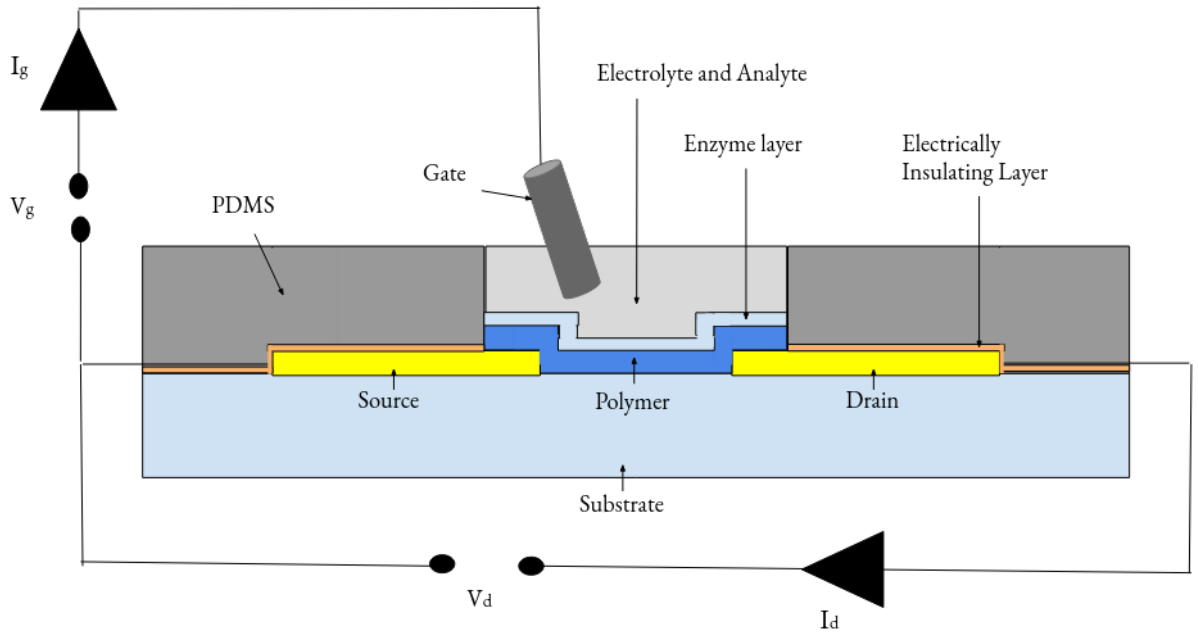
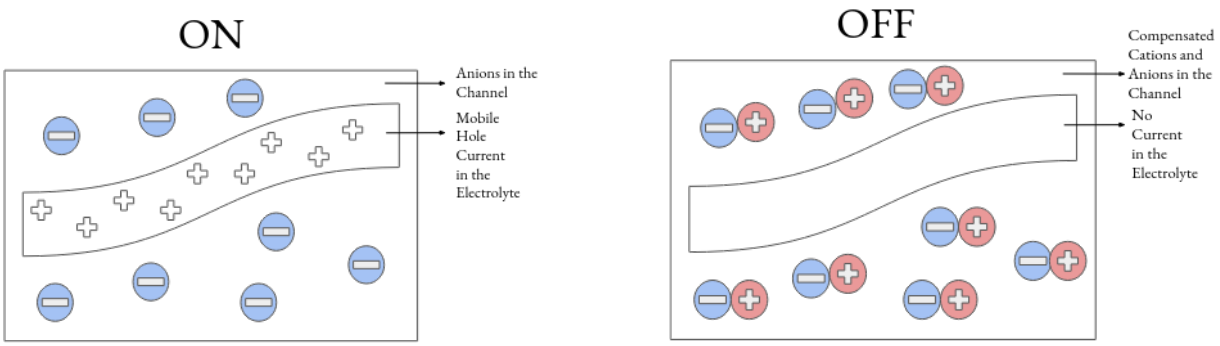


Figure 1: Basic structure of an OECT device, where all relevant parts are labeled. The drain voltage (V_d) measures the potential difference between the source and drain, whilst the gate voltage (V_g) is the difference between the gate and source potentials. Similarly, the drain current (I_d) provides a value for the current between the source and drain, and the gate current (I_g) indicates the current between the gate and source.

Figure 1 shows the basic structure of an OECT device. The base of the transistor is known as the substrate. On top of the substrate lies an electrically insulating layer, which is spread across the entire surface of the substrate, except for the channel. The channel, consisting of a source and drain, is placed on top of this layer. The electrolyte is placed

on top of an organic polymer film, contained within the boundaries of a polydimethylsiloxane (PDMS) buffer. As usual, the latter acts as a physical buffer to make sure that the contact areas, which is where the probes controlling the external current and voltage will be placed, only conduct through the channel rather than the electrolyte directly. The electrolyte will contain a buffer solution, a certain concentration of the analyte, as well as the gate [3].

OECTs can operate in two different modes and types, depletion and accumulation, as well as p-type and n-type, respectively. Figure 2 shows the operation of the p-type depletion mode, which is what the devices developed in this paper will operate in. The device will start by being in the ON mode, turn OFF, and then enter the ON mode again.



(a) The device is turned on (b) The device is turned off

Figure 2: The operation of a device in p-type depletion mode. Figure 2a shows that in the absence of a gate voltage, there will be a flow of current produced by mobile holes, which are positive charge carriers, indicating that it is in the ON state. P-type refers to the cationic nature of the current. In turn, Figure 2b illustrates the effects of applying a positive gate voltage, which will eventually turn the device OFF. The electrolyte on top of the channel injects cations into the anionic channel, thereby dedoping the device since there is a lower number of mobile holes, and hence a lower current. Once there are no cations left in the electrolyte, the device is fully turned OFF because there is no current [3].

1.2 Bernard's Model

Bernard's Model provides a set of assumptions that must hold true for OECTs in order to identify potential errors resulting from factors that are either impossible or difficult to control. One of the assumptions made is that the cations from the electrolyte are injected

into the channel, impacting the conductivity of the entire channel. Another assumption that the model makes is that the OECT is capacitative, meaning that the cations do not react with the charges of the anions in the channel, but rather compensate them. Provided that the OECT adheres to the approximations made in the model, the gate current would reach zero, when the capacitor— which is the channel— is fully charged [3].

