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Development of Nanoelectrodes for Electrochemical Biosensing in Sweat Flow

THE UNIVERSITY
of EDINBURGH

Fiona Moore
2024
Abstract

Developing an electrochemical device capable of measuring biomarkers in sweat would open up a wide range of possibilities for non-invasive health monitoring as well as overcome some of the limitations of sampling in blood, such as low patient compliance and non-continuous measurement. In this work, glucose is used as a proof-of-concept due to its importance for diabetes monitoring, the robustness of the glucose oxidase enzyme, and the wealth of previous research into glucose sensing.

Glucose concentrations in sweat are low compared to those in blood and sweat volume is limited, therefore sensitive measuring techniques that can operate in low sample volumes are important to provide meaningful results. Platinum nanoband electrodes and silver/silver chloride on-chip microelectrodes were fabricated to develop a first-generation glucose sensor. Nanoband electrode arrays have a small active area, which allows low analyte depletion; and highly efficient mass transport, which provides a low limit of detection and high sensitivity as well as a stable electrochemical response under flow conditions. These nanoband electrodes were demonstrated to be capable of measuring at the relevant glucose concentrations in sweat.

Another key challenge is designing an appropriate sweat sampling method to prevent contamination and mixing of sweat at the electrode. To address this, the electrode geometry was developed into a through-flow device with nanoband electrodes embedded into micropores in a silicon nitride membrane. The electrode was electrochemically characterised both experimentally and through COMSOL simulations, giving consistent results. The through-flow electrode operated in a flow insensitive regime across the relevant range of flow rates in sweat and at higher flow rates the response showed reproducible flow sensitivity.

Additionally, fabrication processes were developed to increase the mass manufacturability of certain process steps initially performed at chip-level. This allowed wafer-level electrochemical processing to chlorinate on-chip reference electrodes, and also reproducible microfabrication of protective Nafion membranes with adhesion lasting over six weeks of testing.
Lay Summary

Measuring biomarkers (such as glucose) in blood is painful, therefore people can be unwilling to perform blood tests and they miss out on important information about their health. Additionally, these types of tests don’t allow continuous monitoring, which means trends in glucose levels can't be tracked. Sweat is an alternative fluid that could be used instead of blood. It is easily accessible painlessly from many locations on the body and would allow continuous monitoring. As a result, there is considerable research interest in developing a wearable sweat sensor, but many challenges still remain.

In this work, glucose is used as a proof-of-concept due to its importance for diabetes monitoring and the large amount of previous research. Glucose concentrations in sweat are low compared to those in blood and sweat volume is limited, therefore sensitive measuring techniques are important. Micro- and nanoelectrodes have several benefits over larger electrodes, which allow them to overcome some of these challenges. Nanoelectrodes can be difficult to manufacture reproducibly and in large quantities. Nanoband electrode arrays are a type of nanoelectrode used in this work that can be fabricated reproducibly, and in a way that is compatible with standard manufacturing techniques. These electrodes were used in this work, and shown to be capable of measuring at the relevant concentrations of glucose in sweat.

Another key challenge in developing a wearable sweat sensor is designing an appropriate sweat sampling method to prevent contamination and mixing of sweat at the electrode. A novel electrode geometry was developed to address this additional challenge. This electrode aimed to provide an efficient pathway for sweat to flow from the skin, across the electrode, and out the other side.
Declaration

I hereby declare that the work presented in this thesis and the thesis itself is my own, unless otherwise stated. This thesis has not entirely or in part been submitted for any other degree or professional qualification.

Figure 6.1. was modified from a figure produced by Dr. Ilka Schmüser and Figure 7.1. was produced by Dr. Justin Elliott.

The work presented in Chapter 7 was previously published:


and


The contributions made to these publications is stated in the text of Chapter 7, in sections 7.2.1. and 7.3.

Fiona Moore
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Contents

Abstract i
Lay Summary ii
Declaration iii
Acknowledgements iv
Contents v
List of Figures x
List of Tables xv
Glossary of Terms and Symbols xvi

1. Introduction 1
   1.1. Thesis Structure ................................................................. 3

2. Background 6
   2.1. Introduction ................................................................................. 6
   2.2. Measuring Biomarkers in Sweat .................................................. 6
       2.2.1. Applications for Wearable Sweat Sensing .............................. 6
       2.2.2. Sweat Physiology ................................................................. 7
   2.3. Sweat Collection and Sampling Methods ..................................... 10
       2.3.1. Sweat Stimulation ................................................................. 11
       2.3.2. Current Sweat Collection Methods .................................... 14
       2.3.3. Wearable Collection Methods ............................................ 15
   2.4. Glucose Sensing in Sweat ......................................................... 21
       2.4.1. Detection Method ................................................................. 21
       2.4.2. Electrochemical Glucose Detection ................................. 22
Chapter 1. Introduction

2.4.3. Electrode Material ................................................. 25
2.4.4. Electrode Functionalisation ....................................... 27
2.4.5. Micro- and Nanoelectrodes ....................................... 28
2.5. Summary .................................................................. 31

3. Theory and Methodology ................................................. 41
3.1. Introduction ................................................................ 41
3.2. Electrochemistry Theory ............................................. 41
  3.2.1. Electrode Potential .................................................. 42
  3.2.2. Double Layer .......................................................... 43
  3.2.3. Mass Transport ....................................................... 44
  3.2.4. Electrochemical Cell ............................................... 46
  3.2.5. Reference Electrode .................................................. 46
  3.2.6. Working Electrode .................................................... 47
  3.2.7. Nanoband Electrodes ............................................... 48
3.3. Electrochemical Methods .............................................. 51
3.4. Materials and Equipment Setup .................................... 53
3.5. COMSOL Simulations .................................................. 54
3.6. Fabrication ............................................................... 58
3.7. Electrochemical Cleaning ............................................. 62

4.1. Introduction .............................................................. 65
4.2. Fabrication ............................................................... 67
  4.2.1. Initial Fabrication Process ........................................ 68
  4.2.2. Process Development ............................................... 71
  4.2.3. Nafion Drop-Casting .............................................. 72
Chapter 1. Introduction

4.3. Chlorination Procedure ................................................................. 74
4.3.1. Silver Chloride Structure.............................................................. 74
4.3.2. Chemical Chlorination ................................................................. 76
4.3.3. Electrochemical Chlorination....................................................... 78

4.4. Reference Electrode Performance .................................................. 85
4.4.1. Electrode Testing Procedure ......................................................... 85
4.4.2. Comparison of Chlorination Methods............................................ 87
4.4.3. Electrode Failure ........................................................................... 92
4.4.4. Nafion Coated Electrodes .............................................................. 95

4.5. Performance as a Reference Electrode ............................................ 96
4.6. Effect of Chloride Concentration .................................................. 97
4.7. Summary ......................................................................................... 103

5. Glucose Sensing with Nanoband Electrodes ................................. 107

5.1. Introduction ..................................................................................... 107
5.2. Fabrication ..................................................................................... 110
5.2.1. Design for Glucose Sensing ......................................................... 110
5.2.2. Nanoband Electrode Fabrication Process .................................... 111
5.2.3. Functionalisation for Glucose Sensing ....................................... 113

5.3. Experimental Characterisation ...................................................... 116
5.3.1. Validation of Fabricated Electrodes ............................................. 116
5.3.2. Current Response to Glucose and Cleaning Procedure .............. 118
5.3.3. Measurement Optimisation ........................................................ 124

5.4. Additional Considerations ............................................................ 130
5.4.1. Temperature ............................................................................... 131
5.4.2. Interfering Species ...................................................................... 132
Chapter 1. Introduction

5.5. Summary ........................................................................................................137


6.1. Introduction ..................................................................................................141

6.2. Fabrication ..................................................................................................144
  6.2.1. Deep Reactive Ion Etching (DRIE) ......................................................145
  6.2.2. Potassium Hydroxide (KOH) Etching ...............................................147
  6.2.3. Nanoband Etch Process .....................................................................150
  6.2.4. Cell for Electrode Testing .................................................................154

6.3. Experimental Results ..................................................................................157
  6.3.1. Initial Experimental Results.................................................................157
  6.3.2. Simulation with Ferrocenemethanol (FcMeOH) ...............................160
  6.3.3. Relationship between Current Response and Flow Rate ...............162
  6.3.4. Validation of Theory with Experiment ............................................165
  6.3.5. Characterising Deviations from the Levich Equation ....................167

6.4. Summary ....................................................................................................171

7. Wafer-Scale Processing and Optimisation ...................................................... 175

7.1. Introduction ................................................................................................175

7.2. Wafer-Scale Electrochemical Processing and Characterisation ............177
  7.2.1. System Design and Wafer Fabrication ............................................177
  7.2.2. Wafer-Scale Processing ..................................................................181
  7.2.3. Electrochemical Testing ..................................................................183

7.3. Nafion Fabrication and Optimisation .........................................................184
  7.3.1. Test Structure Design ......................................................................185
  7.3.2. Fabrication ..........................................................................................186
  7.3.3. Nafion Structure Testing ...................................................................191
List of Figures

1.1. Diagram of electrodes developed in this work .................................................. 3
2.1. Diagram of skin showing sweat glands .............................................................. 8
2.2. Diagram of iontophoresis with pilocarpine ...................................................... 13
2.3. Examples of sweat collection for lab analysis .................................................. 15
2.4. Examples of tattoo-based wearable sweat sensors ............................................ 17
2.5. Examples of sensors with microfluidic sweat sampling ..................................... 19
2.6. Diagram of different generations of glucose sensors ....................................... 23
3.1. Diagram showing ferrocenemethanol oxidation process at an electrode .......... 42
3.2. Diagram showing double layer formed at an electrode .................................... 44
3.3. Diagram of an electrochemical cell ................................................................. 46
3.4. Diagram of linear and radial diffusion to a macro- and microelectrode ..... 48
3.5. Diagram of diffusion profile evolution at a nanoband electrode array ............ 50
3.6. Diagram of a characteristic CV form at an electrode under linear and radial diffusion .......................................................... 51
3.7. Diagram of an example calibration curve ......................................................... 52
3.8. Diagram of the environment used for simulations of nanoband and through-flow nanoband electrodes .......................................................... 55
3.9. Simulated current response with different meshes ........................................... 56
3.10 Simulated velocity profile with different meshes ............................................. 58
3.11. Diagram showing lithography steps for positive photoresist ......................... 60
3.12. Diagram showing lithography steps for negative photoresist ......................... 61
3.12. Cleaning CVs on a Pt nanoband electrode ..................................................... 62
Chapter 1. Introduction

4.1. Diagram of an alternative structure Ag/AgCl reference electrode ............. 67
4.2. Diagram of a fabricated Ag/AgCl reference electrode ......................... 68
4.3. Diagram of fabrication flow for fabricated reference electrodes .......... 69
4.4. Images of a finished fabricated silver microdisc electrode ................. 70
4.5. Microscope images of a chlorinated reference electrode before and after testing ............................................................... 72
4.6. Microscope images showing survived and failed drop-cast Nafion ........ 73
4.7. Bar chart showing survival of drop-cast Nafion from testing ............. 74
4.8. SEM image of chemically chlorinated electrode ................................ 77
4.9. SEM image of cross-section of chemically chlorinated electrode ...... 78
4.10. CV of chloride formation and removal on a silver microelectrode .... 79
4.11 Example CAs of silver chlorination ................................................... 80
4.12. SEM images of chemically and electrochemically chlorinated silver .... 81
4.13. SEM images of silver electrodes chlorinated electrochemically with different values of charge .............................................................. 82
4.14. SEM images of cross-sections of silver electrodes chlorinated electrochemically with different values of charge .......................... 84
4.15. Example lifetime graphs of fabricated reference electrodes ............ 86
4.16. Graphs of electrode lifetimes at different chlorination charges .......... 88
4.17. Variation in lifetime for chemically and electrochemically chlorinated electrodes ................................................................. 90
4.18. Variation in potential for chemically and electrochemically chlorinated electrodes ................................................................. 91
4.19. Speciation diagram for AgCl dissolved in NaCl .............................. 94
4.20. Lifetime graph for electrode in 0.1 M KCl saturated with AgCl ........ 95
4.21. CVs comparing fabricated and commercial electrodes .................. 97
Chapter 1. Introduction

4.22. Graph of OCP against log [Cl\(^-\)] ................................................. 99
4.23. Graph of OCP against log [Cl\(^-\)] with activity correction ..................... 101
4.24. Graph of OCP against log [Cl\(^-\)] on Nafion coated electrodes ............... 102
5.1. Diagram of glucose sensing mechanism at a nanoband electrode .............. 108
5.2. Schematic of glucose sensing mechanism at different times .................... 109
5.3. Nanoband electrode mask design ......................................................... 110
5.4. Diagram of fabrication flow for fabricated nanoband electrodes .......... 112
5.5. Microscope images of finished nanoband electrode ................................ 113
5.6. Microscope images of enzyme film ..................................................... 114
5.7. Microscope images of enzyme film with high crystallisation ................... 115
5.8. Simulated CAs for nanoband arrays with and without array overlap ...... 117
5.9. CV of ferrocenemethanol ..................................................................... 118
5.10. Glucose calibration curves before and after electrode cleaning ............... 120
5.11. CAs at varying glucose concentrations .............................................. 121
5.12. Glucose calibration curves on multiple electrodes ................................. 123
5.13. Glucose calibration curve over increased concentration range .............. 124
5.14. CVs with and without glucose at a nanoband electrode ......................... 126
5.15. Glucose calibration curves plotted using CAs taken at 0.5 V and 0.75 V .... 127
5.16. Glucose calibration curves plotted using different time points and the extracted sensitivities ................................................................. 129
5.17. Calibration plot for response of sensor with changing temperature ....... 132
5.18. CVs of ascorbic acid, acetaminophen and uric acid on electrode with and without Nafion coating ................................................................. 134
5.19. CVs of glucose on electrodes with and without Nafion coating ............. 136
6.1. Diagram of PDMS test cell and through-flow electrode ........................... 142
Chapter 1. Introduction

6.2. Diagram showing band electrode in a pipe ........................................143
6.3. Diagram of proposed fabrication flow for through-flow nanoband electrodes using DRIE .................................................................145
6.4. SEM of cross section after DRIE etch ..................................................145
6.5. Diagram showing alignment with DRIE etched channels ......................146
6.6. Diagram of fabrication flow for through-flow nanoband electrodes using KOH etching .................................................................147
6.7. Microscope image of test nanoband cavity etch through a membrane .....148
6.8. Photograph of wafer with KOH etched membranes .............................150
6.9. Diagram of nanoband etch and potential issues ..................................151
6.10. SEM images of test nanoband etch ......................................................153
6.11. SEM image of backside of finished through-flow nanoband electrode ....154
6.12. Photograph of 3D printed mould for flow cell ....................................155
6.13. Diagram and photographs of PDMS flow cell and through-flow electrode .........................................................................................156
6.14. CVs of ferrocenemethanol at different flow rates ...............................157
6.15. Modified Tafel plots at no flow and flow ..........................................159
6.16. Simulated CAs for through-flow nanoband electrodes with and without array overlap .................................................................161
6.17. Simulation results for current response of through-flow electrode with and without array overlap at different flow rates .........................163
6.18. Comparison of simulated and experimental current response for through-flow electrodes at different flow rates .....................................166
6.19. Simulation results for current response of through-flow electrodes with various parameter changes at different flow rates .....................168
6.20. Simulated concentration profiles at electrodes at different time points with and without flow .............................................................171
7.1. Diagram of 3D printer jig for wafer processing
7.2. Mask design for test wafers and cross section of electrodes
7.3. Microscope image of fabricated electrodes
7.4. Chlorination of test electrode using wafer processing setup
7.5. CV of oxygen reduction using wafer processing setup
7.6. Mask design for Nafion test structures
7.7. Microscope images of fabricated Nafion test structures
7.8. Profilometry measurements of spin-coated and drop-cast Nafion
7.9. Microscope images of survived and failed Nafion test structures
7.10. Durability results of Nafion test structures submerged in PBS 1X
7.11. Durability results of Nafion test structures submerged in PBS 1X and agitated by an orbital shaking table
List of Tables

2.1. Analytes detectable in sweat................................................................. 7
2.2. Sweat stimulation and collection methods........................................... 12
2.3. Comparison of optical and electrochemical sensing............................... 22
2.4. First generation glucose sweat sensor characteristics............................. 26
2.5. Comparison of different electrode types................................................ 29
2.5. Comparison of different lithography techniques...................................... 31
4.1. Summary of results for chemically and electrochemically chlorinated electrodes........................................................................................................ 92
5.1. Linear fit from calibration curves of different electrodes......................... 122
5.2. Sensitivity and LOD from calibration curves plotted using CA at 0.5 V and 0.75 V ............................................................................................................. 128
5.3. Current responses to ascorbic acid, acetaminophen and uric acid with and without Nafion coating .............................................................................. 133
5.4. Current responses to glucose with and without Nafion coating ............... 137
6.1. Linear fit for modified Tafel plot............................................................. 160
6.2. Parameters and calculated constants from flow simulations.................... 170
### Glossary of Terms and Symbols