1.3 Sodium L-lactate and Lactate Oxidase

The chosen analyte, supplied by Sigma Aldrich, was sodium L-lactate. The sample's enzyme assay is 98%. Sodium L-lactate and its molecular concentration is a vital biomarker of cellular metabolic states because it is a byproduct of anaerobic glycolysis, which is useful in several fields of medical research such as the biology of cancer as well as immunology [4]. Tumorous cells tend to undergo anaerobic glycolysis more than non-tumorous cells do, meaning that the former should have a higher concentration of sodium L-lactate. The chemical reaction that took place in the biosensor is the sodium L-lactate oxidation reaction, which produces pyruvic acid and hydrogen peroxide [5]. A first generation biosensor, such as the one developed in this study, amplifies the occurring signal when it detects more hydrogen peroxide. One of the enzymes that catalyzes this reaction is lactate oxidase. The specification sheet provided by Sigma Aldrich, the manufacturer, states that the enzyme must be stored in a temperature of 37°C and a pH of 6.5 before and during experimentation [6].

1.4 Types of Data

This study will include three different types of results: the transfer curve, transconductance curve, and dynamic test graph. The transfer curve measures drain current against gate voltage. Small voltage signals applied to the OECT by the gate are transduced into amplified changes in the drain— and voltage— currents. The transduction is described by the transfer curve, and can provide an overall view of the biosensor's functionality. Figure 3 shows whether the current increases or decreases as it approaches the ON mode or the OFF mode, as well as when the device reaches these modes. The rate of change of the

transfer curve provides the efficiency of transduction, known as transconductance, and is used as a metric to evaluate the device's functionality. The high transconductance values associated with OECTs make them an important field of study for biosensors [3]. On the other hand, the dynamic test measures either drain current as a function of time, or gate current as a function of time. The drain current increases proportionally to the increase in sodium L-lactate concentration. The more sensitive a biosensor is, the more amplified the current change should be [7].

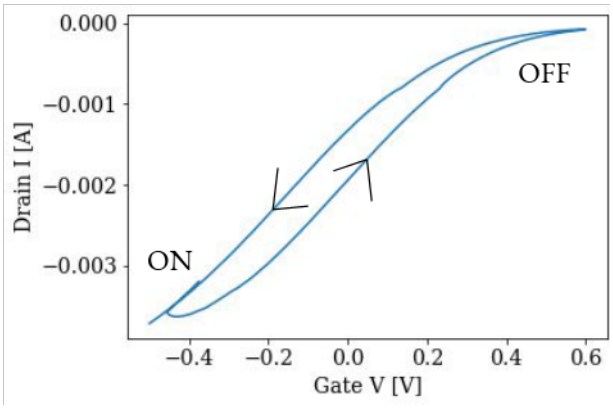


Figure 3: Transfer curve of a transistor operating in p-type depletion mode. It starts by conducting, meaning that they are turned ON, but as a gate voltage is applied, the devices dedope and theoretically stop conducting, which in turn implies that they are in the OFF mode. The Figure uses real data, which is why the drain current comes very close to zero, but doesn't quite reach it.

1.5 Aim of Study

The aim of this study was to develop an organic electrochemical transistor biosensor that successfully detects sodium L-lactate concentration by producing amplified signals, and to analyse its transconductance as well as functionality in achieving said purpose. The biosensor will operate in p-type depletion mode. Only channels of size 10 µm that have undergone identical sample preparation will be utilized, which implies that the tests conducted on each channel can be interpreted as one trial of the biosensor. The channels will be made of gold whilst the gate will be silver, as the latter is more sensitive to different concentrations of the analyte [8].

2 Method

The method is divided into two sections: sample preparation and experimental procedure. The devices, enzymes, and appropriate concentrations of the analyte were prepared before conducting the experiment. The terms 'OECT', 'device' and 'sample' are used interchangeably, and refer to the biosensor.

2.1 Sample Preparation

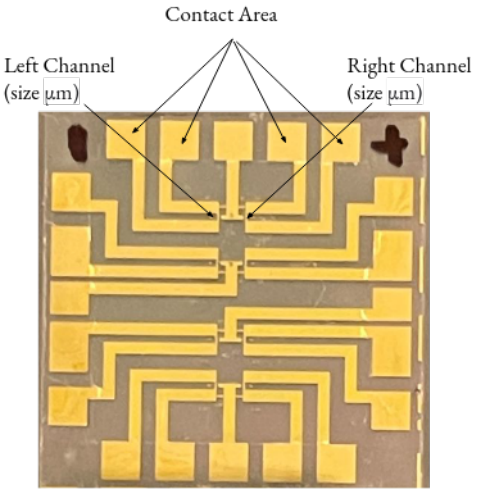


Figure 4: Two channels of size 10 µm and four contact areas are labeled on the device.

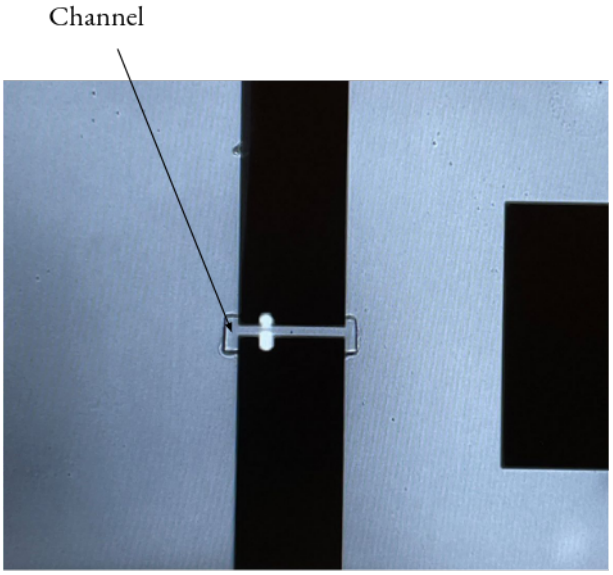


Figure 5: A channel being opened.

Figure 4 shows one of the four samples that was used in the experiment, with the relevant

channels and contact areas labeled. All of the devices had undergone plasma treatment in order to prepare them for the nanoscribe procedure. The channels were opened using a nanoscribe, as showcased in Figure 5, with a laser power of 20 mW for an area of $120 \times 30\mu$m to make sure that the channels are fully opened. The devices were cleaned afterwards to remove any excess ash. The polymer, PEDOT:PSS, was prepared by mixing 950 µL of poly(3,4-ethylenedioxythiophene), 50 µL of ethylene glycol, 10 µL of (3-Glycidyloxypropyl)Trimethoxysilane, and 2.5 µL of 4-Dodecylbenzenesulfonic acid in a test tube. The polymer was spin coated onto the device at 2000 rpm. After the spin coating process, the substrates were placed in an oven of 120°C for 30 minutes.

The samples were treated with plasma for two minutes to create hydroxyl groups that enhance the biosensor's sensitivity. Before the plasma treatment, the contact areas of the devices were covered with tape to keep them as clean as possible. The devices were then placed in a vacuum of 67 cmHg at room temperature with a 1000 µL solution of (3-glycidyloxypropyl)trimethoxysilane, abbreviated as GOPS, for 2.5 hours so that it was spread evenly across the polymer.

The enzyme, lactate oxidase, was prepared in concentrations of 200 units per mL in phosphate-buffered saline (PBS) that immobilized the enzyme and kept it optimally active at a pH of 7.4 [9]. After being refrigerated overnight, 50 µL of the solution was placed on the channels of size 10 µm on each of the four substrates. The substrates were placed in an incubator of 37°C to ensure optimal enzyme activity. A 1M solution of sodium L-lactate was prepared by adding 0.02 g of the salt to 178.5 mL of distilled water. Thus, concentrations of 1, 1×10^{-1}, 1×10^{-2}, 1×10^{-3}, 1×10^{-4}, 1×10^{-5} mol L^{-1} of sodium L-lactate were prepared using the ratio, 1 : 9, of concentrated solution and distilled water.

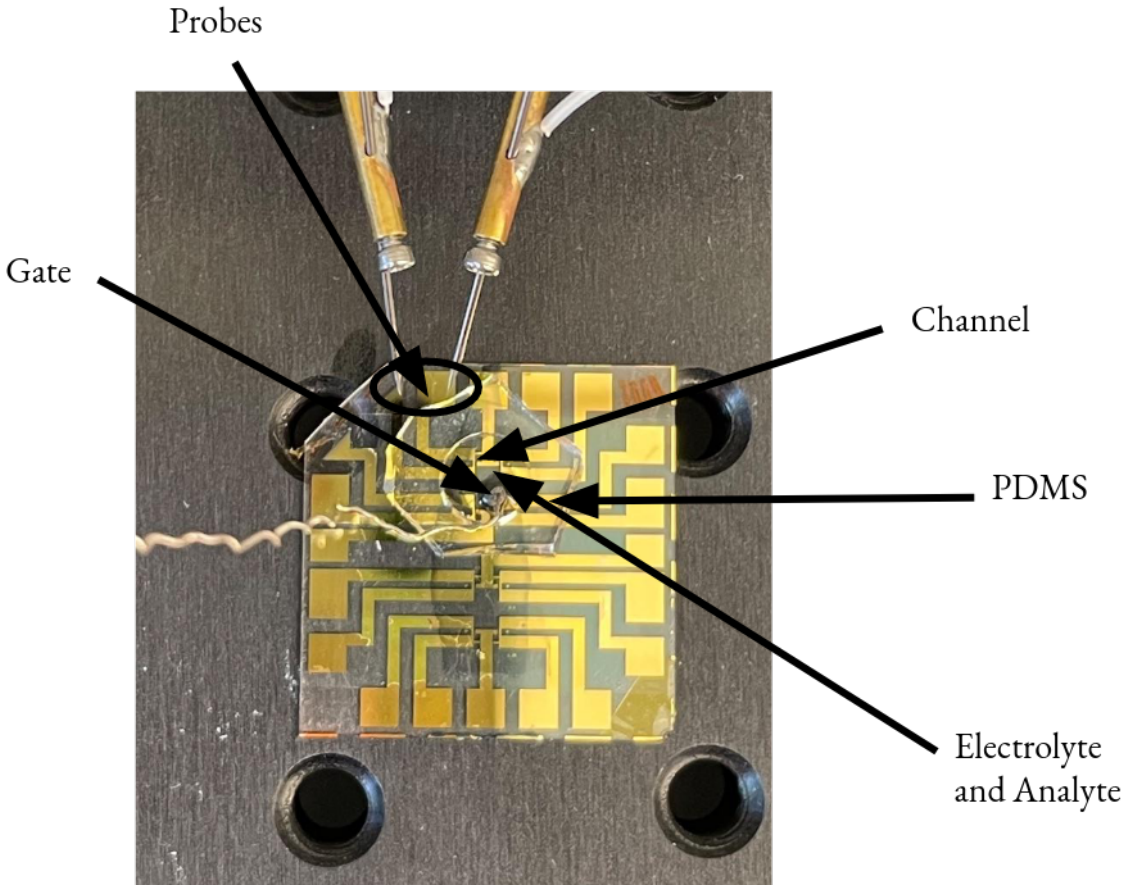


Figure 6: Experimental setup showing the channel, gate, PDMS, electrolyte and analyte solution, as well as the probes connected to the source and drain of the channel.

2.2 Dynamic Test Procedure

As shown in Figure 6, two probes were placed on the contact areas of the source and drain of the selected channel. A PDMS was placed on top of the channel. Thereafter, 50 µL of PBS was placed on the device— within the boundaries of the PDMS— with a pipette, and the gate was placed in the solution. A Keithley 4200A-SCS Parameter Analyzer was utilized to produce transfer curves, where drain current is measured as a function of gate voltage. The gate voltage ranged from -0.5 to 0.6 V, with a step size of 0.004 V. The analyzer operated in linear voltage sweep mode. There were at least three runs performed before the data was saved depending on how consistent the data provided by the analyzer was, which in turn is related to the stability of the polymer and the enzyme activity in the sample. The transconductance curve was derived as the discrete rate of change of the transfer curve. Thereafter, the same instrument was used to obtain dynamic test graphs,