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>1D</td>
<td>One dimension(al)</td>
</tr>
<tr>
<td>1X</td>
<td>One times concentration</td>
</tr>
<tr>
<td>10X</td>
<td>Ten times concentration</td>
</tr>
<tr>
<td>2D</td>
<td>Two dimension(al)</td>
</tr>
<tr>
<td>3D</td>
<td>Three dimension(al)</td>
</tr>
<tr>
<td>a</td>
<td>Activity</td>
</tr>
<tr>
<td>A</td>
<td>Area</td>
</tr>
<tr>
<td>ABS</td>
<td>Acrylonitrile butadiene styrene</td>
</tr>
<tr>
<td>ACT</td>
<td>ACT CMI (photoresist stripper)</td>
</tr>
<tr>
<td>Ag</td>
<td>Silver</td>
</tr>
<tr>
<td>Ag/AgCl</td>
<td>Silver/silver chloride</td>
</tr>
<tr>
<td>AgCl</td>
<td>Silver chloride</td>
</tr>
<tr>
<td>Ar</td>
<td>Argon</td>
</tr>
<tr>
<td>Au</td>
<td>Gold</td>
</tr>
<tr>
<td>AU</td>
<td>Arbitrary units</td>
</tr>
<tr>
<td>B</td>
<td>Dimensionless constant characterised by the</td>
</tr>
<tr>
<td></td>
<td>electrode geometry</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>c</td>
<td>Concentration</td>
</tr>
<tr>
<td>(C_{dl})</td>
<td>Double layer capacitance</td>
</tr>
<tr>
<td>CA</td>
<td>Chronoamperometry</td>
</tr>
<tr>
<td>CE</td>
<td>Counter electrode</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic voltammogram</td>
</tr>
<tr>
<td>d</td>
<td>Double layer thickness</td>
</tr>
<tr>
<td>D</td>
<td>Diffusion coefficient</td>
</tr>
<tr>
<td>DI</td>
<td>Deionised</td>
</tr>
<tr>
<td>DRIE</td>
<td>Deep reactive ion etching</td>
</tr>
<tr>
<td>DSP</td>
<td>Double side polished (wafers)</td>
</tr>
<tr>
<td>e</td>
<td>Charge on an electron</td>
</tr>
<tr>
<td>e^-</td>
<td>Electron</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>$E$</td>
<td>Electrode potential</td>
</tr>
<tr>
<td>$E$</td>
<td>Electric field</td>
</tr>
<tr>
<td>$E^0$</td>
<td>Standard potential</td>
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<tr>
<td>$E'$</td>
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<tr>
<td>$E_a$</td>
<td>Activation energy</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday constant</td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FcMeOH</td>
<td>Ferrocenemethanol</td>
</tr>
<tr>
<td>$G$</td>
<td>Dimensionless constant used as corrective factor to Levich equation</td>
</tr>
<tr>
<td>GOx</td>
<td>Glucose oxidase</td>
</tr>
<tr>
<td>$h$</td>
<td>Channel height</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>HFSIN</td>
<td>High frequency silicon nitride</td>
</tr>
<tr>
<td>HFSIO</td>
<td>High frequency silicon oxide</td>
</tr>
<tr>
<td>HMDS</td>
<td>Hexamethyldisilazane</td>
</tr>
<tr>
<td>$i$</td>
<td>Current</td>
</tr>
<tr>
<td>$i_{\text{lim}}$</td>
<td>Limiting current</td>
</tr>
<tr>
<td>$I$</td>
<td>Ionic strength</td>
</tr>
<tr>
<td>$I^0$</td>
<td>Standard ionic strength, defined at 1 M</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropanol</td>
</tr>
<tr>
<td>$J$</td>
<td>Flux</td>
</tr>
<tr>
<td>$k_B$</td>
<td>Boltzmann constant</td>
</tr>
<tr>
<td>KCl</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>KOH</td>
<td>Potassium hydroxide</td>
</tr>
<tr>
<td>$l$</td>
<td>Litre</td>
</tr>
<tr>
<td>$l_e$</td>
<td>Entry length</td>
</tr>
<tr>
<td>$L$</td>
<td>Characteristic length</td>
</tr>
<tr>
<td>LFSIN</td>
<td>Low frequency silicon nitride</td>
</tr>
<tr>
<td>LFSIO</td>
<td>Low frequency silicon oxide</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LPCVD</td>
<td>Low pressure chemical vapour deposition</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>MF-26A</td>
<td>Type of photoresist developer</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>MNEE</td>
<td>Microscale nanoband edge electrode</td>
</tr>
<tr>
<td>MWCNTs</td>
<td>Multi-walled carbon nanotubes</td>
</tr>
<tr>
<td>( n )</td>
<td>Number tested</td>
</tr>
<tr>
<td>( n )</td>
<td>Number of electrons transferred</td>
</tr>
<tr>
<td>( N )</td>
<td>Number of electrodes in an array</td>
</tr>
<tr>
<td>nLOF 2035</td>
<td>Type of negative photoresist</td>
</tr>
<tr>
<td>OCP</td>
<td>Open circuit potential</td>
</tr>
<tr>
<td>( O_x )</td>
<td>Oxidised species</td>
</tr>
<tr>
<td>( p )</td>
<td>Fluid pressure</td>
</tr>
<tr>
<td>PB</td>
<td>Prussian Blue</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>Pe</td>
<td>Péclet number</td>
</tr>
<tr>
<td>PEB</td>
<td>Post exposure bake</td>
</tr>
<tr>
<td>PECVD</td>
<td>Plasma enhanced chemical vapour deposition</td>
</tr>
<tr>
<td>PPD</td>
<td>Poly-m-phenylenediamine (permselective membrane)</td>
</tr>
<tr>
<td>Pt</td>
<td>Platinum</td>
</tr>
<tr>
<td>PtNPs</td>
<td>Platinum nanoparticles</td>
</tr>
<tr>
<td>( r )</td>
<td>Radius</td>
</tr>
<tr>
<td>( R )</td>
<td>Universal gas constant</td>
</tr>
<tr>
<td>( R_s )</td>
<td>Solution resistance</td>
</tr>
<tr>
<td>( Re )</td>
<td>Reynold’s number</td>
</tr>
<tr>
<td>RE</td>
<td>Reference electrode</td>
</tr>
<tr>
<td>( Red )</td>
<td>Reduced species</td>
</tr>
<tr>
<td>RIE</td>
<td>Reactive ion etching</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>SCE</td>
<td>Saturated calomel electrode</td>
</tr>
<tr>
<td>SHE</td>
<td>Standard hydrogen electrode</td>
</tr>
<tr>
<td>Si</td>
<td>Silicon</td>
</tr>
<tr>
<td>( Si_{3}N_{4} )</td>
<td>Stoichiometric silicon nitride</td>
</tr>
<tr>
<td>SiN</td>
<td>Silicon nitride</td>
</tr>
<tr>
<td>SiRN</td>
<td>Silicon-rich silicon nitride</td>
</tr>
<tr>
<td>SiO(_2)</td>
<td>Silicon dioxide</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal to noise ratio</td>
</tr>
</tbody>
</table>
Chapter 1. Introduction

STD  Standard deviation
STDB  Standard deviation of the blank measurement
SWCNTs  Single-walled carbon nanotubes

\( t \)  Time
\( T \)  Absolute temperature
\( Ti \)  Titanium
\( u_0 \)  Solution velocity at the centre of the channel
\( U \)  Enzyme unit
\( v \)  Flow velocity
\( V_f \)  Volumetric flow rate
\( W \)  Watt
\( WE \)  Working electrode
\( x_E \)  Electrode thickness
\( z \)  Ionic charge number
\( \varepsilon_0 \)  Permittivity of free space
\( \varepsilon_r \)  Relative permittivity
\( \gamma \)  Activity coefficient
\( k \)  Rate constant
\( \nu \)  Kinematic viscosity
\( \mu \)  Fluid dynamic viscosity
\( \rho \)  Fluid density
\( \nabla c \)  Concentration gradient
Chapter 1

1. Introduction

The work reported in this thesis focuses on the challenge of measuring glucose as the analyte of interest in sweat using microfabricated nanoelectrodes. This is informed by the wealth of literature on glucose sensing, and the low cost and high stability of the glucose oxidase enzyme, which make glucose an optimal initial choice as a proof of concept analyte for the sweat monitoring system.

A comprehensive summary of the development of glucose monitoring up to 2018 can be found in [1][2][3]. Analysing biomarkers in blood or urine in laboratories has historically been the gold standard in monitoring and diagnosis. Around the 1980s, electrochemical sensors for at-home testing of diabetes started to become available [4], which provided a material change to the lives of people with diabetes as it allowed them to take control of their own health. These at-home tests are finger-prick tests, where the user draws blood from their finger and applies it to a test strip, which is analysed electrochemically for glucose by insertion into a portable meter. These tests are still the most widely used worldwide, however they have some drawbacks, in particular they are single use and can’t track trends in glucose levels over time, are painful and disruptive, and require multiple tests per day. These issues can cause non-compliance with testing, and therefore worsen health outcomes for people with diabetes.

More recently, wearable continuous glucose monitoring (CGM) or flash glucose monitoring systems have been developed and commercialised, allowing users to track their glucose levels over time [5]. The main companies who have developed commercial products in this area are Abbott Laboratories, Dexcom Inc. and Medtronic who each have commercialised their own wearable glucose sensors. These types of devices generally measure glucose concentration in interstitial fluid (ISF) rather than directly in blood. These CGM devices are particularly important during times when finger-prick testing is difficult or not possible such as during intense activity or sleep.
CGM devices are growing more common, particularly in wealthier countries such as the UK, where many of these monitors have been approved for use by the NHS [6]. Devices such as these allow people with diabetes even greater control over their own health compared to finger-prick tests. The global market size of continuous glucose monitoring systems is large and increasing, it was over $6 billion in 2021 and is predicted to grow at 17% (compound annual growth rate) between 2022 and 2031 to reach $31.7 billion [7].

CGMs and flash glucose monitors which measure in ISF are termed “minimally invasive” devices since they still require penetration of the skin to access the ISF in which they measure, but not as deep as required by blood extraction devices. There have however, been some reports of painful insertion and skin irritation when using these types of devices, particularly in children [8]. As is the case with finger-prick tests, they can also lead to scar tissue development if they are inserted continuously into the same area. Since they operate inside the body, biofouling and the foreign body response are further challenges [9].

Despite the advantages CGMs have brought, it would still be beneficial to develop a wearable sensor which was fully non-invasive and could also allow additional health markers to be measured simultaneously. This would mean measuring in another bodily fluid, with the main identified alternatives being tears, saliva and sweat [10]. The key disadvantages of tears and saliva for continuous monitoring are that their locations (in the eye and mouth) cause considerable challenges including difficulties accessing the fluid and its contamination, e.g. with food [11]; therefore sweat was chosen as the target biological measurement fluid for this work.

Sweat is rich in biomarkers, easily accessible from many parts of the body and shows great promise as a measurement fluid for health analysis. There is therefore a large quantity of literature on sensing analytes for a number of healthcare applications, including glucose, due to its relevance to diabetes monitoring. In addition, studies have shown the potential for glucose to be an analyte of interest in high performance or endurance athletic activities, particularly with respect to optimal fuelling for improved performance [12][13]. Sweat is also currently used as the biofluid to diagnose cystic fibrosis (CF) by measuring chloride concentration. This is not currently performed using a fully wearable device,
however it does show medical acceptance of sweat biomarkers being used clinically, despite the current high costs associated with specialised equipment and skilled users [14][15].

1.1. Thesis Structure

The aim of the work presented in this thesis was to develop and characterise microfabricated reference and working electrodes designed to provide the key components of an electrochemical sensor system, which is applicable to measuring analytes in sweat, with a focus on glucose detection as the proof-of-concept target. This work uses highly sensitive nanoband working electrodes to overcome some of the challenges associated with measuring glucose in sweat. Diagrams of the key electrodes fabricated as part of this work are shown in Figure 1.1.

Figure 1.1: Diagrams (not to scale) of the electrodes developed in this work. (a) Microdisc on-chip reference electrode. (b) Nanoband electrode functionalised for glucose sensing. (c) Through-flow nanoband electrode in PDMS cell for testing.
The goal of the microfabricated reference electrode (Figure 1.1a and Chapter 4) is to achieve an on-chip electrode, which provides a stable reference potential to perform electrochemistry over 24 hours. Micro- and nanoscale electrodes provide several benefits over macroelectrodes including higher sensitivities, lower limits of detection, and increased signal to noise ratios (SNRs), making them attractive for measurements in sweat. Nanoband electrodes are a type of nanoelectrode, where the nanoscale dimension is defined by the deposition thickness of the metal layer rather than its width after patterning and etching. This is significantly less challenging to fabricate and results in highly reproducible electrodes with tuneable geometrical parameters. Nanoband electrode arrays were fabricated and characterised for glucose sensing (Figure 1.1b and Chapter 5), with the aim to measure a linear relationship between current response and glucose concentration across the relevant range in sweat. The response of these electrodes were also characterised to some common interferents.

The nanoband electrodes were then further developed into through-flow nanoband electrodes to support the passage of flowing liquids through the device (Figure 1.1c and Chapter 6), which would make them more suitable for the intended application of sensing in sweat. These electrodes were validated using both simulations and experimental results, with the aim for the device to operate in a flow insensitive regime across the relevant flow rates in sweat. Additionally, the mass-manufacturability of these chips is considered (Chapter 7). In particular, systems and processes for their fabrication were developed and discussed.

References


Chapter 2

Background

2.1. Introduction
This chapter presents some relevant background information and literature relating to developing a sweat sensor. An overview of the applications of sweat sensing is given, as well as the physiology of sweating. Current methods for stimulating and collecting sweat are an important consideration, which will also be discussed. Glucose is used as the proof-of-concept analyte in this work, therefore methods of glucose detection are presented, focusing on electrochemical glucose measurements, electrode materials, and electrode functionalisation. Micro- and nanoelectrodes are evaluated for their potential advantages for glucose sensing in sweat, and methods of fabricating nanoelectrodes are discussed. Finally, a summary of the key challenges and system requirements in presented.

2.2. Measuring Biomarkers in Sweat
2.2.1. Applications for Wearable Sweat Sensing
The aim of this work was to develop an electrode geometry and fabrication processes for use in fluid flow for sweat sensing, although glucose is the analyte discussed in the results of this work, it is important to understand the wider applications of sweat sensing using additional analytes that could be key to future sensors. Due to the large size of the market and prevalence of diabetes, early sweat sensor research was mainly focused on glucose detection, however interest in other analytes has been increasing in recent years and it is now known that there are a wide variety of components in sweat which could be measured for different applications.

Lactate monitoring for sports has been particularly targeted by researchers in recent years [1], but it also has potential applications in critical care [2]. Cystic fibrosis (CF) diagnosis is commonly performed by analysing sweat off-body, but a wearable device could reduce time and expense. Chloride can also be used, along
Chapter 2. Background

with sodium and potassium, to measure hydration status and electrolyte loss which is important in sports, but also in hospitals and in the elderly [3][4]. Other less considered analytes that have been measured in sweat are nerve agents [5] and trace metals [6]. A summary of analytes that have shown potential for measurement in sweat can be found in TABLE 2.1.

**TABLE 2.1**

*Summary of analytes that have been shown to be detectable in sweat, their application, and the measurement method.*

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Application</th>
<th>Current Method</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Blood alcohol content.</td>
<td>Breathalyser or blood test.</td>
<td>[7]</td>
</tr>
<tr>
<td>Sodium</td>
<td>Dehydration, electrolyte loss, help diagnose CF, hypohydration/hyponatremia.</td>
<td>Sweat analysis.</td>
<td>[3][4][8] [9] [10]</td>
</tr>
<tr>
<td>Potassium</td>
<td>Dehydration, electrolyte loss, hypohydration/hyponatremia.</td>
<td>Sweat analysis.</td>
<td>[3][4] [10]</td>
</tr>
<tr>
<td>Chloride</td>
<td>Cystic fibrosis diagnosis, dehydration, electrolyte loss.</td>
<td>Sweat analysis.</td>
<td>[3][4][8] [11]</td>
</tr>
<tr>
<td>Glucose</td>
<td>Diabetes, athletic performance.</td>
<td>Blood test, CGM.</td>
<td>[12][13] [14]</td>
</tr>
<tr>
<td>Lactate</td>
<td>Exertion, athletic performance, critical care, pressure ischemia, tissue hypoxia.</td>
<td>Blood test, sweat analysis.</td>
<td>[15][16] [17][18]</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Stress, low blood glucose levels.</td>
<td>Blood test, sweat analysis.</td>
<td>[19][20] [21]</td>
</tr>
<tr>
<td>Drugs of Abuse</td>
<td>Monitor ingestion of drugs, e.g. cannabis, MDMA.</td>
<td>Blood test, hair analysis, urine test.</td>
<td>[22][23]</td>
</tr>
</tbody>
</table>

2.2.2. Sweat Physiology

It is important to understand the basics of sweat physiology to design effective wearable sweat sensors. There are many fundamental challenges, which must be overcome to develop a viable sweat sensor, some of which will be discussed in more detail in the rest of this chapter. Sweating occurs to regulate
body temperature changes caused by physical or mental exertion or exposure to different environmental temperatures. Along with water, other chemicals and metabolites are excreted in sweat and these can be measured and analysed. Figure 2.1 shows a diagram of an area of skin including the sweat glands. There are different types of sweat glands in different locations on the body, apocrine sweat glands, which secrete into hair follicles, and eccrine sweat glands, which secrete onto the skin surface. Apocrine sweat glands have increased challenges for sweat sensors since they contain more sebum and bacteria and are generally in less accessible locations (such as the armpit), whereas eccrine sweat glands are rich in potential analytes and more easily accessible from many locations across the body (such as the forearm).

Figure 2.1: Diagram of skin showing sweat glands, Created with BioRender.com.

The relationship between the concentration of analytes in sweat and blood varies depending on the analyte and how it reaches the sweat. For some analytes, the concentration in sweat may be considerably higher than in plasma, however for others it may be only 1% of the concentration [24]. Analytes move into sweat predominantly through paracellular partition – which is diffusion or advection between the cells, glucose is thought to be partitioned in this way. During sweat
secretion a negative osmotic pressure is developed, which causes analyte dilution through pulling in significant amounts of water.

This dilution is a key challenge for sweat sensing due to the low concentrations of analytes. For example, the concentration of glucose in sweat is $0.06 - 0.11$ mM for healthy people and $0.01 - 1$ mM for people with diabetes [25]. This means glucose sweat sensors need to be developed with sufficiently low limits of detection and sufficiently high sensitivity to measure in the appropriate range. Other analytes such as lactate are even more complex since they can originate in the blood and partition into sweat, but can also be locally produced in the sweat glands themselves leading to significantly higher concentrations [26].

This partitioning and resulting dilution of the analyte are likely to result in a dependency on sweat rate since this will affect the dilution factor. This is considered to be the case for glucose, and Jajack et al. have shown that it is possible to enhance the partitioning of glucose into sweat [27], although this has similar drawbacks to the pharmacological sweat stimulation methods discussed in section 2.3.1. There is also some evidence that certain analytes may be sweat rate independent leading to opportunities for sweat rate calibration [28]. Currently it is unclear to what extent these analytes (such as $K^+$) are truly independent of sweat rate, but it is clear that many others do depend on it ($Na^+$, $Cl^-$, glucose), which makes sweat rate a key characteristic that may require compensation in measurements.

When monitoring analytes such as glucose where the concentration in blood rather than sweat is clinically useful, the time lag is an important parameter to consider, particularly for diabetes where a delay in measurement can lead to a health emergency for users. This time lag is the delay between the concentration measured in blood and the equivalent concentration measured in sweat. As a comparison, a commercial CGM device was measured to have an overall time delay of $4.5 \pm 4.8$ minutes between the measurement provided by the device and the reference measurement in blood [29]. It can be difficult to measure the time lag between blood and sweat, as was shown in a study that used injected dyes and measured their appearance in stimulated sweat, with time lags between 2 and 5 minutes for the dye to be observed in sweat [30]. Although the sweat was stimulated using pharmaceuticals, this suggests a small time lag between
concentrations in blood and sweat. This has been backed up empirically, for example Moyer et al. [12] who measured a lag of around 8 minutes and also noted that the sampling method was a key variable which affected the correlation and lag time.

The sweat volume provides another key challenge for developing a wearable sweat sensor. Sweat glands each produce only nanolitres of sweat per minute, with one in-vivo study measuring 0.2 – 17.6 nl/min/gland [31]. The sweat gland density is an average of 104 glands/cm² for the forearm [32], which has a relatively high density of sweat glands, but this still leads to a very low volume overall meaning the sampling method is a key issue. This sweat volume challenge can be closely related to the time lag, since the low volume and low flow rate of sweat means additional delay may be required to collect a sufficiently large quantity for analysis.

For some analytes (including glucose) the correlation between blood and sweat is still under debate with some researchers reporting correlations and others finding no correlation [12][33]. Overall, a better understanding of the sweat physiology will help firm up the understanding of the relationship between the concentration in blood and sweat as most of the few studies available are empirical from on-body sweat sensor measurements. Even studies which attempt to directly measure the correlation between sweat and blood note that the challenges in sampling sweat without contamination and in correcting for other factors such as sweat rate, make it challenging to obtain reliable results [12].

2.3. Sweat Collection and Sampling Methods

As stated in section 2.2.2., the way in which sweat is collected and sampled can have an impact on the relationship between how the concentration of analytes in sweat correlate with those in blood. Variation in the method used to collect sweat, the location/timing/duration of collection, sweat storage, and analysis method can all lead to errors or inconsistent results [3]. An important additional part of most sweat collection methods is a cleaning protocol, which is employed to ensure the skin surface is free of contamination (both from the environment and leftover material inside the sweat glands) before collection and also to aid adhesion of collection devices to the skin [34]. This section will cover an overview
of the methods used to stimulate, collect and sample sweat from the body for analysis and how these could affect a wearable sweat sensor.

2.3.1. Sweat Stimulation

Before sweat is sampled or collected, it is important to consider the method of stimulation. This is particularly important for wearable sweat sensing where sweat is likely to be collected over a small area, leading to a very low overall volume. As discussed in section 2.3.1., it has been shown in the literature that sweat glucose can correlate strongly with blood glucose if it is appropriately collected to prevent contamination [12]. The relationship is complicated and dependent on other variables, for example, Count et al. have identified that the sweat and blood glucose relationship will change depending on the sweat rate [35]. This agrees with the physiological information in section 2.2.2. relating to analyte dilution, making sweat rate an important factor to monitor. This also makes the method of stimulating the sampled sweat a key point to consider in on-body experiments. However, sweat rates are extremely low when at rest so the following methods are commonly employed to induce higher sweat rates [3]: pharmacological, temperature, exercise, and reverse iontophoresis. Reverse iontophoresis works by applying a small current across the skin in order to extract charged and neutral analytes via electroosmosis and electrophoresis [36]. The use of these stimulation methods in wearable sweat sensors has been summarised in TABLE 2.2.

These different sweat stimulation methods can also lead to differences in the concentrations of analytes that are measured. For example, reverse iontophoresis can extract analytes both from sweat and ISF at the same time. There are potential differences in the lipid profiles of sweat produced by exercise and stimulated pharmacologically, but it is unclear if this also applies to analytes such as glucose [37]. One study that measured the sweat gland pressure and concentration of K⁺, Na⁺ and Cl⁻ showed there was no difference in the results obtained from stimulated and unstimulated glands [38].

Temperature is an effective method for full-body sweat collection, but it requires strict environmental conditions, which can be controlled during research experiments but not in everyday life. This gives it minimal applicability for use in wearable sensors for continuous monitoring.
### Table 2.2

**Summary of sweat stimulation and collection methods in literature.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sweat Stimulation</th>
<th>Sweat Collection</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, temperature, pH, relative humidity</td>
<td>None</td>
<td>Directly on skin/sweat update layer</td>
<td>[39]</td>
</tr>
<tr>
<td>pH, Cl(^-), K(^+), and Na(^+)</td>
<td>Exercise</td>
<td>Microfluidics and absorbent patch</td>
<td>[40]</td>
</tr>
<tr>
<td>Glucose, pH, temperature</td>
<td>Exercise</td>
<td>Microfluidics</td>
<td>[33]</td>
</tr>
<tr>
<td>Sweat rate</td>
<td>Exercise</td>
<td>Macroduct (microfluidics)</td>
<td>[41]</td>
</tr>
<tr>
<td>H(^+), Na(^+), K(^+), Cl(^-), flow rate</td>
<td>Exercise</td>
<td>Microfluidics</td>
<td>[42]</td>
</tr>
<tr>
<td>Glucose, lactate</td>
<td>Exercise</td>
<td>Microfluidics</td>
<td>[43]</td>
</tr>
<tr>
<td>Glucose</td>
<td>Exercise</td>
<td>Cotton thread and filter paper</td>
<td>[44]</td>
</tr>
<tr>
<td>Na(^+), K(^+)</td>
<td>Exercise</td>
<td>Microfluidics</td>
<td>[45]</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Exercise</td>
<td>Microfluidics</td>
<td>[46]</td>
</tr>
<tr>
<td>Ammonium</td>
<td>Exercise</td>
<td>Tattoo</td>
<td>[47]</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>Exercise</td>
<td>Tattoo</td>
<td>[48]</td>
</tr>
<tr>
<td>Glucose, lactate, Na(^+), K(^+), temperature</td>
<td>Exercise</td>
<td>Sensor directly on skin</td>
<td>[49]</td>
</tr>
<tr>
<td>Lactate, sweat rate</td>
<td>Exercise</td>
<td>Microfluidics</td>
<td>[50]</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>Exercise</td>
<td>Fabric wicking</td>
<td>[51]</td>
</tr>
<tr>
<td>Na(^+), conductivity, pH</td>
<td>Exercise</td>
<td>Fabric wicking</td>
<td>[52]</td>
</tr>
<tr>
<td>Glucose, lactate, sweat rate, pH, Cl(^-)</td>
<td>Exercise</td>
<td>Microfluidics</td>
<td>[39]</td>
</tr>
<tr>
<td>Lactate, pH, temperature</td>
<td>Exercise and iontophoresis</td>
<td>Microfluidics</td>
<td>[53]</td>
</tr>
<tr>
<td>Glucose, lactate</td>
<td>Iontophoresis or exercise</td>
<td>Microfluidics</td>
<td>[54]</td>
</tr>
<tr>
<td>Glucose</td>
<td>Reverse iontophoresis</td>
<td>Tattoo</td>
<td>[55]</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Carbachol</td>
<td>Microfluidic wicking</td>
<td>[7]</td>
</tr>
<tr>
<td>Glucose, Cl(^-), Na(^+)</td>
<td>Acetylcholine, methacholine, or pilocarpine</td>
<td>Directly on skin/absorbent layer</td>
<td>[56]</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Pilocarpine</td>
<td>Tattoo</td>
<td>[57]</td>
</tr>
<tr>
<td>Glucose</td>
<td>Pilocarpine</td>
<td>Microfluidics</td>
<td>[58]</td>
</tr>
</tbody>
</table>
Reverse iontophoresis has been used in wearable glucose sensing, with some of the benefits including non-invasive access to the higher concentration of glucose contained in ISF, the well-studied correlation between ISF and blood, and some interferents being drawn to the opposite electrode from the glucose. However, this is not solely a sweat sensing method and suffers from drawbacks such as the wearer’s sweat mixing with ISF leading to inconclusive measurements, as well as skin irritation and discomfort. Reverse iontophoresis was the method chosen by the commercialised sweat glucose sensing device, the GlucoWatch. The measurements from the watch correlated well with blood glucose concentration, however it was withdrawn from the market due to burning and irritation of users’ skin [59]. For these reasons, this method is not discussed further here and is also not very commonly used in more recent literature.

![Diagram showing iontophoresis used to drive a sweat stimulating drug (pilocarpine) from a hydrogel into the skin to increase sweating rate.](image)

Figure 2.2: Diagram showing iontophoresis used to drive a sweat stimulating drug (pilocarpine) from a hydrogel into the skin to increase sweating rate.

Rather than pulling analytes from the skin in reverse iontophoresis, the same technique can be used in iontophoresis to force a localised sweat stimulating drug (known as a cholinergic) such as pilocarpine through the skin to generate a higher sweat rate, and therefore volume of sweat (Figure 2.2) [56]. This is the method of sweat stimulation which is generally used in cystic fibrosis testing, however the test for CF is performed as a single measurement rather than continuously. This method comes with similar drawbacks to reverse iontophoresis such as skin irritation, and additionally the increased sweat rate only lasts a couple of hours which means it must be re-stimulated regularly to be able to monitor analyte levels continuously (and generally re-stimulation of the same area is not immediately possible). Some of these problems have been alleviated by use of lower doses to minimise skin irritation, or the use of alternative drugs such as
carbachol, which is more slowly metabolised meaning the sweat stimulating effect occurs for a longer period of time [60].

One of the most common methods in published work on sweat sensing relies on subjects taking part in intense physical exercise to induce higher sweat rates [49]. Whilst this method has led to results with high correlations to reference measurements, it is not feasible for use in continuous monitoring and can result in an unstable or unpredictable sweat rate which can affect device functionality (particularly if an acceptable time lag is reliant on a high sweat rate). However, it can be a very suitable option for sweat sensors with applications for athletic performance. In addition, intense physical activity is likely to increase body temperature and this can have an effect on sensor response, particularly enzymatic sensing methods (discussed further in section 2.4.4.).

Some of these sweat stimulation techniques are more suited to certain applications than others, in particular short-term or single point measurements are much easier to achieve if a higher sweat rate is necessary. The ideal solution for wearable sensors would be to measure continuously without the need to stimulate a higher sweat rate whilst also having the capability to deal with higher sweat rates when they occur naturally. However, as discussed in section 2.2.2, the sweat rate is so low at rest (nl/min) and variable between individuals or when stimulated that it is extremely challenging to measure effectively in sweat.

2.3.2. Current Sweat Collection Methods (sweat in lab)

The whole body washdown has historically been the accepted method to collect and analyse sweat, it is thought to be the most accurate method to collect full body sweat without interfering with the normal sweating process. This method encloses the subject in a controlled environment and measures the total sweat produced by the body. The sweat and dilution liquid (normally DI water) are collected and analysed. Simple methods are used to quantify fluid loss by weighing participants at two time points and attributing weight loss to fluid lost during sweating. This is only useful for calculating sweat loss or approximate sweat rate however. Towel blotting is another common method where sweat produced during the experiment is periodically absorbed onto a towel [61] [62] [3]. These methods are commonly used both during exercise and at elevated temperatures to induce sweating. It is clear that these methods are inconvenient, expensive and do not lend themselves well to continuous monitoring applications. They involve storing
and moving samples to be tested in other locations with trained professionals and expensive lab equipment. Obtaining sufficient volumes and preventing evaporation or degradation of analytes is also a challenge.

Regional sweat collection is also possible, using absorbent patches (usually covered to minimise evaporation) either to correlate to whole body sweating or to analyse local sweat (Figure 2.3a) [65]. This method is much more convenient for sweat collection, but still requires the sample be removed from the body and taken to a lab for analysis making it non-continuous and expensive. Gauze or filter paper, or the Macroduct® system (Figure 2.3b) are also common regional sweat collection methods, and are particularly commonly used in cystic fibrosis diagnosis [66], where they form part of the guidelines [11], as well as being employed in research [41]. Some less common regional sweat collection methods include sweat collection capsules similar to Macroduct® [67], and sweat collection on cotton socks and gloves [68].

In all of these methods, once the sweat has been collected and taken to the lab it must be analysed. with commonly using methods including liquid chromatography–mass spectrometry, gas chromatography–mass spectrometry and nuclear magnetic resonance spectroscopy [69]. These techniques are highly accurate but are expensive and incompatible with wearable devices.

2.3.3. Wearable Sweat Collection Methods

Sample collection and external analysis can be avoided by measuring in-situ, developing a wearable device which could measure the analytes of interest in sweat has the potential to be cheaper and more convenient than current sweat
Chapter 2. Background

sampling methods. For example, a portable Nanoduct® system has been developed for cystic fibrosis diagnosis that shows comparable or improved results over the current diagnosis system, showing the potential for such systems to be developed for use in medical testing and diagnosis of diseases [70], [71].

An important consideration for wearable sensors is that occlusive collection methods (which cover the skin surface in non-breathable material and lack ventilation) can lead to blocking of sweat pores and therefore a reduced or suppressed sweat output (hydromeiosis) [3]. This is less vital during short collection periods where the collection vessel does not become saturated, but can become particularly important for longer or continuous monitoring devices that are more at risk of saturation if there is no exit route for the sweat [72]. It is also important to minimise any changes to the natural sweating process while measuring to ensure the sweat collected is not significantly different from the sweat produced without measurement interference. It has been suggested by Hoovels, et al. that an ideal method would be to isolate the sweat collection in a way which allows natural sweating but prevents outside contamination and sweat gland blocking [1]. TABLE 2.2 shows a summary of different sweat collection methods. The most commonly used are microfluidics or sensors directly on the skin which will be discussed further below.

Sensor Directly on Skin

Much of the initial research in wearable sweat sensing did not employ any specific sweat collection method and sensors were placed directly on the skin to measure analytes of interest. A key example of this is the work by Joseph Wang’s group who developed wearable sensors based on temporary tattoos [73] to measure a variety of analytes including glucose (Figure 2.4a) [55], lactate [74], sodium [48], along with various others (Figure 2.5b) [47][6][57]. The advantage of this type of device is that the analyte is available almost immediately on excretion since there is no volume space between the skin and sensor. Additionally, by leveraging the already well-known technology of temporary tattoos, the printing materials can be adapted to produce a device which is highly conformal with the skin surface and unobtrusive to the wearer, at a low cost and high compatibility with mass production. This type of sensor featured in these early sweat sensor publications and helped establish the potential of sweat as an analyte fluid for wearable sensors. However, they are compatible with short-term
single-use applications rather than long-term continuous monitoring, and since they are exposed on the skin surface they can easily be affected by evaporation and other external factors.

![Figure 2.4: Tattoo-based wearable sweat sensors. (a) Adapted with permission from [55], copyright 2014 American Chemical Society. (b) Adapted with permission from [57], copyright 2016 American Chemical Society.](image)

**Microfluidics**

Using a type of microfluidic device is one of the most common sweat collection methods for wearable sweat sensors. It has some advantages over other systems like tattoo sensors where the sensing element is directly on the skin because it allows the flow of sweat to be directed and controlled. It also helps to minimise external contamination and can reduce sweat mixing on the skin surface if designed appropriately.

It is clear from the expected sweat rates (nl/min, section 2.2.2.) that any form of volume collection method, including microfluidic systems, will lead to an increased time lag between analyte excretion and measurement. Microfluidic devices add “dead volume” meaning time is lost while the analyte traverses the channels to reach the sensing element. In the case of glucose, any time delay between blood glucose levels changing and being available to the user could be life-threatening to a diabetic person so it is crucial to be able to make measurements quickly. With this in mind, microfluidic systems aiming to measure these analytes need to minimise the volume of sweat they require to function. As a comparative example, a study measured the total delay between a commercial CGM and finger-prick testing as 10.9 ± 1.1 minutes [75]. The additional problem caused by filling volumes (even microfluidic ones) is that it can allow the sweat to mix inside the filled volume meaning the real-time measurements of analytes in the sweat will be slightly adjusted due to the mixing inside the chamber. This
problem is worse for larger volumes and could be compensated for if the sweat/filling rate is known. The microfluidic filling time can be reduced through the design of the system, for example using multiple collection channels feeding into one sensing chamber as done by Sempionatto et al. (Figure 2.5b) [45], however the filling time for their sensor was still over 5 minutes.

In order to collect sweat in a microfluidic system, a force is required to push the sweat into the channels. In a microfluidic sweat system this is likely to be a mixture of the secretion force from the sweat glands and capillary action in the microfluidic channels. Sweat glands have a natural pumping action and a pressure is formed causing sweat excretion. Sweat gland pressure was measured by Choi et al. using a wearable microfluidic system, they measured 2.4 – 2.9 kPa in healthy young adults with the highest values measured on the forearm [76]. The maximum hydrostatic pressure of sweat glands has been measured as 70 kN/m² [77] and the minimum as 33.3 kN/m² [38]. The simplified form of the Young-Laplace equation is: \( \Delta P = \frac{2\gamma}{r} \), where \( P \) is pressure, \( \gamma \) is surface tension, and \( r \) is radius. This equation can be used to estimate a minimum radius for a microfluidic device, using the surface tension of sweat as 69.25 mN/m [78]. This gives a minimum radius of 1.98 – 4.16 \( \mu \)m for these maximum and minimum pressures, implying that sweat generation pressure should be sufficient to fill microfluidic devices with a radius of around 5 \( \mu \)m or larger. Microfluidic channels have been shown to fill from sweat gland pressure and capillary action empirically in the literature, for large microfluidic channel radiiuses, including some of those in TABLE 2.2.

Crespo and co-workers developed a flexible microfluidic cell for sweat collection and analysis (Figure 2.5a) [33][40][53]. Sweat is collected and flows through a channel across the electrodes (2 mm diameter) and exits through an outlet. If placed on the back during a medium intensity exercise, it takes around 12 minutes to completely fill the sensing zone and for sweat to be renewed [40]. This filling time is similar to those recorded by other sweat collection methods based on microfluidic channels [46]. The filling time can be reduced by separating the different sensors into individual identical channels so the flow does not need to pass all the electrodes in a row [53].
Chapter 2. Background

A Mean Absolute Relative Difference (MARD) can be used to measure the average difference between a measurement from a device and a reference measurement (usually from a blood glucose measurement). A MARD of less than 10% is considered to signify good analytical performance and be acceptable for insulin dosing decisions in CGMs [79][80]. In the amperometric glucose measurements in [33], a maximum error of under 10% was measured for the highest glucose concentrations (200 μM) due to differences in flow rate. However, this is solely the error in response due to flow and the total error for the system...
as a whole is likely to be higher. This makes the response of the sensor under changing flow rate an important parameter to consider. It is important to note that the error in measurement with flow rate discussed here is an error due to the response of the sensor, which is a separate challenge to the physiological dependence of the concentration on sweat rate discussed in section 2.2.2.

Karyakin’s group developed wearable sweat sensors based on flow systems [50][81]. One of their key findings was that by applying a coating to the electrode the dependence upon flow could be reduced at a certain range of flow rates, and this could be compared to an uncoated electrode with a high flow rate dependence as a method of measuring flow rates [50]. This is an important development since flow rate measurements in sweat are not often reported and most current methods of measuring sweat rate only have a limited working time (Figure 2.5c) [42].

The effect of skin roughness can also contribute to contamination because it leaves large gaps (µL in volume) between the skin and sensor surfaces which must be filled before sweat can reliably reach the sensor. These volumes can allow evaporation to occur and can become collection points where old sweat or interferents build up and contaminate the newer sweat. It is important for sweat to reach and leave the sensor quickly to prevent contamination from both the skin surface and old sweat mixing with newer sweat. There are some notable innovative designs which have been used to try to deal with the extremely low sample volumes available in sweat. These include using an oil layer to “fill” any gaps between the skin surface and the sensing element, this ensures the sweat can progress directly to the sensor rather than filling unnecessary space [82]. Innovatively designed wicking in hexagonal channels has also been used to try to move sweat onto a sensor allowing the wicking area to be large but the volume of liquid to be very small [83]. These papers were some of the key motivations to exclude any large microfluidic devices or channels and move towards developing a sensor which could allow fluid flow without the addition of large filling volumes.

Overall, there are various methods of stimulating higher sweat rates, most of which are currently unsuitable for continuous monitoring for durations of longer than a couple of hours. Ideally a wearable sensor would be able to measure at resting sweat rates without additional stimulation, however there is promising research using pharmacology to stimulate higher sweat rates. Various different
methods of sampling sweat have also been developed: from collecting sweat in towels to microfluidics, and placing sensors directly on the skin surface. Due to the low volumes of sweat produced, the ideal sampling method would not require a large volume to be filled before reaching the sensor surface, and would additionally provide some protection to the sensor from contaminants, including the surrounding environment.

2.4. Glucose Sensing in Sweat

As discussed in section 2.2.1., there is a wide range of analytes which could be detected in sweat. In this work glucose is used as a proof-of-concept analyte for the sensing system. The background information below covers glucose detection and was used to inform design choices in the experimental work which was undertaken. Some of this background information can be applied to other analytes, in particular those used with oxidase enzymes. This section will cover methods of electrochemical glucose detection, the types of electrode that can be used, and how they can be functionalised to measure glucose, as well as using micro/nanoelectrodes as a potential solution to some of the challenges discussed in this chapter.

2.4.1. Detection Method

The main detection methods which are used in wearable sweat glucose sensors are optical or electrochemical detection. An example of optical methods is colorimetric detection where a functional layer is used whose colour varies with changes in glucose concentration [84][85]. Colourimetric detection can be convenient and simple but is generally less widely used, as quantitative information requires additional analysis equipment (such as a camera). Quantitative data is particularly important for conditions like diabetes, where a precise value can ensure the correct course of action is taken. They also may be only single-use, once the dyes or colour changing element has reacted it often cannot change continuously so a new device would be needed.

Electrochemical sensors are usually the preferred method of detection in wearable sweat sensors, and numerous review articles are available on the topic [86][87][88][89]. Overall, they are highly accurate, inexpensive and easy to miniaturise, and can also have well defined fabrication methods ensuring enhanced manufacturability. For these reasons, this work will be focused on
electrochemical sensors for glucose detection. A comparison table summarising some key differences between optical and electrochemical sensors can be found in TABLE 2.3.