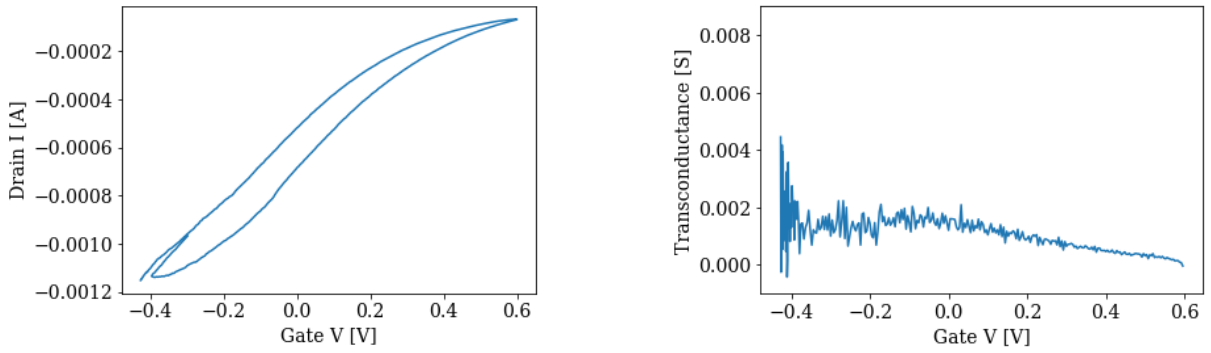
which measure drain current and gate current as functions of time. The sodium L-lactate solutions were added manually using a pipette, 5 µL at a time, increasing the electrolyte concentration and amplifying the produced signals.

3 Results

The first two types of data collected in this experiment were the transfer curve and transconductance curve. These graphs provide a solid understanding of the OECT's effectiveness and functionality. The third result, the dynamic test, displays the ability of the biosensor to amplify signals as the concentration of sodium L-lactate increases. The data is described and analysed in the discussion, under the subheading of "Interpretation of Results".

3.1 Transfer and Transconductance Curves

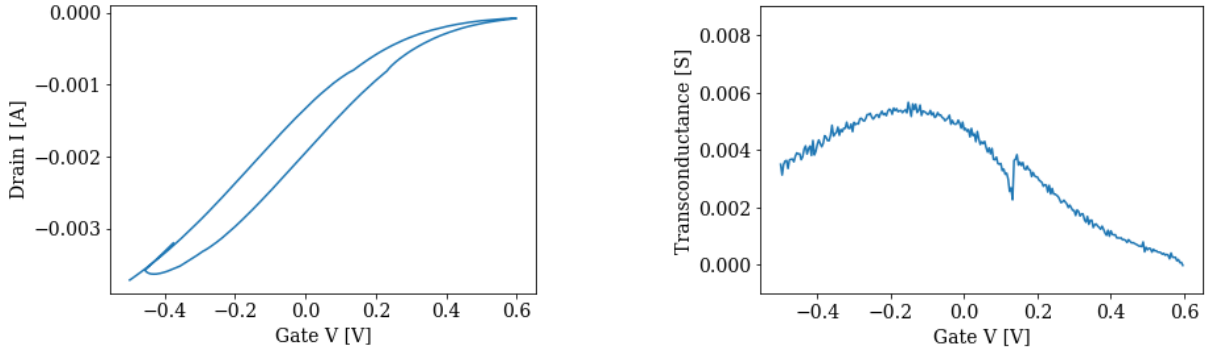
The transfer and transconductance curves provide an understanding of the device's transduction. The graphs for each sample and channel are listed below.



(a) Transfer curve

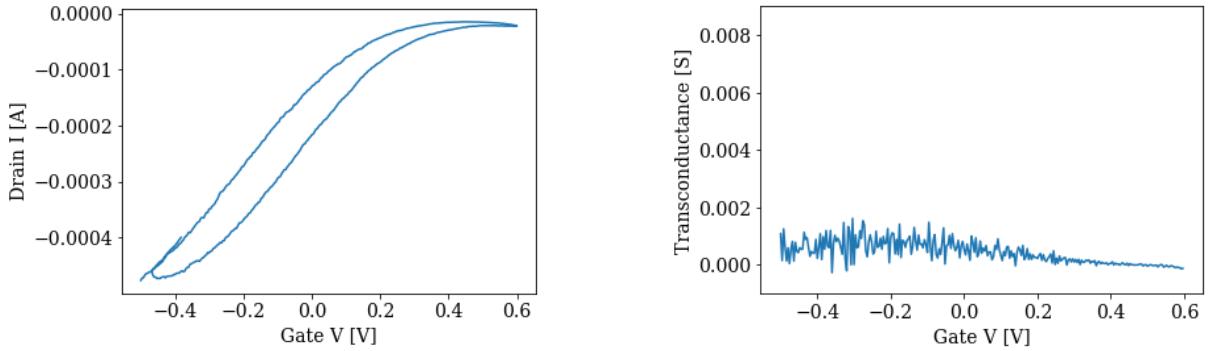
(b) Transconductance curve

Figure 7: Transfer and transconductance curves for sample 1, left channel.



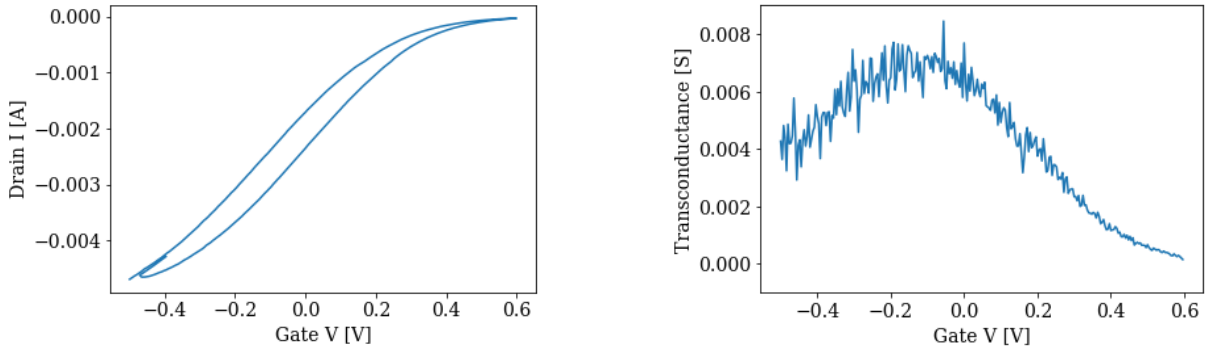
(a) Transfer curve (b) Transconductance curve

Figure 8: Transfer and transconductance curves for sample 1, right channel.