**TABLE 2.3**

Comparison of key glucose sweat sensor parameters for optical and electrochemical sensing techniques.

<table>
<thead>
<tr>
<th></th>
<th>Optical</th>
<th>Electrochemical</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sweat sampling</strong></td>
<td>Requires defined volumes</td>
<td>Requires constant flow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>due to analyte depletion</td>
</tr>
<tr>
<td><strong>Skin irritation</strong></td>
<td>Possible</td>
<td>No</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Lifetime</strong></td>
<td>High (not reliant on reagents)</td>
<td>Limited (e.g. by enzyme lifetime)</td>
</tr>
</tbody>
</table>

2.4.2. Electrochemical Glucose Detection

More general information on electrochemical theory and methods can be found in Chapter 3. It is not a simple matter to measure glucose directly using electrochemistry. The majority of ongoing research and commercialised systems (including widely available glucose test strips for finger prick testing) use an enzymatic approach, which involves functionalising an electrode with an enzyme, commonly glucose oxidase (GOx). The first such approach was proposed by Clark and Lyons in 1962 [90]. This allows glucose to be indirectly detected by electrochemically measuring oxygen or hydrogen peroxide. The flavin adenine dinucleotide (FAD) redox active centre of GOx performs the oxidation of glucose (2.1) and is reduced to FADH$_2$ in the process. FADH$_2$ is then oxidised back to FAD by a mediator, in (2.1) this mediator is oxygen, which occurs naturally in many aqueous systems. Artificially mediated enzyme systems are also used to shuttle electrons between the FAD centre and the electrode. Enzymatic glucose sensors can be split into first, second and third generation sensors depending on the type of mediation used (Figure 2.6).
Figure 2.6: Diagram showing working mechanism of different generations of enzymatic glucose sensor.

First generation glucose sensors (Figure 2.6(a)) are mediated with oxygen which is a natural substrate, as shown in (2.1). The oxygen regenerates the redox active centre so the enzyme can continue to oxidise more glucose. Hydrogen peroxide is produced from this reaction which can be measured electrochemically at an electrode. Glucose dehydrogenase is an alternative enzyme that it is not dependent on the concentration of dissolved oxygen, however it tends to be less selective and less stable than GOx, and requires a high overpotential unless used in a mediated system [91].

Second generation sensors (Figure 2.6(b)) replace the oxygen mediator with an artificial mediator. This ensures the reaction will not become dominated by the oxygen concentration rather than glucose since the artificial mediator concentration can be controlled during functionalisation. The enzyme mediator is measured electrochemically and this often leads to a decrease in the required measurement potential. Second generation sensors can be more complicated to develop than first generation since they require the additional mediator component, which can make them more costly. They can also suffer from stability issues due to leaching of the mediator and there can be competition at the enzyme from dissolved oxygen in the solution and the artificial mediator.

Third generation sensors (Figure 2.6(c)), also known as direct electron transfer, rely on directly measuring the redox centre of the enzyme at the electrode (e.g. without another mediator) however they still present a lot of
research challenges and there are mixed results from direct electron transfer with GOx [92]. A review of electrochemical glucose biosensors can be found in [93] and includes further details about the different generations of glucose sensor.

Additionally, there is some research focusing on non-enzymatic glucose sensors [94][95], their key advantage is that they have no reliance on the lifetime of a biological enzyme component. This type of sensor works by directly oxidising the glucose rather than relying on an enzyme interaction. This non-enzymatic process is highly dependent on the electrode material and geometry. Non-enzymatic glucose sensors have not reached the maturity necessary for commercialisation, mainly due to lack of selectivity, slow kinetics and lack of functional systems applicable to biological fluids (for example many have a requirement for an alkaline electrolyte) [96].

Overall, most commercial sensors use some type of first or second generation enzymatic glucose detection, and this work will focus on first generation glucose sensing due to its low cost, simplicity to implement and compatibility with miniaturisation.

First generation glucose sensors work using the following reaction:

\[
glucose + O_2 \xrightarrow{\text{glucose oxidase}} \text{ gluconolactone} + H_2O_2
\] (2.1)

The gluconolactone hydrolyses spontaneously to produce gluconic acid. Hydrogen peroxide can be oxidised (or reduced) at an electrode by applying an appropriate potential and the reaction produces an electric signal which can be measured:

\[
H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-
\] (2.2)

First generation glucose sensors are very well-characterised, simple and cheap to produce, and easily miniaturised. Oxidising the hydrogen peroxide at the electrode during the measurement has the added advantage of producing some of the oxygen required to regenerate the enzyme. The main disadvantages of the sensors are that if there is insufficient oxygen concentration they can become dependent on this rather than the glucose concentration, and the relatively high potential required for oxidising hydrogen peroxide can allow other interfering species to be detected. There are many constituents of sweat, some of which may interfere with electrochemical glucose measurements [97].
Chapter 2. Background

The key interferents that have been identified are uric acid, ascorbic acid and acetaminophen (paracetamol) [55]. Due to the concentration ranges of glucose in sweat, it should be possible to avoid oxygen dependency, which is more of a concern in blood where glucose concentrations are likely to be significantly higher than oxygen concentrations. However, avoiding interferents requires additional development of the 1st generation glucose sensor and this is commonly done by either adding a selective membrane above the electrode (e.g. Nafion) to screen out interferents, or to incorporate a hydrogen peroxide electrocatalyst such as Prussian Blue (PB) onto the electrode which will allow the measurement to take place at a lower potential and therefore avoid other interfering species [98]. Nafion is a perfluorinated polymer that can be used as a permselective membrane, which makes it attractive as a barrier for negatively charged interferents such as ascorbic acid and uric acid. A more detailed overview of Nafion can be found in [99]. Since PB can have stability issues in aqueous solutions [54] and commercial membranes are available to buy and can be easily drop-cast onto electrode surfaces, the use of a Nafion membrane was deemed the best option for this work.

2.4.3. Electrode Material

Electrode types can generally be split into screen printed, microfabricated or textile-based. Screen-printing is usually the cheapest fabrication method and uses well established techniques, however the quality between electrodes can be highly variable leading to poor reproducibility. Textile electrodes can easily bend and conform to the skin, are breathable, and can be incorporated into clothing or dressings/plasters [100][101]. Developing the electrodes into a sensor can be difficult since they tend to be applied over larger areas or applied directly over the skin making it hard to control the sweat flow or environmental contamination. Microfabricated electrodes tend to offer the best reproducibility since they can often make use of standardised microfabrication processes which allow precise reproducible control over their dimensions. Using well-established silicon based-technologies also provides benefits for initial prototyping of sensors for the same reasons.
As discussed in section 2.4.1., when using a first generation glucose sensor, the electrochemical reaction of interest is the oxidation of hydrogen peroxide. This means it is necessary to find an electrode material at which hydrogen peroxide can be oxidised that is also non-toxic/biocompatible. As shown in TABLE 2.4,
electrodes used for first-generation sweat glucose sensors are commonly fabricated from carbon or gold with a PB coating. PB is commonly used due to its high electrocatalytic activity towards hydrogen peroxide which is around three orders of magnitude greater than that of platinum, and its higher selectivity for hydrogen peroxide compared to oxygen [98]. The mechanism of the measurement allows the required electrode potential to be reduced close to 0 V (vs Ag/AgCl) and avoids the unwanted oxidation of some of the most common interferents.

However, pH variations can cause degradation of the PB, and variations in the background electrolyte (e.g. K⁺ concentration) can affect measurements since the PB redox reaction (which is utilised to measure hydrogen peroxide) relies on charge compensation from ionic species in the electrolyte [54]. This is due to the participation of cations (such as K⁺) in the PB redox reaction [104]. PB also requires additional steps in the fabrication process which increases the complexity of the design as it is not as simple as metal deposition. Platinum is a noble metal, it is biocompatible, has standard fabrication processes and is highly stable, it also has a relatively high catalytic activity for hydrogen peroxide and was historically a popular electrode material for its detection [105][106][107]. The sensitivity of platinum metal can be increased, for example by using nanoparticles of platinum [54]. The sensitivity can be increased in a similar way by fabricating nanoscale electrodes rather than coating with nanomaterials, this will be described in more detail in section 2.4.5 and Chapter 5.

2.4.4. Electrode Functionalisation

In order to fabricate a first generation electrochemical glucose sensor, the working electrode must be functionalised with an enzyme. Some of the methods used to achieve this can be found in TABLE 2.4, where the papers chosen focused on examples of first generation glucose sensing in sweat. The functionalisation method used in this work will be discussed further in Chapter 5.

Methods of enzyme immobilisation include physical adsorption, entrapment, and covalent bonding [108]. Physical adsorption can retain high enzyme activity since there are usually few conformational changes to the enzyme structure, however it is not commonly used since it is the weakest immobilisation method and tends to have poor stability. Entrapment means the enzyme is captured inside another material, for example, a physical barrier is used to hold the enzyme in place while allowing the substrates and products to pass through. Nafion membranes or
mixing the enzyme with carbon paste (e.g. for screen-printing) are examples of the entrapment method. Covalent bonding is a very commonly used method which results in strong enzyme immobilisation. This can be done using a cross-linker, such as glutaraldehyde, which has been used for decades due to its low cost and low complexity. This method can lead to conformal changes in the enzyme which can result in a loss of activity, however this is usually balanced against the increased stability. Additionally, glucose oxidase is a comparatively low cost enzyme with high activity so it is a good candidate for this technique.

Membranes can have additional benefits outside of enzyme encapsulation, such as increasing biocompatibility, stability, and linear range of the sensor by acting as a barrier to the electrode [109]. Generally for glucose in sweat, the concentration ranges are low enough that it is usually not necessary to use diffusion barriers to extend the linear range of the sensor but some researchers may choose to use an electrode coating to improve the stability and biocompatibility, or decrease the effect from interferents.

Although glucose oxidase is highly stable, there are other parameters such as temperature and pH which can affect the concentration of glucose measured. This is both for the physiological reasons discussed in section 2.2.2. and also since the enzyme performs optimally under certain conditions and deviations from these conditions will lead to reduced enzyme performance. However, it has been shown that by additionally measuring these parameters, and modelling their effect on glucose concentration, it is possible to compensate for changes quite successfully in software [49]. This is something that is becoming more common in the literature, where initially the majority of publications addressed single analyte detection, it is now more common to see devices which aim to measure multiple parameters simultaneously [33] [40] [39].

2.4.5. Micro- and Nanoelectrodes

An obvious limitation of the small sample volume is the limited amount of analyte it contains. Macroelectrodes will convert more of this analyte in the process of measuring, which could lead it to become depleted when monitoring continuously, resulting in a lower signal overall. Due to their larger size macroelectrodes will intrinsically need a larger sample volume than micro- or nanoelectrodes which adds more time delay to build up this volume. Nanoelectrodes have the benefit of depleting an analyte less while measuring, due
to their small size. Nanoelectrodes have several other benefits over macroelectrodes including higher sensitivity and lower limits of detection [110], which should allow accurate measurement at the relevant low concentration levels of glucose in sweat. A table summarising the key differences between macroelectrodes, nanoelectrodes and nanoband electrode arrays for sweat glucose sensing can be found in TABLE 2.5. Further information on the advantages of micro- and nanoelectrodes can be found in Chapter 3 and a review of electrochemical nanosensors can be found in [111].

**TABLE 2.5**

*Comparison of key parameters for different electrode types for glucose sensing in sweat.*

<table>
<thead>
<tr>
<th></th>
<th>Macroelectrodes</th>
<th>Nanoelectrodes</th>
<th>Nanoband Electrode Arrays</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>Medium</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td><strong>LOD</strong></td>
<td>Medium</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Analyte depletion</strong></td>
<td>High</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Current</strong></td>
<td>High</td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td><strong>Fabrication</strong></td>
<td>Simple, reproducible</td>
<td>More complex, can be variable</td>
<td>Medium, reproducible</td>
</tr>
</tbody>
</table>

As well as the physiological dependence of glucose concentration on sweat rate (section 2.2.2) there is an additional dependency of the electrochemical measurement systems on the flow rate (section 2.3.3 and Chapter 6). The current measured electrochemically is dependent on the transport of material to the electrode. As the flow rate changes, the transport of material will change with it, leading to more or less material reaching the electrode in the same amount of time and therefore a variation in the measured current. Another potential advantage of using micro- and nanoelectrodes is that, due to their improved diffusional mass transport efficiency, they are an ideal choice to decrease this electrochemical sensitivity to flow [112].

Currently most of the published literature uses macroelectrodes for sweat sensing systems and only a few have used microelectrodes [113]. The use of nanoelectrodes is usually restricted to the addition of nanomaterials or
Chapter 2. Background

nanoparticles to larger electrodes and these are often employed as non-enzymatic sensors and rarely to sweat sensing applications [96][114]. Nanoelectrodes have been used for glucose sensing for other target applications, such as measurements of glucose in saliva using gold nanowires [115].

Existing methods for fabricating nanoelectrodes can be simple, such as ensembles of nanotubes [116]. However, there is generally little control over the geometrical parameters such as the number or distribution of elements. In comparison, utilising lithographical techniques results in electrodes of controllable size, spacing and distribution. Electrodes fabricated using these techniques will produce a more reproducible response, which can also be tuned during the electrode design phase.

It is difficult, and therefore expensive, to define a nanoscale dimension in the horizontal plane using photolithography since there is an inherent limit of the wavelength of light. Resolutions of 3 – 4 nm are achievable in industry with extreme UV tools, but these tools cost upwards of $100M. Typically the lowest photolithographic resolution that can be achieved by facilities outside of integrated circuit foundries is of the order of 100 nm, but there can be issues with reproducibility, and variations in other parts of the process such as the photoresist can make this worse [117]. Other lithographic methods are available, including e-beam and nanoimprint [118], however these have other disadvantages. E-beam lithography is expensive and a slow process at the wafer-level. Nanoimprint lithography has disadvantages including pattern distortion due to thermal expansion of the template and substrate during printing, and removing the residual resist without affecting the integrity of the pattern [119]. Although the throughput is considerably higher than e-beam lithography, it has also still not reached the high throughput of conventional photolithography for wafer patterning. A comparison of these different lithography techniques can be found in TABLE 2.6.

In comparison to these techniques, thin-film deposition processes are well established, meaning the nanoscale dimension of the electrode can be controlled by the thickness of the metal layer [120]. This method can be used with a process called nanoskiving [121] to fabricate nanoelectrodes with well-defined geometries. Electrodes fabricated with this process have been used to electrochemically detect
hydrogen peroxide and glucose, with high sensitivities and low limits of detection (LODs) reported [115][122].

**TABLE 2.6**

*Comparison of different lithography techniques for fabrication of nanoelectrodes.*

<table>
<thead>
<tr>
<th></th>
<th>Photolithography</th>
<th>Nanoimprint Lithography</th>
<th>E-beam Lithography</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resolution</strong></td>
<td>~100 nm</td>
<td>A few nm</td>
<td>Single nm’s</td>
</tr>
<tr>
<td><strong>Reproducibility</strong></td>
<td>High</td>
<td>Medium (pattern distortion)</td>
<td>Medium (stitching errors)</td>
</tr>
<tr>
<td><strong>Throughput</strong></td>
<td>High (&gt;100 wafers per hour)</td>
<td>Medium (&lt; 10 wafers per hour)</td>
<td>Low (hours per wafer)</td>
</tr>
</tbody>
</table>

This work will focus on electrochemical detection of glucose using nanoband electrodes (Microscale Nanoband Edge Electrodes, MNEEs) which have been developed and fabricated at the University of Edinburgh’s cleanroom facilities [123][124][125]. The nanoscale dimension of these electrodes is defined using thin-film metal deposition, and a microscale cavity, which can easily be defined using conventional photolithography, is etched to expose the side profile of the deposited metal layer resulting in a nanoband electrode (Figure 1.1b and Chapter 5). This type of electrode has advantages over other types of nanoelectrodes due to the reproducibility and simplicity of the wafer-scale fabrication process, and the high level of control over the geometry. In addition to the expected reduction in flow sensitivity, the geometry of the nanoband electrodes makes them compatible with development into a novel through-flow electrode design which will be discussed in more detail in Chapter 6. Further information about the fabrication processes used in this work can be found in Chapter 3 and the fabrication runsheets can be found in Appendix B.

**2.5. Summary**

Concentrations of analytes such as glucose are significantly lower in sweat than blood and therefore can be challenging to measure accurately. Nanoband electrodes, which have a high sensitive and low LOD, are promising candidates to measure at the low concentrations of glucose in sweat. A first generation glucose sensor will be developed in this work, due to its robustness and low-cost. This
type of sensor can be negatively impacted by common interferents, which are found at similar concentrations to glucose in sweat. Nafion will be used as a protective membrane as it is widely available commercially and has shown good results in the literature for screening such interferents from the electrode.

Sweat can be contaminated by the skin surface or the surrounding environment, making it preferable for the device to be encapsulated. It is also beneficial to maintain a high degree of control over the movement of sweat from its source to the sensing element to prevent mixing and control additional time lag, which can result in lower accuracy of measurements. Therefore, a device with minimal free space between the skin surface and the electrode is desired. A modified through-flow design based on nanoband electrodes may mitigate the requirement for additional microfluidic systems, and a miniaturised on-chip reference electrode will ensure a compact sensor design.

Sweat flow rates are extremely low, meaning the measurement volume and the quantity of analyte are very small. Nanoband electrodes should deplete this limited quantity of analyte to a lesser extent. Sweat rates may also vary during a measurement and the concentration of certain analytes (including glucose) are physiologically dependent on the sweat rate. Additionally, the response of electrochemical sensors is often dependent on flow rate, due to the dependence on mass transport. The geometry and highly efficient diffusional mass transport to nanoband electrodes make them a promising candidate to mitigate the electrochemical dependence on flow rate. A flow insensitive electrode could be combined with a flow sensitive electrode to extract the flow rate, which may allow compensation for the physiological dependence of concentration on sweat rate.

In addition, commonly used techniques, such as drop-casting or electrochemical processing, are less compatible with standard wafer processing methods. It is important to consider the mass-manufacturability of the fabricated devices during the design and development process.

References


Chapter 2. Background


A. Jajack, M. Brothers, G. Kasting, and J. Heikenfeld, “Enhancing glucose flux into sweat by increasing paracellular permeability of the sweat gland,” 2018.


Chapter 2. Background


Chapter 2. Background


Chapter 2. Background


Chapter 2. Background


Chapter 2. Background


Chapter 3

Theory and Methodology

3.1. Introduction

This chapter provides an overview of the electrochemical, simulation and fabrication theory and techniques, which were used in this thesis. First the key electrochemistry theory is discussed, followed by the electrochemical methods, and the materials and equipment used in the work. The model used to perform simulations of the response of the fabricated electrodes is presented. Next, the background and fundamental fabrication processes for the electrodes are discussed. Finally, the electrochemical cleaning procedure for the fabricated electrodes is described.

3.2. Electrochemistry Theory

In electrochemistry an externally applied electrical signal causes a chemical reaction, or a chemical reaction produces an electrical signal which can be measured. These interactions take place in an electrochemical cell which is composed of an electrolyte, through which charge may move (usually in the form of ions), and the electrodes which control and/or measure the flow of charge (usually in the form of electrons). The general form of the equation for a reversible n-electron charge transfer process is:

$$O_x + ne^- \rightleftharpoons Red$$

where $O_x$ is the oxidised species, $Red$ is the reduced species, and $n$ is the number of electrons ($e^-$) transferred. A detailed overview of electrochemistry theory can be found in [1].

In this work, the electrochemical cell is composed of an aqueous electrolyte (consisting of deionised water and dissolved ions), and metal electrodes which are the interfaces for the charge to flow between the electrolyte and the electronic circuitry, to which they are connected. The electrical signals can be monitored and/or controlled using a potentiostat.
Chapter 3. Theory and Methodology

Ferrocenemethanol (FcMeOH) is an exemplar redox active species used in parts of this thesis, a diagram showing the oxidisation of FcMeOH can be seen in Figure 3.1. When a potential is applied to the working electrode (WE), at which FcMeOH ions are oxidised to FcMeOH\(^+\), the resulting electron (e\(^-\)) transfers into the electrode and can be measured as a current, known as the Faradaic current.

![Diagram showing example oxidation process of FcMeOH to FcMeOH\(^+\). This reaction occurs when the electrode is set to a potential, at which the FcMeOH oxidises.](image)

3.2.1. Electrode Potential

For a reversible process, the Nernst equation can be used to describe the relationship between the ratio of oxidised and reduced species and the electrode potential:

\[
E = E^0 - \frac{RT}{nF} \ln \left( \frac{a_{\text{Red}}}{a_{\text{Ox}}} \right) = E^0 - \frac{RT}{nF} \ln \left( \frac{\gamma_{\text{Red}} C_{\text{Red}}}{\gamma_{\text{Ox}} C_{\text{Ox}}} \right)
\]  

(3.1)

where:

\(E\) is the electrode potential,
\(E^0\) is the standard potential,
\(R\) is the universal gas constant,
\(T\) is the absolute temperature,
\(n\) is the number of electrons transferred,
\(F\) is the Faraday constant,
\(a_{\text{Red}}\) and \(a_{\text{Ox}}\) are the activities,
\( \gamma_{\text{red}} \) and \( \gamma_{\text{ox}} \) are the activity coefficients, and \( C_{\text{red}} \) and \( C_{\text{ox}} \) are the species concentrations at the electrode surface for the reduced and oxidised species respectively.

Often, the activity coefficients are constant during an experiment or can be assumed to be approximately equal to each other, this means the Nernst equation can be simplified to:

\[
E = E' - \frac{RT}{nF} \ln \left( \frac{C_{\text{red}}}{C_{\text{ox}}} \right)
\] (3.2)

where:

\( E' \) is the formal potential, and the remaining parameters are as defined previously.

Due to the assumptions made about the activity coefficients, the formal potential may be slightly offset from the standard potential.

3.2.2. Double Layer

When a potential is applied to the electrode, an electric field is generated at the interface of the electrode and solution and charge will accumulate at the electrode surface. This will cause ions in the solution to align themselves as shown in Figure 3.2. This layer of redistributed charged ions is referred to as the double layer and prevents further migration of ions, which is caused by the electric field generated from the applied potential. A double layer capacitance is generated from the separation between the charges accumulated on the electrode and the charges accumulated in the solution at the electrode interface.

The capacitance of this double layer leads to a charging current when a potential is applied, which can obscure the Faradaic current. The charging current can be calculated using:

\[
i_c = \frac{E}{R_s} e^{-\frac{t}{C_{\text{dl}}}}
\] (3.3)

where:

\( R_s \) is the solution resistance,
\( t \) is time, and
\( C_{\text{dl}} \) is the capacitance of the double layer given by:
\[ C_{dl} = \varepsilon_0 \varepsilon_r \frac{A}{d} \]  

(3.4)

where:

\( \varepsilon_0 \) is the permittivity of free space,

\( \varepsilon_r \) is the relative permittivity of the measurement solution,

\( A \) is the electrode area, and

\( d \) is the thickness of the double layer.

Figure 3.2: Diagram showing the double layer formed at an electrode. Negatively charged ions are attracted to the positively charged electrode when a potential is applied.

3.2.3. Mass Transport

The species of interest (and their resulting products) usually need to be transported to (or from) the WE during an electrochemical measurement. There are 3 methods of mass transport: diffusion, convection and migration. Diffusion occurs due to differences in concentration throughout the measurement environment, which sets up a concentration gradient. This commonly occurs at the electrode surface as species are oxidised or reduced during an electrochemical measurement. For the example in Figure 3.1, FcMeOH ions diffusion from the bulk solution towards the electrode surface where they are then oxidised and the
Chapter 3. Theory and Methodology

FcMeOH\(^+\) ions produced will diffuse from the electrode into the bulk solution. Convection can be subdivided into natural convection (e.g. caused by density gradients) and forced convection (e.g. fluid flow driven by stirring or pumping). Migration occurs due to the influence of an electric field.

These three mass transport methods can be summarised using the Nernst-Planck equation [2][3]:

\[
J = -D \nabla c + cv + \frac{Dze}{k_B T} c E
\]

\(Total\;flux = Diffusion\;+\;Convection\;+\;Migration\)  

where:

- \(J\) is the flux of species,
- \(D\) is the diffusion coefficient,
- \(\nabla c\) is the concentration gradient,
- \(c\) is the concentration,
- \(v\) is the flow velocity,
- \(z\) is the ionic charge number,
- \(e\) is the charge on an electron,
- \(k_B\) is the Boltzmann constant, and
- \(E\) is the electric field.

The diffusion term in this equation is also known as Fick’s first law. A supporting electrolyte at a significantly higher concentration than the electroactive species was used in this work. This means mass transport by migration can be assumed to be negligible due to the formation of the double layer, which shields the electroactive species. Excluding the migration term leads to:

\[
J = -D \nabla c + cv
\]

The flux can be related to the current by:

\[
|i| = nF AJ
\]

where \(J\) is the flux of electroactive species to the electrode surface, and the remaining parameters as defined previously.
3.2.4. Electrochemical Cell

A 3-electrode electrochemical cell was used in this work, and consists of a working electrode (WE), counter electrode (CE) and reference electrode (RE) as shown in Figure 3.3. The potential is set/measured across the WE and RE, and the current is set/measured between the WE and CE. The WE is where the reaction of interest occurs, and the RE provides a stable, known potential against which the potential can be set or measured. The CE supplies the necessary current to the WE which is required by the reaction, this means the CE must be sufficiently large compared to the WE to ensure it does not limit the supply of current.

![Diagram of an electrochemical cell](Image)

*Figure 3.3: Diagram of an electrochemical cell with counter electrode (CE), working electrode (WE) and reference electrode (RE) connected to a potentiostat. The potential is measured between the WE and RE, and the current is measured between the WE and CE.*

3.2.5. Reference Electrode

A reference electrode is an electrode with a reproducible and consistent potential, against which the reaction at the working electrode can be set or measured. To be suitable for use as a RE, the electrode must:

- hold a stable and reproducible potential over the experiment duration,
- not contaminate or be contaminated by the measurement solution,
- allow an electrical/ionic conduction path between itself and the measurement solution, and
there are many different types of RE and the potentials set by these electrodes are defined against the standard hydrogen electrode (SHE), which has unit activity at 25°C and is not practical for use in most systems. Other common REs are the saturated calomel electrode (SCE) and the silver/silver chloride (Ag/AgCl) electrode. An Ag/AgCl reference electrode was used in this work as they are widely available, do not have the toxicity concerns of SCEs, and can be miniaturised. The potential of the Ag/AgCl electrode is set by the reaction of silver chloride (AgCl) and silver (Ag) in a chloride containing solution:

$$\text{AgCl} + e^- \rightleftharpoons \text{Ag} + \text{Cl}^-$$

The potential of the reference electrode varies with the concentration of Cl\(^-\) in the solution according to the Nernst equation (3.1) and is 0.197 V vs. SHE at 25°C in a saturated KCl solution. Commercial Ag/AgCl reference electrodes often consist of an internal compartment, which is filled with a solution containing KCl at a constant and predetermined concentration and saturated AgCl. These electrodes also have an ion permeable frit, through which the reference electrode can interact with the measurement solution. The filling solution and frit allow the RE to maintain a fixed potential, regardless of the composition of the measurement solution.

3.2.6. Working Electrode

The working electrode is the electrode at which the reaction of interest occurs and its current response is proportional to the electrode area. The diffusional mass transport profile to the electrode also varies depending on the size of the WE. At macroelectrodes, the diffusion profile is linear (Figure 3.4a). The current response over short time periods at the WE is given by the Cottrell equation [4]:

$$i(t) = \frac{nFACvD}{\sqrt{\pi t}}$$

Microelectrodes and nanoelectrodes are typically defined as having one dimension which is on the order of micrometres or nanometres respectively. As the dimensions of the WE are decreased, the behaviour begins to change from that of a macroelectrode. The diffusion-limited current response decreases due to the decrease in area, however the current density increases. There are many
benefits to decreasing the electrode size including higher sensitivities, lower limits of detection, decreased \(iR_s\) drop, smaller capacitive charging current relative to the Faradaic current, and higher signal to noise ratio (SNR). These benefits occur due to the radial diffusion profile at a micro/nanoelectrode, which is more efficient compared to the linear diffusion profile at a macroelectrode (Figure 3.4b). The radial profile leads to a steady-state, diffusion limited current, which is independent of time.

The Saito equation can be used to calculate the limiting current at a microdisc electrode when under diffusion control [5]:

\[
i_{\text{Lim}} = 4nFCDr
\]  

(3.10)

where \(r\) is the electrode radius, and all other parameters as defined previously.

3.2.7. Nanoband Electrodes

Nanoband electrodes reported in [6][7][8] were used as the WE in parts of this work, the benefits of nanoband electrodes over other types of nanoelectrodes can be found in Chapter 2. Further information about the specific fabrication processes can be found in Chapters 5 and 6, and about the fabrication methods in general in section 3.6. Due to their small size single nanoelectrodes produce a very small current but they can be arrayed to give an overall larger signal. The use of standard fabrication processes allows arrays with controlled size and spacing to be created.

The Saito equation (3.10), which was shown previously, can be generalised to give the limiting current at micro/nanoelectrodes of different geometries, including nanoband electrode arrays [9]:

\[\text{Figure 3.4: Diagram showing (a) primarily linear diffusion at a macroelectrode and (b) primarily radial diffusion at a micro/nanoelectrode.}\]

\[\text{3.2.7. Nanoband Electrodes}\]

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The Saito equation (3.10), which was shown previously, can be generalised to give the limiting current at micro/nanoelectrodes of different geometries, including nanoband electrode arrays [9]:

\[\text{Figure 3.4: Diagram showing (a) primarily linear diffusion at a macroelectrode and (b) primarily radial diffusion at a micro/nanoelectrode.}\]
where:

\[ i_{\text{lim}} = B N n F c D r \]  

\[ (3.11) \]

\( B \) is a dimensionless constant characterised by the electrode geometry,

\( N \) is the number of electrodes, and

all other parameters as previously defined. For nanoband electrodes, \( r \) is defined as the radius of the nanoband cavity rather than the electrode radius.

The key difference in the diffusional mass transport to nanoband arrays compared to single nanoelectrodes is the overlap of the diffusion profiles from adjacent electrodes. The spread of the diffusion layer over time at a nanoband electrode array is shown in Figure 3.5 and proceeds as follows:

- Initially the material at the electrode surface is reacted and planar diffusion occurs (Figure 3.5a).
- The diffusion layer spreads outwards from the electrode surface, forming a radial diffusion profile around the microdisc area of the cavity (Figure 3.5b and Figure 3.5c). A limiting current will be measured while the electrodes are operating under the radial diffusion conditions in Figure 3.5c.
- The diffusion layer spreads from the region surrounding the electrode to the centre of the space between adjacent electrodes. At this point the diffusion layers of adjacent electrodes will overlap with each other (Figure 3.5d).
- Finally, the diffusion profile transforms from radial to a linear response across the area of the array footprint (Figure 3.5e).

The time, \( t \) for the diffusional profile to spread a specified distance, \( L \) can be calculated using:

\[ t = \frac{i^2}{2D} \]  

\[ (3.12) \]

This equation can be used to tune the geometry of a nanoband electrode array to ensure the steady-state region occurs in a specified time period. The mass transport at nanoband arrays needs to be considered when designing experiments and devices to account for the interaction of the diffusion profiles from adjacent electrodes.
Figure 3.5: Diagram (not to scale) showing evolution of diffusion profile over time at a cross-section of nanoband electrodes in an array.
3.3. Electrochemical Methods

In chronoamperometry (CA) the potential is set and the current response is measured over time. The current response will contain contributions from the charging current and the Faradaic current. Often the potential is first set to a potential at which the current response is zero (or close to zero) and then stepped to the potential at which the reaction of interest occurs. Cyclic voltammetry can be used to identify suitable potentials for the CA measurement.

In cyclic voltammetry, the electrode potential is swept in both directions between two values and the resulting current response is measured. Example cyclic voltammograms (CVs) are shown in Figure 3.6. A peak current is observed at electrodes operating under linear diffusion (such as macroelectrodes) and a wave shaped current response is observed at electrodes operating under radial diffusion (micro- or nanoelectrode), which is characteristic of their steady-state time-independent response. Often a pre-conditioning step is used prior to running the measurement. The potential is held at a specific value, usually to result in zero current flow or to reduce/oxidise any products formed from the previous measurement.

![Figure 3.6: Diagrams showing the characteristic shape of a CV at an electrode under linear diffusion (left) and an electrode under radial diffusion (right). Current magnitudes are arbitrary and are not to scale.](image)

It is often desirable to measure an analyte at multiple concentration values. These measurements can be combined to form a calibration curve, which relates the measured signal to the concentration. This relationship can then be used to evaluate an unknown concentration of the analyte from the measured signal. In this work the signal will be the current response from CA measurements at
different glucose concentrations. Calibration curves for first generation glucose sensors consist of a linear region followed by a plateau region caused by the dependency on the dissolved oxygen concentration or Michaelis-Menten kinetics (further information in Chapter 5), an example diagram of a calibration curve can be seen in Figure 3.7.

The limit of detection (LOD) can be calculated from the calibration curve using:

\[
LOD = \frac{3.3 \, STD_B}{Sensitivity}
\] (3.13)

from [10], where:

\( STD_B \) is the standard deviation of the blank (measurement with no analyte in the solution) and

\( Sensitivity \) is the gradient from the linear region of the calibration curve.

![Figure 3.7: Diagram showing an example of a calibration curve with an initial linear region followed by a plateau. The sensitivity can be measured using the linear region of this graph.](image)
3.4. Materials and Equipment Setup

Electrochemical measurements were performed using Autolab PGSTAT128N potentiostats, which were controlled via a laptop running NOVA 1.11 software. Data was plotted using MATLAB R2018b and analysed using either Microsoft Excel or MATLAB. Unless otherwise stated, the reference electrode used in this work was a single junction commercial Ag/AgCl (3 M KCl) electrode and a platinum coated silicon chip was used as the counter electrode. The fabricated electrodes were used as the WE or in some cases the RE depending on the experiment (stated in the text where appropriate). Electrical connection was made to the bond pads of the fabricated electrodes either directly with crocodile clips, or using edge connectors.

Electrochemical experiments were performed in a Faraday cage to minimise the effect of external noise. The magnetic stirrer/hotplate was unplugged during electrochemical measurements to eliminate the noise it produced. The majority of experiments were performed in the cleanroom at the Scottish Microelectronics Centre at a temperature of 21°C (±1°C). Some experiments were performed in the Pyrochemical Research Laboratory in the School of Chemistry at an average temperature of 25°C, stated when relevant.

Glassware was initially rinsed in concentrated sulphuric acid and deionised water before use to minimise risk of contamination. Between experiments glassware was rinsed using acetone, isopropanol (IPA) and deionised (DI) water (>18.2 MΩcm). Phosphate buffered saline (PBS) was purchased as a 10X concentrated solution and diluted with DI water to PBS 1X, this diluted solution contains a phosphate buffer concentration of 0.01 M, a sodium chloride concentration of 154 M, and has a pH of 7.4. Measurement solutions were prepared using DI water and a background electrolyte of either 0.1 M KCl or PBS 1X, unless stated otherwise. The key analytes used in this work were FcMeOH and D-glucose (referred to as glucose), the concentrations were varied and are stated in text where relevant. Perfluorinated resin solution containing Nafion was used as purchased or diluted with DI water (stated when relevant), this solution will be referred to as Nafion solution in the text. A list of chemicals and suppliers can be found in Appendix A.
3.5. COMSOL Simulations

COMSOL Multiphysics 5.6 version 5.6.0.401 was used for the simulations in this work. Simulations were performed for nanoband electrodes and through-flow nanoband electrodes, further details about these electrodes and the simulation results will be discussed in the relevant chapters (Chapters 5 and 6). In this section, the model used to simulate the current response at the electrodes is presented.

COMSOL uses finite element analysis for its simulations, meaning the simulation environment is made up of small regions (mesh elements), which form the mesh for the model when combined together. The equations describing the diffusional mass transport and the velocity profile are calculated at each of the mesh nodes, at specified time points to generate the overall simulated result from the model. The flux due to diffusion is calculated using Fick’s first law, from the Nernst-Planck equation (3.5), and Fick’s second law, which is given by:

$$\frac{\partial C}{\partial t} = D \nabla^2 C$$

(3.14)

The velocity profile is calculated in a similar way at each node using the Navier-Stokes equations for incompressible flow ($\nabla \cdot \mathbf{v} = 0$):

$$\rho \left( \frac{\partial \mathbf{v}}{\partial t} + \mathbf{v} \cdot \nabla \mathbf{v} \right) = -\nabla p + \nabla \cdot (\mu (\nabla \mathbf{v} + (\nabla \mathbf{v})^T)) + \mathbf{F}$$

(3.15)

*Inertial forces = Pressure forces + Viscous forces + External forces*

where:

- $\rho$ is fluid density,
- $p$ is fluid pressure,
- $\mu$ is fluid dynamic viscosity.

External forces (for example gravity) can be considered negligible.

The simulation environment used for the simulations in this work is shown in Figure 3.8 for both the through-flow nanoband electrode (Figure 3.8a,b) and the standard nanoband electrode (Figure 3.8c). The concentration at the inlet boundary is assumed to be fixed to the bulk concentration and the concentration at the electrode is assumed to be zero, meaning the analyte is immediately oxidised (or reduced). The electrode was simulated with 2D rotational symmetry to
generate results for the 3D structure. The symmetry boundary was located in the centre of the nanoband pore and the right edge of the simulation environment is located at the half way point of the edge-to-edge separation between adjacent electrodes in the array. Simulations were also performed for a single electrode with the simulation boundary on the right hand side extended to 2 mm. This was beyond the expansion of the diffusion layer during the simulation time, and therefore overlap of the diffusion profile did not occur during the simulation.

Figure 3.8: Diagram (not to scale) of the simulation environment for (a) the through-flow nanoband electrode, and (b) magnification of the region surrounding the electrode. (c) Magnification of the simulation environment for the standard nanoband electrode in the region surrounding the electrode. Inlet boundary is set to the bulk concentration, the membrane boundary is set to zero flux along with the upper boundary in (c), and flux is allowed at the left and right boundaries. The axis of rotational symmetry is labelled and shown by the dotted line, this allowed a 2D simulation environment to be used.
A line integration was performed along the electrode along with rotational symmetry from 2D to 3D, to obtain the total flux to the electrode. In the flow simulations a coupled solver was used to first simulate the velocity profile and then to simulate the diffusion of analytes throughout the simulation environment. The through-flow nanoband and standard nanoband electrode simulations used the same environment and mesh settings where possible to maintain consistency. Simulations of the standard nanoband electrodes were performed under diffusional mass transport conditions only and the insulation layer was extended across the nanoband pore as the electrode was exposed on only one side of the device (Figure 3.8c).