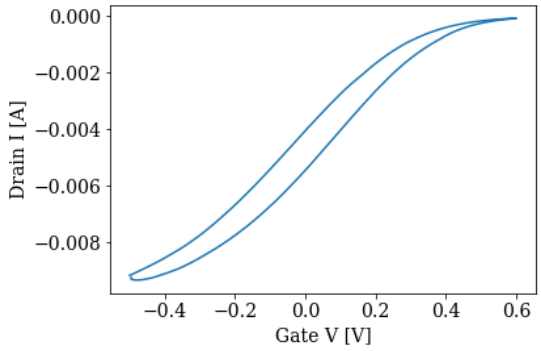
(a) Transfer curve (b) Transconductance curve

Figure 9: Transfer and transconductance curves for sample 2, right channel.

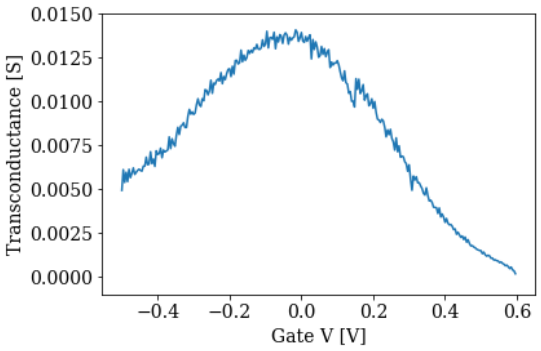


(a) Transfer curve (b) Transconductance curve

Figure 10: Transfer and transconductance curves for sample 3, left channel

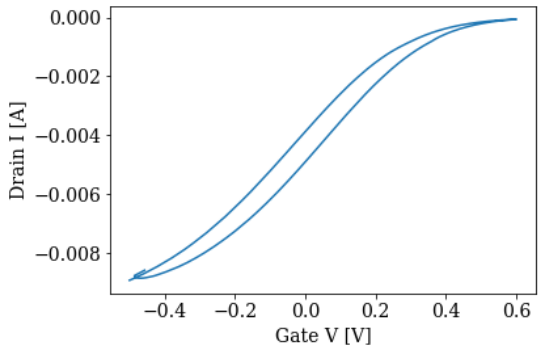


(a) Transfer curve

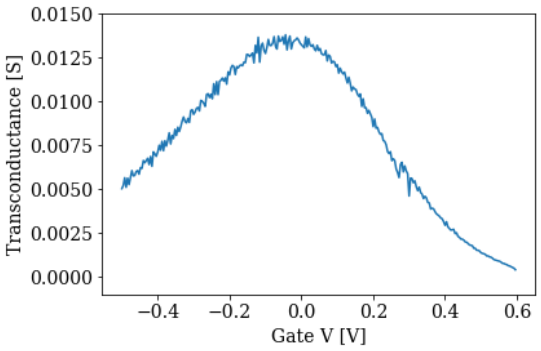


(b) Transconductance curve

Figure 11: Transfer and transconductance curves for sample 3, right channel.

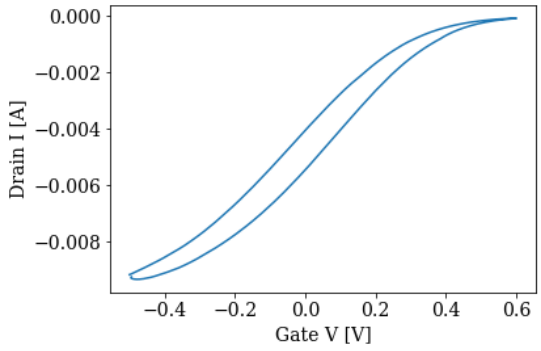


(a) Transfer curve

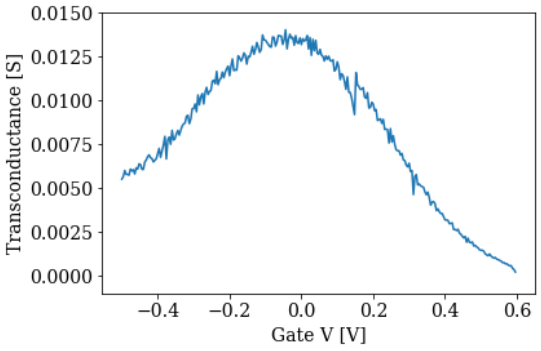


(b) Transconductance curve

Figure 12: Transfer and transconductance curves for sample 4, left channel.



(a) Transfer curve



(b) Transconductance curve

Figure 13: Transfer and transconductance curves for sample 4, right channel.

3.2 Dynamic Test

The dynamic test is a measure of the biosensor's ability to amplify the current signal produced by the change in sodium L-lactate concentration. The tests for each sample and channel are compiled below.

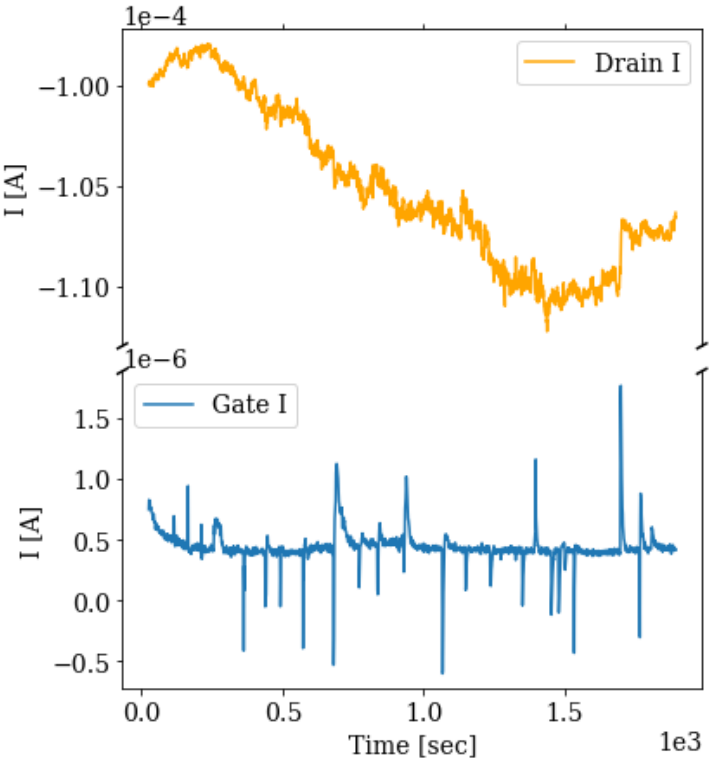


Figure 14: Dynamic test for sample 1, left channel.