![Simulation of current response](image)

*Figure 3.9: Simulation of current response (with 0 mlh^{-1} (0 m^3s^{-1}) flow) using an unsuitable mesh (red cross), a partially optimised mesh (orange plus), and the final optimised mesh (blue dot). Note y axis is magnified and current values at the lowest time points have therefore been excluded.*

The density of the mesh elements is a key parameter to control when setting up the simulation model. A higher meshing density is required in regions where the concentration profile (or velocity) changes rapidly. For these electrodes, this was the region surrounding the electrode surface and cavity. The mesh used in the simulation model was developed by changing the growth rate of meshing.
elements and defining additional meshing boundaries. The mesh growth rate is a multiplication factor that defines the change in the size of adjacent mesh elements. Meshing boundaries are regions of the simulation environment where the mesh properties (such as the maximum mesh element size) can be set. The mesh was adjusted until differences in the resulting simulated flux were limited to the third significant figure at both no flow and the highest flow rate tested.

Initially the growth rate between mesh elements was set to fractions of a percentage of change. However, this did not appear to be suitable since the mesh was still coarse close to the electrode and the resulting concentration profile and extracted current response were not smooth, as shown in Figure 3.9. Additional meshing boundaries were added through the environment to help control the growth rate and density of the meshing elements. The number of points across the electrode was set to 500. The electrode and insulator vertical wall were set to a maximum mesh element size of 1 nm. The smallest meshing box was set to a maximum element size of 10 nm, the box boundary lines were also set to this size. The second meshing box was set to a maximum element size of 100 nm as well as its boundary lines. The largest box was set to a maximum element size of 1 μm. The maximum growth rate was set to 1.05 except on the largest box where it was set to 1.005. The remaining geometry was set to the predefined “normal” element size. Setting the element size and growth rate on the boundary edges meant the meshing elements would grow smoothly from these points into the next meshing region. The concentration profile transitioned smoothly using the optimised mesh, along with the extracted current response as shown in Figure 3.9, and further increasing the meshing density appeared to result in negligible change.
Figure 3.10: Simulation result showing the velocity profile (a) without additional meshing in the pore channel showing rough transitions in the velocity profile, and (b) with additional meshing showing smooth transitions in the velocity profile.

The velocity simulations required an additional meshing boundary inside the nanoband pore to produce a smooth transition in the velocity profile, Figure 3.10 shows the simulation results with and without this additional boundary. The figure shows the asymmetrical and uneven nature of the velocity profile without the additional meshing boundary (Figure 3.10a), and the resulting symmetrical and smooth velocity profile with additional meshing (Figure 3.10b). In the additional meshing region and along its boundary, the mesh was set to a maximum element size of 10 nm. This was not required for the transport of species simulation so was removed for the second step of the simulation, the remaining mesh boundaries and sizes were kept consistent.

3.6. Fabrication

In this work various devices were fabricated, an overview of these devices is given in Chapter 1. The specific fabrication process for each type of electrode is discussed in the corresponding chapter. Additionally, runsheets for these fabrication processes can be found in Appendix B. These fabrication flows follow similar processing steps and this section will contain more general background information on the electrode fabrication using the tools available in the cleanroom at the University of Edinburgh. The electrodes used in this work were fabricated on n-type silicon wafers with <100> orientation, which were insulated with 500 nm thermally grown silicon dioxide (SiO₂). The full specifications of the purchased silicon wafers can be found in Appendix A. The process steps that will
be discussed in this section are the insulator deposition, metal deposition, photolithography, and wet and dry etching processes.

Insulators deposited using Low Pressure Chemical Vapour Deposition (LPCVD) tend to be of better quality than those deposited using Plasma Enhanced Chemical Vapour Deposition (PECVD), and entire cassettes of wafers can be insulated under the same conditions in one batch with a high throughput [11]. LPCVD insulators can be grown or deposited on both sides of the wafer simultaneously, which is beneficial for balancing stress across the wafer and for processing the double-sided wafers used in Chapter 6. Although it was not relevant for this work, an advantage of PECVD is the lower temperature requirement, which can make it compatible with a wider range of underlying materials. The thickness and uniformity of the insulating layers were measured using reflectometry.

The metal layers were deposited using electron-beam evaporation, which results in high density and high purity metal layers. This type of deposition method was used in this work due to the availability of the deposition tool with the required metals in the cleanroom. Other metal deposition methods were not used, but would likely be suitable. In electron-beam evaporation a magnetic field is used in a high vacuum chamber to direct an electron beam onto a metal target in a crucible, which becomes heated and evaporates. The evaporated metal travels through the chamber until it reaches the wafer, where it condenses as solid metal. A quartz crystal is used to monitor the amount of metal deposited on the target by the change in frequency. The metal layers were measured using profilometry after deposition and patterning. A titanium seed layer was often used to improve the adhesion of other metals to the insulator (in this work, platinum or silver). The metals were deposited using the same tool without breaking the vacuum. Therefore, the Ti layer was not able to form a surface layer of oxide before the second metal was deposited. Any Ti exposed during etching will immediately form an oxide layer in the ambient environment and under the experimental conditions, ensuring the electrochemical response occurs only on the intended layer [12].
Both positive and negative photoresists were used for fabricating the electrodes, and diagrams showing both of these techniques can be seen in Figure 3.11 and Figure 3.12. In this work, positive photoresist was patterned and used for etching of insulators and metal layers, whereas a negative photoresist (designed for lift-off processes) was used for silver lift-off. Chapter 6 additionally discusses the use of negative photoresist for dry etching. Positive or negative photoresists can be used for both etching and lift-off, however some photoresist formulations are optimised for specific processes. Unless stated otherwise, the choice to use negative or positive resist in this work was informed by standard practices developed by previous cleanroom users and/or the type of photomask, which was already available.

Wafers were exposed to a hexamethyldisilazane (HMDS) atmosphere for 10 minutes to aid adhesion before the photoresist was spin-coated. Photoresist was then exposed using UV light through a photomask. This photomask was either a physical quartz mask used with a mask aligner (Karl Suss MA8) or a virtual mask used with a virtual mask aligner tool (Durham Magneto Optics, MicroWriter ML3). The virtual mask aligner uses direct-write lithography to project and expose the desired pattern directly into the photoresist. This is considerably slower than exposure using a physical mask, however it allows different mask designs to be tested and modified quickly and at a comparatively low cost. Once the photoresist has been developed and the wafer etched (if required), the photoresist was stripped from the wafers using ACT CMI or Microposit Remover 1165 at 60°C with sonication. The wafers were then soaked in IPA followed by DI water, to remove any residue from the resist stripper.
Chapter 3. Theory and Methodology

Figure 3.12: Diagram showing lithography steps for negative photoresist. (a) A cleaned wafer with negative photoresist spin-coated onto the wafer, (b) resist is exposed through a photomask with the mask shielding areas of the wafer and the exposed sections are cross-linked, (c) the resist, which was not cross-linked, is then removed during development leaving the wafer ready for further processing. Negative resist commonly forms an overhang after development due to the cross-linked process.

A hard mask can be used when it is not possible to adequately pattern and protect the underlying layers using photoresist. Hard masks can be patterned using the photolithography and etching techniques discussed in this section, and used to replace or supplement the photoresist layer when it is incompatible with the subsequent processing steps. This could be due to various factors, such as chemical incompatibility or low selectivity of an etch process. For example, photoresist is not compatible with high temperature potassium hydroxide etching and instead a hard mask can be created from insulator layers (e.g. silicon nitride).

Wet and dry etch processes were used to remove unwanted material from the wafers. The dry etching processes used a plasma tool, which etched the material using a physical or chemical process. Reactive ion etching (RIE) and argon milling were both used in this work. RIE is used for the insulator etches and uses a combined physical and chemical process, the composition of gases in the RIE depends on the target material. Argon milling is used to etch the Pt and Ti layers in the nanoband electrode etch and is purely a physical process. As previously, the choice of etch was informed by standard practice developed in the cleanroom and in many cases it would be possible to use either a dry or wet etch to achieve the same result. However, in processes such as the nanoband cavity etch in Chapter 4, an anisotropic etch (meaning material will only be removed in one direction) is required to achieve the desired profile, and therefore RIE was chosen rather than wet etching.

Finally, wafers were coated with a protective layer of resist before being diced into individual chips with a diamond dicing saw. This protective coating was removed after dicing by rinsing in acetone, IPA and DI water.
3.7. Electrochemical Cleaning

Planar electrodes are commonly cleaned mechanically, followed by an electrochemical cleaning step. Nanoelectrodes are particularly sensitive to contaminants, therefore care must be taken to ensure a high level of cleanliness. Due to their geometry they are difficult to clean using mechanical polishing and therefore must be cleaned electrochemically. A benefit of electrochemical cleaning using cyclic voltammetry is the ability to monitor any additional unexpected peaks which may appear in the scan. Platinum nanoband electrodes were cleaned in 0.1 M KCl by cycling electrochemically into the solvent limits, which leads to oxygen and hydrogen evolution. Around 15 scans were usually required before the observed change in current was negligible.

![Cleaning CVs at 100 mVs⁻¹ of a Pt nanoband electrode in 0.1 M KCl, the first scan (red, line), the second scan (orange, dotted) and the final scan (blue, dashed) are shown. The potential is initially cycled in the direction of the positive potential, and the peak at the negative potential end is due to hydrogen evolution and the peak at the positive potential end is due to oxygen evolution.]

Care must be taken when electrochemical cleaning not to generate excessive current at the electrode. The maximum value will vary between electrode geometries but leads to excessive generation of bubbles on the electrode.
surface, which can cause irreparable damage to the devices through delamination of the insulator. The potential limits for the electrochemical cleaning were set to produce a maximum current magnitude of approximately 100 – 300 μA (equivalent to 29 – 87 x 10² Am⁻²). This ensured the electrodes were not damaged by bubble formation, but sufficient bubbles were generated to ensure effective cleaning occurred. Example CVs during the cleaning procedure are shown in Figure 3.13. Nanoband electrodes are susceptible to localised pH effects, which is why the potential window widens during the cleaning scans.

The gases produced during electrochemical cleaning (predominantly hydrogen gas from hydrogen evolution) can be absorbed into the Pt layer, changing the current measured at the electrode [13][14]. After the cleaning has been completed these gases will slowly diffuse back out of the devices over the course of several hours, at which point the current response will remain stable [9]. This process means it is necessary to electrochemically clean the electrodes at least several hours prior to performing experiments to ensure measurement conditions are consistent throughout the experiment.

References


Chapter 3. Theory and Methodology


Chapter 4

Microfabricated On-Chip Reference Electrodes

4.1. Introduction

One of the challenges in developing on-chip electrochemical systems is the need for a miniaturised solid-state reference electrode. A wearable glucose sensor will require a miniaturised on-chip reference electrode to function, therefore the development of this electrode is a key component of the sensor system. The advantages of solid-state reference electrodes compared to traditional glass tube reference electrodes is that they can be low cost, miniaturised and used in different spatial orientations in a system, such as integrated on a wearable or implantable device [1]. Due to the size constraints of miniaturisation it may be difficult to maintain the same components in these reference electrodes, as are found in macroscale electrodes, such as a filling solution and ion permeable frit. This means miniaturised reference electrodes must be constructed using new approaches. The lack of compartmented filling solution means the electrode potential is set by the nature and concentration of the redox-active agents in the measurement solution. This may necessitate a greater understanding or control of the variation in the composition of the solution.

Bare Pt or Ag electrodes are often used in on-chip systems as they are simple to fabricate and tend to require minimal additional process development. At these electrodes, the potential is controlled by electrochemical processes that are not well understood, which means the accuracy of the system is generally lower compared to other reference electrode systems [2]. Although, if the experimental conditions can be well-controlled, the potential of the Ag or Pt reference electrode can remain constant during measurements [3]. Due to the variability of both the measurement environment and composition of sweat, this type of on-chip electrode was not selected for this work.
A commonly used on-chip reference electrode for biosensors is the silver/silver chloride (Ag/AgCl) reference electrode, which is well studied and has an established route to on-chip fabrication [4]. Ag/AgCl reference electrodes are suitable for use in sweat sensing; they require measurement solutions with chloride to function and sweat naturally contains chloride at concentrations of around 20 – 50 mM (an average of 30 mM on the forearm) [5]. On-chip Ag/AgCl reference electrodes are limited by the volume of silver chloride that can be converted from the available silver layer [4]. As a result, the stability and lifetime of such electrodes can be significantly decreased, representing a major obstacle in device development.

An on-chip Ag/AgCl reference electrode, which has microscale dimensions and a thin-film silver thickness of 500 nm or less (as presented in this chapter) has not been found in the literature. An on-chip micro Ag/AgCl electrode was constructed in [6], however the silver layer was 1 μm thick, which is at least double that used in this work, and its lifetime performance was not characterised as it was not intended for use as a reference electrode. It was characterised using electrochemical impedance spectroscopy and it was suggested the low impedance of the micro reference electrode made it a promising candidate as a working electrode for high sensitivity impedance-based biosensing. Another comparable example is the thin-film (500 nm silver) macro reference electrode reported by Lim et al. [7]. The electrode was coated with a Nafion membrane and measured to last 18 days, however the measurement at day 18 showed quasi-stable behaviour, indicating that the potential was no longer being set by the Ag/AgCl redox reaction. The electrode lifetime is therefore not known as it was unclear at what point the electrode deviated from true reference behaviour. This is one of many papers that use a Nafion membrane to extend the lifetime of the fabricated reference electrode [4][8][9].

An interesting alternative reference electrode structure shown in Figure 4.1 is presented in [10], where a 300 nm silver layer is sandwiched between insulating layers and the chloride is converted horizontally. This structure is similar to the nanoband electrodes presented in Chapters 5 and 6. In this way, the effective “thickness” of the silver occurs horizontally rather than vertically and provides an alternative to a thick silver layer. This electrode requires a large footprint area to produce a sufficient exchange current, and would require significant development
of the fabrication process so was not considered in detail in this work. However, it is an interesting concept, which could be compatible with the electrode geometry presented in Chapter 6.

![Cross-section showing formation of AgCl at a conventional thin-film electrode](image1)

![Cross-section showing formation of AgCl at novel alternative structure suggested by Suzuki et al. [10].](image2)

Figure 4.1: (a) Cross-section showing formation of AgCl at a conventional thin-film electrode. (b) Cross-section showing formation of AgCl at novel alternative structure suggested by Suzuki et al. [10]. Arrows show direction of AgCl formation. 1, adhesive layer; 2, gold layer; 3, silver layer; 4, upper polyimide layer; 5, AgCl layer. Figure adapted from [10] with permission from Elsevier.

This chapter investigates different fabrication methods with the aim to develop a procedure for producing an on-chip microscale Ag/AgCl reference microelectrode with a reproducible functional lifetime of at least one day (24 hours). This was chosen as a reasonable initial lifespan for use as a sensor in a wearable device as it would allow a user to change the sensing device once per day. This chapter discusses fabrication of silver microelectrodes, optimisation of the chlorination of the silver layer and characterisation of the electrode performance. Additionally, Nafion is investigated as a coating membrane to improve the performance of the fabricated reference electrodes.

### 4.2. Fabrication

A diagram of the reference electrodes fabricated and characterised in this chapter can be seen in Figure 4.2 and consist of a silver microdisc electrode, which is chlorinated to form a silver chloride layer. Electrodes are tested both with and without the addition of a Nafion membrane coating the electrode.
4.2.1. Initial Fabrication Process

The fabrication of the reference electrodes followed the procedure developed by C. Dunare [11] with adaptations discussed below. These were found to be required during electrode testing in order to improve device robustness. A diagram showing the key processing steps can be seen in Figure 4.3 and process runsheets can be found in Appendix B. A silicon wafer was insulated with 500 nm of thermally grown silicon dioxide, after which a 50 nm thick Pt layer was deposited on top of a 10 nm Ti adhesion layer (Figure 4.3a) and wet etched to the electrode outline with aqua regia (Figure 4.3b). The wafer was then insulated with silicon nitride (Figure 4.3c), which was etched using RIE to expose the bond pad and a platinum microdisc electrode of either 30 or 50 μm diameter (Figure 4.3d). Negative resist was spin-coated and patterned for the silver lift-off process. As the cleanliness of the substrate is extremely important for adhesion of the silver layer, a 5 minute oxygen plasma step was then used, followed by silver layer deposition by e-beam evaporation. Fabricated on-chip reference electrodes in the literature often use silver layer thicknesses in the μm range [12]. However, the thickness of the silver layer used in this work was limited by the metal deposition tool and operator time constraints. The initial batch of wafers had a silver layer thickness of 500 nm and the subsequent batches had to be reduced to 250 nm due to timing constraints from the tool and operator.
Figure 4.3: Cross-section showing key processing steps in fabrication of reference electrodes (not to scale). (a) Silicon wafer insulated with silicon dioxide. 10 nm Ti and 50 nm Pt deposited. (b) Pt and Ti wet etched. (c) Pt layer insulated with silicon nitride. (d) Electrode bond pad and Pt microdisc exposed by etching through SiRN. (e) Ag patterned and deposited using lift-off process. (f) Ag chlorinated to form AgCl. Dotted line indicates location of cross-section in Figure 4.2.

The silver layer needed to slightly overlap the surrounding insulator to ensure no Pt was exposed to electrolyte, which should prevent mixed potentials from forming. The silver layer was patterned to have a 10 μm larger diameter than the electrode, resulting in 40 and 60 μm diameter silver discs (as shown in Figure 4.3b). The wafers were then submerged in a bath of ACT CMI photoresist stripper at 60°C until all the silver was removed from the unwanted areas (Figure 4.3e). This typically took around 1-2 hours, with the addition of 1 minute of sonication every fifteen minutes of submersion. The silver layer was found to be removed from the electrode if longer sonication times were used. It can also be seen in the SEM image of Figure 4.3c that this deposition process resulted in a dense layer of silver without pinholes or defects. Additionally, there is good adhesion to the underlying platinum layer prior to chlorination. The surface of the silver was roughened by exposure to the ACT used in the lift-off process. A number of lift-off procedures have been investigated, and ACT was identified as the best choice of resist stripper since it resulted in the least amount of surface roughening of the silver layer [13].
After silver deposition, it is necessary to limit exposure to air to reduce the formation of oxides or sulphides on the surface of the silver [3][14]. Therefore, wafers and chips were stored under vacuum until ready for further processing. The wafers were diced either before electrochemical chlorination or after chemical chlorination, both of which are discussed in section 4.3. A finished silver electrode can be seen in Figure 4.4. Once the electrodes were chlorinated (Figure 4.3f) they
were protected from exposure to light as silver chloride is photosensitive [15]. This also presents a problem for any visual characterisation method of the silver chloride. For example, it must be considered when using the SEM for imaging as the AgCl surface can be reduced back to Ag while under the focus of the electron beam. This also means that using the EDX for material composition analysis is not very practical to provide meaningful quantitative results as the amount of chloride can decrease during the measurement. As the final step, a Nafion membrane was drop-cast on some of the electrodes. The method developed for this deposition process is given in section 4.2.3.

4.2.2. Process Development

Issues were encountered during testing which necessitated improvements to the initial fabrication process detailed above. From Figure 4.5b it can be seen that the ring of silver which overlaps the insulating layer has lost adhesion during the measurement and that the entire outer ring structure has become detached. In these initial electrodes there was no adhesion layer under the silver (since silver adheres well to platinum). To prevent loss of adhesion, a 10 nm titanium adhesion layer was deposited before the silver deposition.