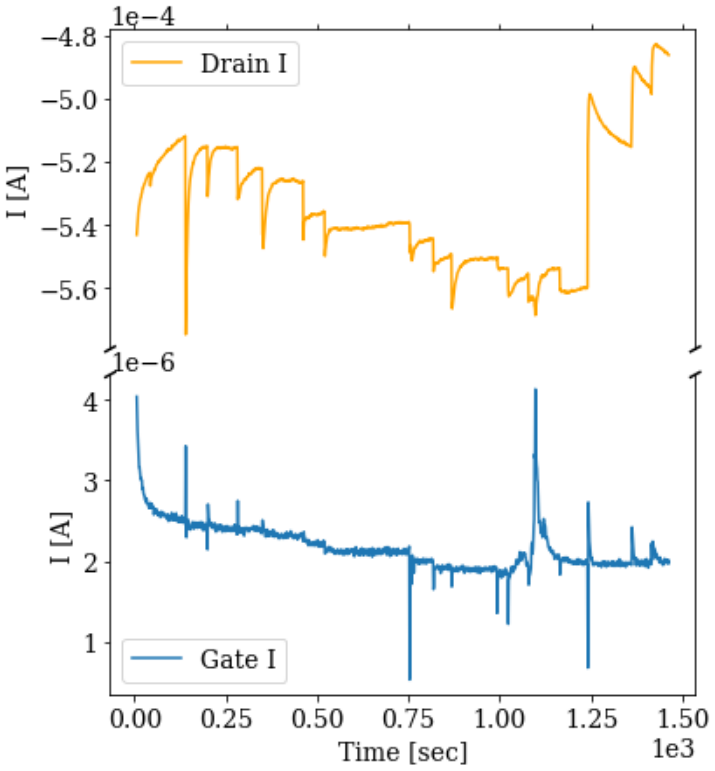


Figure 15: Dynamic test for sample 1, right channel.

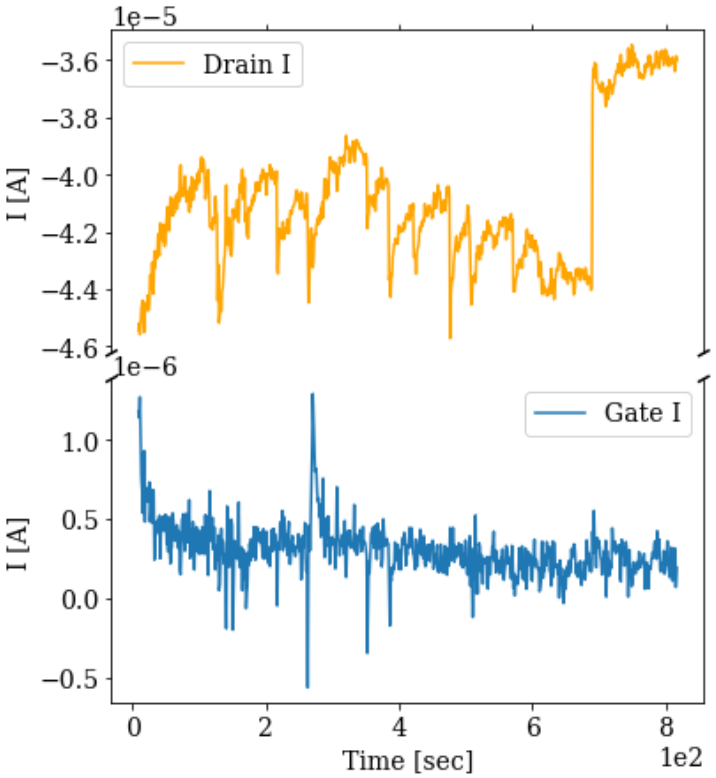


Figure 16: Dynamic test for sample 2, right channel.

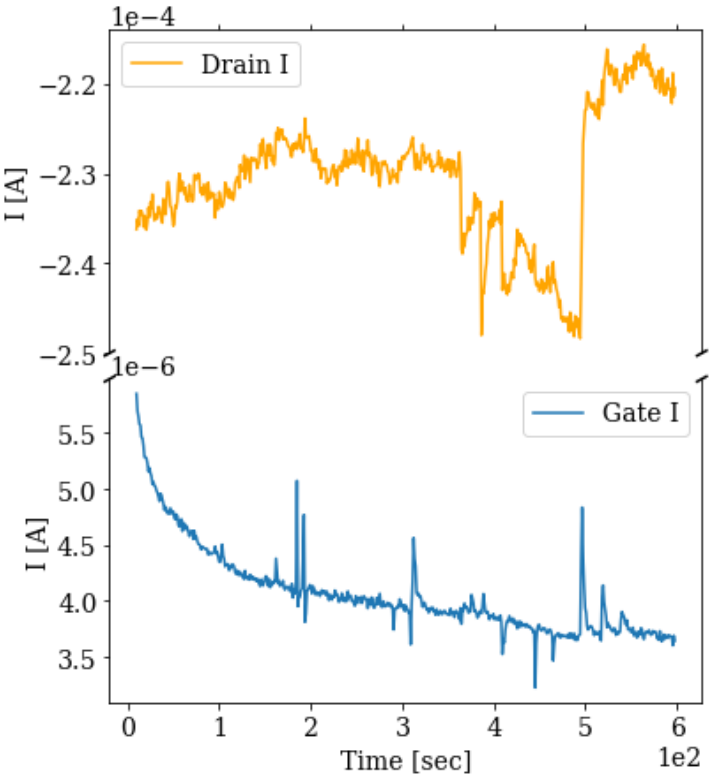


Figure 17: Dynamic test for sample 3, left channel.