There was also an issue with the physical and electrical connection on some of the electrodes between the central disc and the ring overlapping the insulating layer, this problem is related to the detachment of this ring during testing. It is most noticeable when chlorinating electrochemically and can be seen in Figure 4.5a where the centre of the electrode appears dark due to chlorination while the outer ring remains silver indicating it was not chlorinated. This can lead to a difference in the depth of chlorination between electrodes chlorinated with a fixed charge (section 4.3.3), due to the reduced volume and surface area of Ag and will therefore affect the reproducibility of the chlorination process.

The initial wafers tested were fabricated with a top insulator consisting of PECVD nitride and oxide, however subsequent wafers were fabricated using LPCVD SiRN. Nitride deposited using LPCVD is a higher quality of insulator and can therefore be made thinner (300 nm) than the combination of PECVD nitride (500 nm) and oxide (100 nm). The initial insulator was thicker than the deposited silver, meaning the silver layer on the Pt was not physically connected to the silver layer on the insulator. Decreasing the insulator thickness allows the silver layer to be continuously connected across the Pt and insulator (this can be seen in Figure
4.2. This should additionally help prevent the detachment of the outer ring since it will be better adhered to the bulk of the silver layer.

Figure 4.5: Microscope images of a chlorinated electrode (a) before and (b) after testing. Images show (a) discontinuity between the electrode centre and outer ring and (b) outer ring migration during testing.

4.2.3. Nafion Drop-Casting

Nafion was used to extend the lifetime of the fabricated reference electrodes. More specifically, the Nafion was used to mitigate the dissolution of the negatively charged \( \text{AgCl}_2^- \) complex from the electrode, this is discussed further in sections 4.4.3 and 4.4.4. This section discusses the drop-casting procedure, which was used to deposit the Nafion membrane over the electrode area and measure its adhesion to the substrate over time. Nafion adhesion has been measured previously and Nafion was found to adhere strongly to Pt and various PECVD insulators over several weeks of testing [16]. However, those substrates were treated using a silane adhesion promoter solution and the Nafion was thermally annealed. There was a concern that these additional process steps would interfere with the glucose sensing layers on the final device, and therefore in this work Nafion was drop-cast onto untreated substrates and was not thermally annealed. Success or failure of Nafion adhesion to the substrate was measured over the testing period to ensure the layer remained intact for a sufficient duration to be used as a protective layer for AgCl.

Nafion solution was diluted 1:4 with deionised water to increase the viscosity, which helped to control the spread of the drops [17]. This diluted Nafion was then drop cast (pipetted) onto wafers as 2 \( \mu l \) drops, which were left to dry overnight before testing. The test samples consisted of 76 drops spread over a wafer surface. The wafers were submerged in PBS 1X for adhesion testing, to
simulate operation in sweat, and analysed after one, two and four weeks for membrane survival. Survival or failure of the Nafion membranes was determined optically using a microscope. Figure 4.6 shows examples of surviving and failed structures on a PECVD high frequency silicon oxide (HFSIO) substrate. The PECVD HFSIO substrate showed some evidence of optimum Nafion adhesion in [16], therefore this substrate was tested along with LPCVD SiRN, as this was the top insulator used in the fabrication process. It is possible to create a thin layer of silicon oxide on the surface of a silicon nitride layer through oxygen plasma treatment to increase the number of oxygen groups [18]. LPCVD SiRN was treated with oxygen plasma to form an oxidised SiRN substrate. Both PECVD oxide and oxidised SiRN could be incorporated into the fabrication process if it could be shown that they significantly improve the Nafion adhesion.

![Survived and Failed Structures](image)

*Figure 4.6: Example microscope images of surviving (left) and failed (centre, and right) drop-cast Nafion films on PECVD HFSIO.*

It can be seen from the graph in Figure 4.7 that the oxidised SiRN substrate performed very poorly with not even half of the Nafion drops surviving the first week. This could potentially be due to some kind of smoothening effect on the surface from the plasma treatment leaving less surface area for the Nafion to adhere to. The SiRN substrate provided the highest survivability in these tests, despite the literature results suggesting optimal performance from the PECVD HFSIO substrate [16]. However, this paper compared PECVD nitrides rather than the LPCVD SiRN used here, which may account for the difference. Additionally, the silane treatment used in the experiments of [16] may have had a greater effect on the silicon oxide substrates compared to the silicon nitride, and this step was omitted here. The results show the SiRN provided the optimal Nafion membrane survival, 97% of the Nafion structures on SiRN remained after 1 week. This survival rate was determined to be sufficient for use as a reference electrode.
coating, and therefore there was no need to make any further changes to the fabrication process.

Figure 4.7: Bar chart showing percentage survival for drop-cast Nafion \( (n = 76) \) on PECVD HFSIO, LPCVD SiRN, and LPCVD oxidised SiRN over 1, 2, and 4 weeks of submersion in PBS 1X.

4.3. Chlorination Procedure

4.3.1. Silver Chloride Structure

Chlorination of the silver layer is a key process step in developing an Ag/AgCl reference electrode and controls how the outer AgCl layer is formed by progressively converting the part of the Ag layer in contact with solution. The chlorination procedure is particularly challenging for on-chip reference electrodes like those discussed in this chapter due to both the small diameter and thickness of the silver layer. The resulting structure (and porosity) of the AgCl as well as the depth of chlorination into the silver will affect how the electrode performs.

Chemical and electrochemical chlorination were explored as methods of fabricating the Ag/AgCl reference electrodes. Chemical chlorination is the preferred method for mass production since an entire wafer can be chlorinated in
one step and all the electrodes will experience the same chlorination conditions. The AgCl structure and depth can be controlled by changing the current density and the charge passed during chlorination. For chemical chlorination this would mean changing the concentration of the oxidising agent and the chlorination time. Electrochemical chlorination allows a higher level of control since the current density and charge passed can be set directly using a potentiostat. This level of control may be necessary to achieve the desired Ag/AgCl performance, but would require the electrodes to be processed individually.

The formation of AgCl into the silver layer does not proceed uniformly, the silver chloride forms nucleation sites at the silver grain boundaries and spreads from these points [15]. This means chlorination is not a diffusion controlled process and that not only is the resulting AgCl structure dependent on the chlorination parameters, it is also dependent on the structure of the underlying silver layer. For example, silver layers formed from porous Ag$_2$O that are then chlorinated led to more porous AgCl layers [19]. Since the e-beam evaporated silver used for these electrodes did not show a high porosity after fabrication (section 4.2.1), the chlorination step was likely to have the biggest impact on electrode porosity, and therefore performance.

The porosity of the AgCl layer is a key parameter to control, with longer chlorination times and higher current density leading to higher porosity [20]. Highly porous electrodes will have a high surface area and therefore a higher exchange current density, which can lead to better potential stability [3]. However they tend to have longer settling times due to Cl$^-$ diffusing slowly out from within the pores, and also shorter lifetimes [19]. If the silver layer is thin, chlorination that results in a highly porous AgCl structure may expose the underlying metal layers to the electrolyte, which can lead to the development of mixed potentials at the electrode [21]. This means a balanced approach must be taken when considering the structure of the AgCl formed.

In order to create a true reference electrode, at least some silver must remain on the chip to allow the appropriate reactions to occur to set the potential. However, there is literature showing that the percentage of Ag converted to AgCl is important. Janz and Ives suggested 10-25% of the silver should be converted to silver chloride to obtain good reproducibility and stability [15]. Brewer et al [22]
compared electrodes fabricated by different labs and found some evidence of an optimum at around 15-20% AgCl, however the authors also stated that more data was needed to support this statement. Koudelka found the AgCl layer lost adhesion to the underlying silver layer when the conversion of silver to silver chloride was higher than 50% and suggested 30% as a suitable silver chlorination percentage. Their paper compared thin-film and bulk silver structures and found the chlorination percentage was most important for thin-films [23], which may be why chlorination percentage is not often discussed in the literature. The optimal ratio of silver to silver chloride varies between papers and it is possible it also depends on the quality and thickness of the underlying silver layer. However, it does seem clear that there is a relationship between the amount of silver converted and the performance of the fabricated reference electrode.

4.3.2. Chemical Chlorination

Various oxidising agents such as KCrO$_3$Cl [24] and NaOCl [25] have been used in the literature to chlorinate silver electrodes, but FeCl$_3$ is the most common chemical chlorination method [4][12][1]. The end result is the same – silver chloride is grown from the silver layer. In this work FeCl$_3$ was used to chlorinate the silver electrodes, following the process in [11], as it is inexpensive, widely available and commonly used. The following reaction occurs at the Ag electrode during chlorination:

$$\text{Ag} + \text{FeCl}_3 \rightarrow \text{AgCl} + \text{FeCl}_2 + e^- \quad (4.1)$$

The fabricated silver electrodes used in this section have a thickness of 500 nm and were chlorinated using a fresh solution of 50 mM FeCl$_3$ in DI water. The wafers were submerged in the solution for 60 s and then gently rinsed in DI water. Figure 4.8 shows an example SEM image of such a chemically chlorinated electrode. The structure of the AgCl is highly porous, particularly away from the edges of the electrode. The concentration of FeCl$_3$ or the chlorination time could be decreased to try to decrease the AgCl porosity. However, this was not attempted as part of this work due to the decision to focus on electrochemical chlorination (discussed in section 4.4.2) and the lack of reproducibility of the chemical chlorination process discussed below.
Chapter 4. Microfabricated On-Chip Reference Electrodes

Figure 4.8: SEM image of a chemically chlorinated electrode showing high AgCl porosity.

To identify how much of the silver had been converted, an electrode was cleaved with the dicing saw and imaged using the SEM (Figure 4.9). However, it was hard to measure accurately from a cleaved cross-section as the cut is not straight and it has smeared and delaminated parts of the structure. Using FIB milling instead of dicing the cross-section, then imaging in the SEM would allow the silver and silver chloride layers to be more easily identified and measured. It is not really possible to analyse the image in Figure 4.9 quantitatively but it does appear that the majority of the Ag may have been converted into AgCl. This would also be consistent with the results presented in [11], where 500 nm thick silver test structures were chlorinated with 50 mM FeCl₃ for 60 s and the measured sheet resistance indicated full chlorination.

Chemical chlorination can be less challenging for thicker films of silver as the risk of fully chlorinating the silver layer is decreased, however thicker films of silver were not available in the cleanroom. Reproducibility of AgCl depth using chemical chlorination is challenging on thin-films of silver [11]. Since this work was completed, there has been some research towards developing a more controllable and reproducible chemical chlorination process on thin-film microstructures by varying the chlorination time and FeCl₃ concentration [26]. The
pH of the chlorination solution was also lowered by adding 10 mM HCl to increase the stability of the FeCl$_3$. However, the results still showed a high level of variability in the depth of chlorination of the Ag layer. At the time, it was decided electrochemical chlorination should also be investigated as it would offer a higher degree of control over the chlorination process.

![Figure 4.9: SEM image of a cross-section of a chemically chlorinated electrode. Cross-section created by dicing with the dicing saw, which has caused smearing of the layers.](image)

4.3.3. Electrochemical Chlorination

Due to the limited thickness of silver (250 nm), conversion of the entire layer is a concern. However, one of the key benefits of electrochemical chlorination is that the chlorination process can be terminated by setting a cut-off charge so over-chlorination of the electrode can be avoided.

Chloride formation can be controlled electrochemically by applying either a constant potential or constant current. Brewer et al [27] found better reproducibility using constant potential. Huang et al [20] used a constant current and found that the current density used to chlorinate was related to the size and porosity of the resulting AgCl grains. Longer chlorination times and higher current densities both created larger grains and pores in the resulting layer, and this had a negative impact on the adhesion of the film. The literature on this subject is
underdeveloped, it is likely that the potential or current chosen to perform the chlorination is more important than the method itself. The constant potential method was chosen for this work and the potential was selected to result in a smaller current density, this should mean the rate of AgCl formation would not be dependent on surface area.

To choose an appropriate potential to perform the chlorination, CVs were recorded on a silver microdisc in a 0.1 M KCl solution. In Figure 4.10, the formation of AgCl can be seen in the oxidation peak around 0.13 V, as well as the reduction of this AgCl back to Ag at around −0.02 V. There are small changes in the peak shapes, which are consistent with slight changes to the nature of the surface due to repeated formation and reduction of silver chloride. It was decided to use 0.11 V as the chlorination potential (dashed line in Figure 4.10), as this is close to the onset of AgCl formation and should produce a small current, which will result in a less rapid chlorination process.
Figure 4.11: Example CAs of silver chlorination at 0.11 V on 60 µm electrodes and corresponding charge passed, showing typical variability in current from electrochemical chlorination.

The charges consistent with full chlorination can be calculated as 2.95 and 6.64 μC for the 40 and 60 µm diameter electrodes respectively. However, the chlorination process can be cut off once a certain amount of charge has been passed, which should allow the percentage of chlorination to be controlled and the electrodes to be chlorinated with greater reproducibility.

Figure 4.11 shows example CA measurements of electrodes during chlorination and the resulting charge passed. The current response is variable between electrodes, which is consistent with a surface nucleation and growth process rather than mass transport. This type of process would be expected to have some variability. However, the wide range of current responses is surprising since the silver layer should be very reproducible between electrodes due to the repeatability of e-beam metal deposition in the cleanroom. The current does generally reach a peak, thought to occur once all the easily accessible silver has been converted, after which the solution must penetrate deeper into the Ag/AgCl structure to reach bare Ag to chlorinate resulting in a lower current. If the
Chlorination is continued, the current eventually drops back to zero once there is no further accessible silver left to chlorinate. The high level of variation in the current responses may indicate different AgCl structures formed.

Figure 4.12: SEM images (planar view) of surface of AgCl electrochemically chlorinated with 4.4 μC (left) and chemically chlorinated (right).

Comparing the SEM images in Figure 4.12 for an electrode which has been electrochemically chlorinated with 4.4 μC of charge (66% conversion) and one which has been chemically chlorinated shows that the generated structures are quite different. The AgCl produced from electrochemical chlorination is much less porous and consists of quite evenly sized and densely packed grains whereas the chemical chlorination resulted in a very porous structure. As discussed in section 4.3.1, the current passed during chlorination can affect the AgCl grain structure. The current passed during electrochemical chlorination can be seen in Figure 4.11 and is somewhat variable between electrodes indicating the resulting AgCl structure will also be slightly different. The current density for the chemically chlorinated electrode was not measured, but the porous structure would indicate a higher current density. This decreased porosity for the electrochemically chlorinated electrodes could lead to improved results.

As discussed in section 4.3.1, an optimum chlorination value below 50% of the silver layer thickness has been suggested [23]. It has been assumed that the maximum chlorination charge results in total chlorination of the electrode and a lower value of charge would chlorinate the electrode to a lesser extent. Therefore, SEM images were taken of the electrodes at different levels of chlorination charge to identify the effect on the amount of silver converted.
Chapter 4. Microfabricated On-Chip Reference Electrodes

Figure 4.13: SEM images (planar view, tilted at 54.0°) of 60 μm electrodes electrochemically chlorinated with 1 μC (15%), 3 μC (45%), and 6 μC (90%) of charge.

SEM images of the surface of three 60 μm diameter electrodes can be seen in Figure 4.13, these electrodes were chlorinated with 1, 3 and 6 μC of charge (calculated as 15%, 45% and 90% chlorination respectively). The SEM images
appear to match with the assumptions about electrode chlorination percentage. At the lowest charge the AgCl grains are spread out in small clusters across the surface of the electrode and do not form a continuous layer. At 3 μC of charge, the grains now cover the entire surface of the electrode. Finally, at the highest charge (close to full conversion) the AgCl grains have formed large clumps and it appears as if sections have already fallen off the electrode surface since areas have become uncovered.

FIB milling was used to remove a small area of the electrode to allow cross-sections at the centre of the electrode to be imaged and these can be seen in Figure 4.14. In all images, the side profile of the AgCl looks porous and "stringy". This is due to the FIB decomposing the structure of the AgCl on exposure as it cut through the adjacent material. This can make it easier to differentiate between Ag and AgCl in the cross-section images since the AgCl is visibly affected. The cross-section images show similar results to the SEM images of the electrode surface. At the highest chlorination charge (90%), it is clear that the chlorination has penetrated through the silver down to the platinum layer, consistent with a near 100% conversion of Ag to AgCl. The AgCl layer is uneven with some extremely large structures and other smaller pieces of AgCl. The AgCl has not adhered well to the underlying Pt, it is highly porous with a lot of gaps at the boundary layer. This poor adhesion may lead to decreased lifetimes at higher chlorination charges. In comparison, at the lowest chlorination charge (15%) only some of the Ag has been chlorinated, the AgCl has formed in patches across the surface and most of the cross-section is still silver.

Finally, in the cross section of the electrode chlorinated with 3 μC (45%) the AgCl appears to have penetrated partially through the silver layer, with a visible layer of silver remaining. The chlorination is still uneven in places, with some areas of AgCl penetrating further into the silver than others. The boundary layers appear better connected than that seen at the higher chlorination charge. Overall, it seems that the AgCl covers the entire electrode surface and there is still a layer of Ag remaining between the Pt substrate and the AgCl. This is the expected structure for a functional reference electrode, and therefore the electrode which should have the optimum performance.
Figure 4.14: SEM images of cross sections milled using FIB, images are tilted at 54.0°. Note the small white dots are contamination from the SEM chamber. Images show electrodes chlorinated electrochemically to charge cut-off values of 1 µC (15%), 3 µC (45%), and 6 µC (90%).
Chapter 4. Microfabricated On-Chip Reference Electrodes

4.4. Reference Electrode Performance

4.4.1. Electrode Testing Procedure

Electrodes were tested in 50 ml of 0.1 M KCl in DI water. This volume is large enough compared to the total amount of silver chloride on the electrodes such that the solution would not become saturated by dissolution of the electrode during testing. The setup for the testing procedure used the standard electrochemical cell described in Chapter 3 with the fabricated reference electrode connected as the working electrode and a standard commercial Ag/AgCl (3M KCl) reference electrode connected as the reference electrode. The open circuit potential (OCP) was measured to evaluate the lifetime, using chronopotentiometry with the current set to 0 A and the cell turned off to ensure no current was passed.

Typical lifetime graphs showing OCP over time for a chemically and electrochemically chlorinated electrode can be seen in Figure 4.15. The graphs show a short settling time of less than 10 minutes, then a very stable reference electrode potential for a significant time period followed by a sharp change indicating electrode failure. The failure mechanism was variable between all the electrodes. The two types of failure response were seen on both chemically and electrochemically chlorinated electrodes and are not indicative of a particular chlorination procedure. This indicates that both types of chlorination methods produce electrodes which are suitable for use as reference electrodes. The change to a lower potential that occurs in some cases appears to be relatively stable, however it is likely to occur once the environment local to the electrode no longer contains sufficient AgCl to fix the potential reliably (this is consistent with the Nernst equation since a decrease in the concentration of oxidised species would lead to a decrease in potential). This can be matched to a Pourbaix diagram, such as in [28]. The cause of the high potential after failure is currently unclear. However, in both cases it is not guaranteed that the reaction setting the potential will remain stable therefore both are undesirable for a reference electrode.
Figure 4.15: Example lifetime graphs showing OCP over time for chemically chlorinated (top) and electrochemically chlorinated (bottom) electrode. Inset with the first hour magnified.