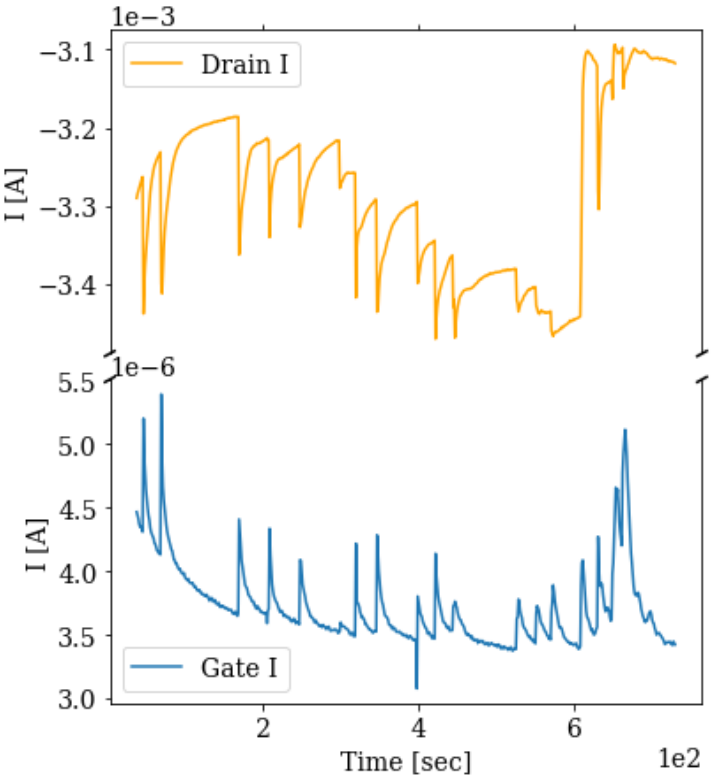


Figure 18: Dynamic test for sample 3, right channel.

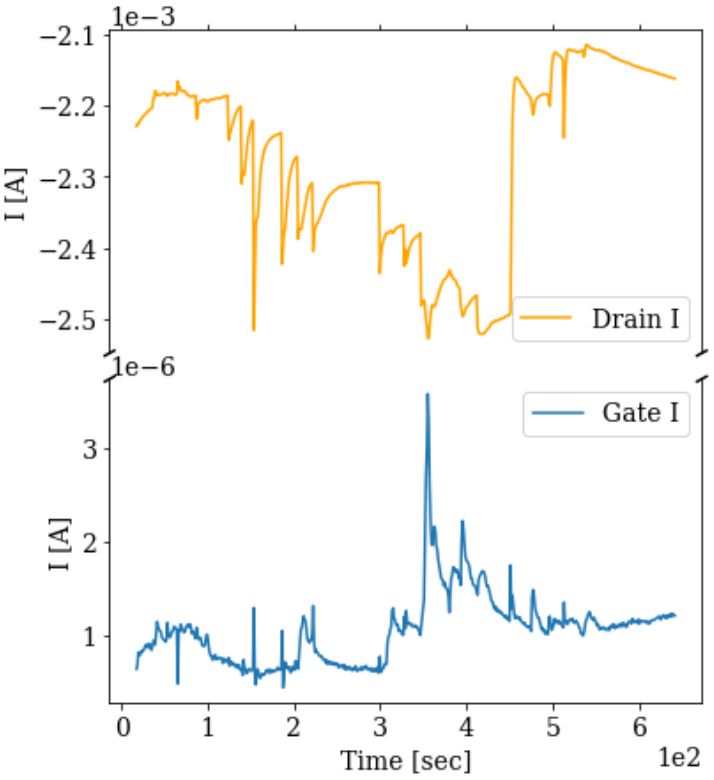


Figure 19: Dynamic test for sample 4, left channel.

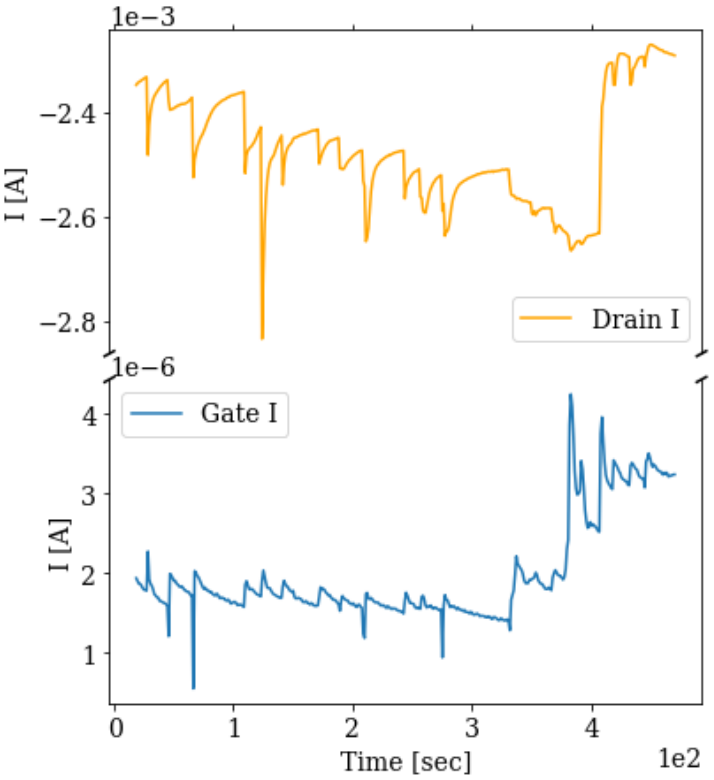


Figure 20: Dynamic test for sample 4, right channel.

4 Discussion

In addition to the analysis of the results, this section will also include a discussion of their validity, as there are several factors that can affect the data's reliability, precision, and accuracy. Thereafter, areas of improvement will be identified, so that when a study with the same purpose is repeated, the data will be more valid.

4.1 Interpretation of the Results

4.1.1 Transfer Curves and Transconductance Curves

It is clear that the transfer curves— part a) of Figures 7 to 13— behave in a similar manner. One can compare the behavior of the devices when they start by being in the ON mode, enter the OFF mode, and then turn ON again. When turning OFF, the current is very close to zero, meaning that the devices are working as they should. However, when turning ON again, some of the devices return to their initial current value— specifically Figures 11, 12, and 13— whilst the rest of the channels have varying ending-and-starting current values. There is an absolute error produced by the instrument used to collect data— Keithley 4200A-SCS Parameter Analyzer— of 1×10^{-13}, which emphasizes the precision of the results.

When it comes to the transconductance— part b) of Figures 7 to 13— , the operation of the device is more successful if the curves are smoother, as it indicates that the device is stable, and if the stationary point is at a higher value, as this is the transconductance value that can be compared across all samples. Since transconductance is the discrete rate of change of the transfer curve, it is natural that Figures 11, 12, and 13, which have similar transfer graphs, also have like transconductance curves. Since those Figures portray expected behavior, such as the current nearly reaching zero in the OFF mode and returning to a very similar value in the ON mode, the y-value of the stationary point is also very high— at around 0.015 S. On the other hand, Figures 8 and 10 act in a somewhat predictable manner, which means that they have lower transconductance values of around

0.005-0.008 S. Figures 7 and 9 have rather unstable transfer and transconductance curves in comparison to the rest, with their low transconductance values lying in the range 0.000 to 0.002 S.

4.1.2 Dynamic Test

Channels with high transconductance values are more sensitive to changes in sodium L-lactate concentration. Figures 11, 14, 15, and 16 display rather stable gate voltage baselines, indicating that the devices are stable. There is also noticeable change in both the drain and gate currents, which is proportional to the change in concentration majority of the time. Figure 16, on the other hand, shows excellent signal amplification when it comes to drain current as both the signal change and the baseline increases proportionally to time, and therefore, the concentration of sodium L-lactate. Figure 14 has both a stable baseline and enough sensitivity to display decent signal change, but the same cannot be said for the drain current as there is no obvious signal amplification to indicate the time of when the concentration increased. By contrast, Figure 17 has a rather unstable baseline for both the drain and gate currents. The signal change is more noticeable for the gate current, and less so for the drain current.

4.2 Validity of Results

4.2.1 Transfer Curve

As mentioned in the method, each of the transfer curves were measured 3 or more times, depending on how stable the device, and the data provided by it, were. Some channels had more runs than others because they were less stable. The overall stability of the channel can be affected by several factors, such as the consistency in the performances of the polymer, as well as enzyme activity. Seven trials were conducted, as seven channels of the same size that had undergone identical sample preparation were tested. Due to the high number of trials and similar data behavior, the results are deemed as rather reliable. The fact that the channels follow a general trend in functionality also serves the purpose of confirming that the results are indeed precise. When it comes to accuracy, it

is obvious that there is some systematic error as, in all of the channels, the drain current never reaches the value of 0 A in the OFF mode, and when turning back ON, the drain current in several of the channels does not quite return the original starting value, as it theoretically should. The systematic error can be attributed to the approximations made in Bernard's Model that are applied to OECTs. Hence, it can be concluded that there is some systematic error present, but the data is still mostly predictable.

4.2.2 Dynamic Test

The dynamic test results are quite reliable. This is because the baselines for each channel was very stable. However, in the process of making the electrolyte more concentrated, some channels only showed signal change in the gate current, whilst the current change in other channels was noticeable in both the drain and gate currents. Even though all the tests were conducted within the same day and in two sittings, the differences can be attributed to human error or environmental factors. The position at which the sodium L-lactate solution is added to electrolyte to make it more concentrated, the location of the gate, as well as any changes that occur for these two aspects can also affect the result. The gate occasionally came in contact with the pipette, thus moving it and affecting the current. Hence, the results are bound to random error. However, the results were rather accurate because the same change in concentration was tested at least three times to see if it would provide a similar, proportionate change in current. Therefore, since there were several smaller trials conducted in the dynamic test of each channel, the data is reliable. The accuracy of the results can be commented upon in the perspective that most of the channels showed a change in current as the concentration of sodium L-lactate increases, meaning that the majority of the channels behave accurately. However, the signal change is not proportional to the change in concentration, which makes the results less accurate. Nonetheless, this can be attributed to leakages of the electrolyte as its volume exceeds the threshold of the PDMS buffer. When it comes to precision, it is important to note that, in most cases, there was an increase in current when the sodium L-lactate concentration increased. Since most of the data shows this, it is safe to conclude that the results are

precise.

4.3 Future Studies

The transfer curve data was collected over the period of two days. Since environmental factors, such as temperature, can impact the biosensor, it would be optimal to conduct the tests in one sitting so that the surroundings change as little as possible. In addition, since changing the concentration of sodium L-lactate in the electrolyte required manual work, the automization of this process using a coded program would greatly improve the accuracy of the results and prevent random human error from affecting the data. The significance of using biosensors to detect lactate concentration lies in the fact that it can help piece together a fuller picture of energy metabolism by providing information about the specific cellular metabolic status. This is applied in medical research, such as those concerning cancer, immunology, brain cells. Furthermore, once a sensitive enough biosensor has been developed, it can even be used to diagnose these diseases.

4.4 Conclusion

The results showed that the OECT biosensor developed in this study was successful. Some channels provide dynamic test graphs where the change was more obvious, and others less so. Specifically, the right channel of sample 3 was the most precise biosensor as it had a high transconductance value at the stationary point, and the drain current came very close to zero when turning OFF and returned to a nearly identical value when it, once again, entered the ON mode as seen in Figure 11. As seen in Figure 18, it also accurately amplified the changes in both gate and drain current, in a manner that was proportionate to the change in lactate concentration.

References

[1] N. Bhalla, P. Jolly, N. Formisano, and P. Estrela, "Introduction to biosensors," *PubMed Central*, Jun 2016.

[2] G. Rattu, N. Khansili, V. Maurya, and P. Krishna, "Lactate detection sensors for food, clinical and biological applications: a review," *Environmental Chemistry Letters*, vol. 19, pp. 1–18, 10 2020.

[3] J. Rivnay, S. Inal, A. Salleo, R. M. Owens, M. Berggren, and G. G. Malliaras, "Organic electrochemical transistors," *Nature Reviews Materials*, vol. 3, no. 2, pp. 1–14, 2018.

[4] K. Schmiedeknecht, A. Kaufmann, S. Bauer, and F. Venegas Solis, "L-lactate as an indicator for cellular metabolic status: An easy and cost-effective colorimetric l-lactate assay," *Plos one*, vol. 17, no. 7, p. e0271818, 2022.

[5] Y. Lin, "Organic electrochemical transistors for the detection of xanthine," 2022.

[6] S. Aldrich, "Product specification: Lactate oxidase," *Sigma Aldrich*.

[7] P. Li and T. Lei, "Molecular design strategies for high-performance organic electrochemical transistors," *Journal of Polymer Science*, vol. 60, no. 3, pp. 377–392, 2022.

[8] R. Tiwari, S. Singh, P. Lohia, and D. Dwivedi, "Comparative study between silver and gold metal film-based surface plasmon resonance biosensor with platinum diselenide," in *VLSI, Microwave and Wireless Technologies: Select Proceedings of ICVMWT 2021*, pp. 329–335, Springer, 2022.

[9] S. Aldrich, "Product specification: Phosphate buffered saline," *Sigma Aldrich*.