An issue with some electrodes never reaching a stable potential was identified during electrode testing. The electrodes had all been identically processed as they were fabricated on the same wafer, and the location of the chip on the wafer did not seem to be correlated to whether or not it reached a stable potential during testing. In the case of electrochemical chlorination, the OCP of
the electrodes could be measured prior to chlorination and a potential below approximately -0.1 V or above 0.1 V was an indication that the electrode would either not chlorinate successfully or never reach a stable potential during testing. It was found that in some cases the electrode could function correctly if it was disconnected from the electrochemical cell and reconnected, or cycled into hydrogen evolution to clean the electrode surface. This was similar to the standard electrode cleaning protocol described in Chapter 3, but oxygen evolution could not be used without changing the electrolyte because the higher potential would chlorinate the electrode. This cleaning procedure sometimes resulted in electrodes returning to an OCP within the -0.1 V to 0.1 V range in which case they could then be chlorinated successfully. This indicates there may have been a contamination problem from the fabrication process as well as a connectivity problem to the electrode bond pad. These issues would be worth investigating further in future to increase the device yield.

Example lifetime graphs such as those shown in Figure 4.15 were used to determine appropriate conditions for data analysis. A stable potential was observed to have been reached after 10 – 15 minutes of starting the measurement and the average potential was taken over the subsequent 10 minutes. Electrodes were often stable immediately (in most cases there was little or no settling time since all electrodes were submersed in the measurement solution for a few minutes before the measurement was started), however this time was kept consistent for data analysis. Electrode failure was defined as the point at which the potential of the electrode deviated more than 10 mV from the initial stable reference potential, ignoring noise spikes if they occur. The lifetime data was obtained using a methodology written and implemented using Matlab, and the results were checked manually against the plotted data to confirm no errors had occurred in the processing.

4.4.2. Comparison of Chlorination Methods

One of the benefits of using the electrochemical chlorination method was that different percentages of electrode chlorination could be tested by using a selection of charge cut-off points during chlorination. The depth of AgCl formed should remain constant, however as shown earlier in Figure 4.11 the chlorination current did vary significantly between electrodes (close to an order of magnitude in some cases) so this may still lead to variation in the AgCl structure even at the
same chlorination percentage. A graph of different chlorination charges and the resulting electrode lifetimes can be seen in Figure 4.16. The different markers represent two different fabrication batches of electrodes. There was no clear difference found between electrodes from different batches indicating the fabrication process was robust. There was still quite a high level of lifetime variation throughout the chlorination charges, however it seemed likely that there may be a peak in lifetime for both electrode sizes so additional electrodes were measured at these points.

Figure 4.16: Graphs of lifetime for different values of charge used to electrochemically chlorinate electrodes of 40 µm (top) and 60 µm (bottom) diameter. Each point is an individual electrode that was tested. Different colours/markers represent two different batches of wafers. Dotted line represents the target lifetime of 24 hours.
The peak average lifetimes occurred at around 34% chlorination (1 μC) for the 40 μm electrodes and 45% chlorination (3 μC) for the 60 μm electrodes. From section 4.3.3 these percentages are estimates from the maximum charge needed to convert all the silver, which is 2.95 μC and 6.64 μC respectively. However, the values of charge used in the experiments were based on choosing a spread of values across the range, but the number of repeats at each charge value were too low to generate sufficient data to obtain a precise optimum chlorination charge. The results shown here should be taken as an indication of optimum lifetime, and appear consistent with the literature that chlorination at a point around 50% or below produces a more reproducible electrode. This is also consistent with the SEM images in section 4.3.3, which suggested 3 μC charge on the 60 μm diameter electrodes would result in an optimum lifetime.

The average lifetime of the 60 μm electrodes with 3 μC (45%) chlorination was 25 ± 7 hours. The average lifetime is close to the target lifetime of 24 hours, however this means only around half of the electrodes were suitable for the application. The average lifetime of the 40 μm diameter electrodes with 1 μC (34%) chlorination was 16 ± 6 hours, which is less than the target and makes the 60 μm electrodes preferable for this application. It was thought that the variability in lifetime may have been due to the variability in the chlorination current. The experiment was not performed as part of this work, but it may be worth investigating if setting the current instead of the potential during chlorination could decrease the variability in the lifetime response.

The lifetime results from the chemically chlorinated electrodes of both sizes varied from 0.12 hours (less than 8 minutes) to over 40 hours. The sample number was small and the variation is so high that it is impossible to conclude anything meaningful other than that the electrode performance was not reproducible. The SEM images in section 4.3.2 showed a highly porous AgCl layer and little remaining silver, with detachment of sections of silver chloride material from the electrode being more likely to occur for deeper and more porous silver chloride. These experimental results are consistent with the highly variable response expected from an electrode with this structure.

A slightly lower number of electrodes was measured compared to the electrochemical chlorination data and the lifetime results of both chlorination
methods can be seen in Figure 4.17. The range in lifetimes is still quite large for the electrochemically chlorinated electrodes, particularly for the 40 μm electrodes. However, the minimum value could be considered an outlier since the other 12 data points are considerably higher. The overall variability in lifetime of the electrochemically chlorinated electrodes is much lower for both electrode sizes. The result from t-testing showed the difference between the chemically and electrochemically chlorinated data sets was not significant (P > 0.05), however the sample size was small so this may change if more electrodes could be tested. Ultimately, the significantly higher variation in lifetime of the chemically chlorinated electrodes meant they were less preferable as reference electrodes.

![Figure 4.17](image)

*Figure 4.17: Variation in lifetime for chemically (left) and electrochemically (right) chlorinated to electrodes of 40 μm and 60 μm diameter. Electrochemically chlorinated electrodes were chlorinated with 1 μC (34%) and 3 μC (45%) respectively.*

The potentials recorded from the chemically and electrochemically chlorinated electrodes are shown in Figure 4.18. Using the Nernst equation and correcting for activity, the expected potential at 0.1 M [Cl\(^-\)] is 76.6 mV at 293.15 K. The variation in potential is quite significant for the chemically chlorinated electrodes at 56 ± 8 mV for the 40 μm electrodes (n = 8) and
62 ± 5 mV for the 60 μm electrodes (n = 6). This is over 15 mV variation for the smaller electrodes, which is considerable for electrodes fabricated and tested in the same way. Additionally, neither of the potentials are within error of the Nernst equation, which indicates that mixed potentials may have formed due to the high porosity exposing the underlying Pt layer to the solution.

The average potential for the electrochemically chlorinated electrodes was 72 ± 4 for the 40 μm electrodes (n = 24) and 73 ± 4 for the 60 μm electrodes (n = 22). Taking the optimum chlorination charges leads to potentials of 74 ± 3 mV and 74 ± 3 mV for the 40 (n = 13) and 60 μm (n = 8) electrodes respectively. The standard deviation on these values has decreased slightly and both potentials are within error of the expected value from the Nernst equation, unlike the chemically chlorinated electrodes. The standard deviation on the chemically chlorinated electrodes was also much higher indicating they were less reproducible.

Figure 4.18: Variation in potential for electrodes that were chemically chlorinated (left), electrochemically chlorinated (centre), and only those electrochemically chlorinated with 3 μC (45%) charge (right).
The results from both chlorination methods are summarised in TABLE 4.1. Overall, electrochemical chlorination produced electrodes with less variability and therefore a higher reproducibility than chemical chlorination. These electrodes generated a potential consistent with the Nernst equation. A chlorination charge of around 3 μC should be chosen for the 60 μm electrodes as it resulted in an average lifetime of greater than 24 hours. However, since this is an average some of the electrodes do not meet the 24 hour target lifetime and it would therefore be desirable to further increase the lifetime and/or reproducibility.

**TABLE 4.1**

*Summary of results for chemically chlorinated electrodes and electrochemically chlorinated electrodes.*

<table>
<thead>
<tr>
<th>Diameter /μm (Charge)</th>
<th>Number of electrodes</th>
<th>Potential /mV</th>
<th>Lifetime mean /hours</th>
<th>Lifetime range /hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>8</td>
<td>56 ± 8</td>
<td>14 ± 12</td>
<td>0.1 – 33</td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>62 ± 5</td>
<td>17 ± 13</td>
<td>0.3 – 40</td>
</tr>
<tr>
<td>40 (1 μC)</td>
<td>13</td>
<td>74 ± 3</td>
<td>16 ± 6</td>
<td>0.9 – 24</td>
</tr>
<tr>
<td>60 (3 μC)</td>
<td>8</td>
<td>74 ± 3</td>
<td>25 ± 7</td>
<td>15 – 33</td>
</tr>
</tbody>
</table>

4.4.3. Electrode Failure

Understanding the electrode failure mechanism is important, since this can allow methods to increase the lifetime to be identified. The most likely failure mechanisms are dissolution of AgCl complexes and mechanical delamination of sections of AgCl from the electrode into the solution. The same advantages of microelectrodes as working electrodes, can also be disadvantages as reference electrodes. The enhanced radial diffusion profile leads to more efficient diffusion of AgCl complexes from the area surrounding the electrode surface and into the bulk solution. Dissolution of material occurs over time and should lead to a fairly reproducible electrode lifetime as this process can be expected to be reasonably consistent between electrodes. In contrast delamination is more difficult to predict and is likely to lead to a more variable lifetime.

It is likely that a mixed failure mechanism will occur. As discussed in section 4.3.1 the chlorination process can affect the lifetime of the electrodes; higher porosity and chlorination depth can both lead to decreased lifetimes. In these cases the predominant failure mechanism is likely to be delamination. This is
supported by the results in section 4.4.2 where the electrodes consistent with a higher chlorination depth (chlorinated electrochemically with higher charge or chemically chlorinated) showed a higher variability in lifetime. The electrodes with a lower chlorination depth (chlorinated electrochemically with lower charge) are more likely to fail dominated by dissolution. This section will discuss the failure mechanism of the electrodes electrochemically chlorinated with 3 μC charge as these were shown to give the optimum results in section 4.4.2. The failure mechanism of these electrodes is more likely to be dominated by dissolution than delamination and this will be further investigated both theoretically and experimentally below.

The effect of activity has been neglected in the calculations in this section, which allows them to be simplified to provide estimates. The time for all the AgCl to dissolve from the electrode can be calculated as the theoretical lifetime for the fabricated electrodes. The solubility products can be used to give the concentrations of the dissolved species once equilibrium is reached in the solution. Using this as a concentration source at the electrode surface and assuming the species will then diffuse into the bulk solution, the current (which is proportional to the flux) can be calculated using the Saito microdisc equation (3.10). This current can be converted into a dissolution time (lifetime) by using the charge passed to electrochemically chlorinate the electrode.

From the speciation diagram in [29] shown in Figure 4.19, AgCl$_2^-$ will be the dominant species dissolved in a 0.1 M [Cl$^-$] solution. A value of 3 μM was taken from [30] as the concentration of AgCl$_2^-$ at the electrode surface in 0.1 M KCl. For 60 μm diameter electrodes this gives a limiting current of 0.035 nA. For a chlorination charge of 3 μC an estimated lifetime of 23.99 hours was calculated, which is the desired minimum reference electrode lifetime. Although it is only an estimate, this value is within error of the experimentally measured lifetime in section 4.4.2, which indicates the results are consistent with a dissolution failure mechanism.
If the measurement solution were saturated with AgCl and its complexes, this should prevent further AgCl from dissolving from the electrode. This experiment provides a method of confirming if dissolution was the failure mechanism, which would then allow strategies to be identified to mitigate the dissolution process. AgCl was added to the testing solution to create a solution of 0.1 M KCl saturated with AgCl species. A silver electrode was electrochemically chlorinated using a charge of 3 μC and the electrode lifetime was then evaluated using the standard testing procedure with this saturated measurement solution. This can be seen in Figure 4.20, and the electrode lasted approximately 180 hours before failure. This is significantly longer than the lifetime that was estimated above and measured experimentally in section 4.4.2, and therefore supports the theory that the dominant failure mechanism is dissolution.
In summary, the dominant failure mechanism of the electrodes electrochemically chlorinated with 3 μC charge appears to be dissolution of the Ag/AgCl layer into the bulk solution. The species dissolving into the solution is predominantly negatively charged AgCl$_2^-$, the additional dissolved species consist of slightly more than 10% AgCl$_3^{2-}$ and slightly less than 10% AgCl [29]. The majority of these dissolving species are negatively charged, which provides opportunity for reducing or preventing their diffusion with Nafion, which is a negatively charged membrane.

4.4.4. Nafion Coated Electrodes

The predominant failure mechanism of the electrochemically chlorinated electrodes was dissolution of AgCl. Since Nafion is a negatively charged membrane, it should mitigate the dissolution of the negatively charged AgCl complexes, predominantly AgCl$_2^-$ at 0.1 M [Cl$^-$]. It may also help to prevent some delamination of AgCl by acting as a physical barrier layer, but it will be primarily used here for its charge screening properties.
The 60 μm electrodes which were chlorinated to 3 μC gave the highest lifetimes as well as the most reproducible results, therefore these were used for the Nafion lifetime experiment. Nafion was drop-cast using the method described in section 4.2.3 and the electrodes were tested as before. Three Nafion coated reference electrodes were tested, and their lifetimes were found to be 201 hours, 66 hours and 180 hours. Therefore the lowest lifetime recorded with Nafion was still almost double the highest recorded lifetime without Nafion, and well above the target of 24 hours. The potential was 77.6 ± 0.4 mV, which is consistent with the Nernst equation (76.6 mV).

Due to the small number of data points, it is not possible to draw meaningful conclusions about the method of failure in terms of failure of the Nafion membrane (section 4.2.3) or dissolution of the Ag/AgCl material. The electrodes were not removed from measurement solution immediately after they had failed, this meant identifying membrane failure optically was not possible. In the adhesion testing (section 4.2.3), more than 90% of the drop cast membranes survived at least 2 weeks making membrane failure less likely over these timescales (201 hours is 8.4 days). It is desirable to repeat these experiments with a greater number of electrodes to be able to draw more meaningful conclusions. However, it does appear that the Nafion coating has led to a significant improvement in the electrode lifetime.

4.5. Performance as a Reference Electrode

A fabricated reference electrode coated with Nafion was used as the reference electrode to run CVs with a platinum nanoband working electrode in 1X PBS. This was compared to the same experiment using a commercial Ag/AgCl reference electrode, and the results are shown in Figure 4.21. The CV was run for 30 cycles to ensure the response remained stable during a longer measurement (only one scan is shown in Figure 4.21 since a mild cleaning affect is seen in subsequent scans, as discussed in Section 3.7). Investigating the onset of the oxygen reduction reaction at around −0.1 V, there is a separation of around 60 mV between responses using the different reference electrodes. PBS 1X has a higher chloride concentration of 154 mM so the offset is slightly different from that measured in 0.1 M KCl. The expected value in PBS 1X compared to 3.5 M KCl, adjusting for activity, is 65.6 mV so the separation between the CVs is consistent with this offset value. Additionally, the peak current from the oxygen reduction
reaction is within 1% between the two measurements, which was as expected since the current at the WE should be unaffected. Overall, this shows that the on-chip reference electrode can be used in place of a commercial Ag/AgCl reference electrode for electrochemical measurements, as long as the offset from the commercial electrode is determined and considered during measurements to ensure the appropriate potential is set/measured.

![Figure 4.21: CVs in PBS 1X for a Pt nanoband working electrode using a fabricated on-chip reference electrode (blue) and commercial Ag/AgCl 3M reference (red). Scans were recorded at 100 mVs⁻¹.](image)

4.6. Effect of Chloride Concentration

In all experiments reported in this chapter the electrodes were tested in 0.1 M KCl, however the relevant concentration ranges of chloride in sweat are around 0.02 – 0.05 M, and average 0.03 M on the forearm [5]. While this is a lower concentration value than that used for testing, the solubility of AgCl and its complexes increase with higher chloride concentrations [31]. This occurs because the percentage of AgCl species changes from around 80% AgCl₂⁻ at 0.1 M [Cl⁻] to around 50% at 0.01 M, with the remaining 50% consisting mostly of AgCl [29].
AgCl is highly insoluble so this could increase the lifetime of the reference electrodes assuming a predominant dissolution failure mechanism. However, it may reduce the effectiveness of the Nafion screening membrane since a lower proportion of the species would be negatively charged. Unfortunately, this experiment was not performed due to time constraints, but it could provide confirmation that the lifetime should not be significantly affected. Additional parameters that should be considered in future are pH (4 – 7.4 [32]) and temperature as variations in these will affect the solubility of the silver chloride or the performance characteristics of the on-chip reference electrode.

In addition to the lifetime, the electrode potential also changes with chloride concentration, where this change in potential is defined by the Nernst equation (3.1). For the Ag/AgCl reference electrode:

\[
E = E^0 - \frac{2.303RT}{nF}\log_{10}(a_{Cl^-})
\]  

(4.2)

with

\[a_{Cl^-} = \gamma_{Cl^-}[Cl^-]\]

where:

- \(E\) is potential,
- \(E^0\) is the standard potential,
- \(R\) is the universal gas constant,
- \(T\) is absolute temperature,
- \(n\) is the number of electrons transferred,
- \(F\) is the Faraday constant,
- \(a_{Cl^-}\) is the chloride activity, and
- \(\gamma_{Cl^-}\) is the activity coefficient.

The activity of pure metals and solids are equal to 1, so the activity coefficients of AgCl and Ag have been set to 1. In this section the measured OCP is considered to be equivalent to \(E\) from equation (4.2).

The OCP was measured over a range of chloride concentrations and compared to the Nernst equation. This was repeated for electrodes coated in
Nafion. Since Nafion is a negatively charged permselective membrane a Donnan potential may also be generated that could affect this relationship [33]. OCP was recorded at each chloride concentration for three difference fabricated reference electrodes. The OCP was taken after 5 – 10 minutes of stabilisation time and the results from the three electrodes were averaged to give the final value.

![Graph showing OCP against log to base 10 of [Cl\(^-\)].](image)

**Figure 4.22:** Graph showing OCP against log to base 10 of [Cl\(^-\)]. (blue cross) NaCl only (slope = \(-49.7 \pm 0.9\), intercept = \(40.9 \pm 1.2\), \(R^2 = 0.9997\)); and (red circle) NaCl with NaNO\(_3\) ionic strength compensation (slope = \(-59.2 \pm 2.2\), intercept = \(29.0 \pm 2.4\), \(R^2 = 0.9959\)).

The relationship between potential and chloride concentration can be calculated from the Nernst equation as \(-58.2\) mV/dec. The measurements at varying concentrations of NaCl, shown in Figure 4.22, give a slope of \(-49.7 \pm 0.9\) mV/dec, which is close to \(10\) mV/dec different from that calculated by the Nernst equation. In reality, it is not just the concentration of Cl\(^-\) added to the solution which sets the potential, but the activity. Therefore, this experiment was repeated using additional NaNO\(_3\) to maintain a constant ionic strength of \(240\) mM, matching the highest concentration of NaCl measured. The results of this
are also shown in Figure 4.22 and this resulted in a slope of $-59.2 \pm 2.2$ mV/dec which is consistent with the value calculated from the Nernst equation. This supports an activity effect as the cause of the differences in slope in the experimental data.

The Debye–Hückel (limiting) law applies at low concentrations and can be used to correct for activity effects. The Debye–Hückel equation (assuming the solvent is water at 298 K) is:

$$\log_{10} \gamma_{\pm} = -0.509 \frac{|z^+_{-}|}{\sqrt{I/I^0}}$$

where:

$\gamma_{\pm}$ is the mean ionic activity coefficient,

$z_+$ and $z_-$ are the cationic and anionic charge numbers respectively,

$I$ is the ionic strength, and

$I^0$ is defined as 1 M.

Using (4.2) with the Debye–Hückel limiting law, $\frac{2.303RT}{nF} \log_{10} \gamma_{\pm}$ can be subtracted from the measured OCP to correct for activity effects. The resulting data is show in Figure 4.23 and is still linear, which shows the limiting law can be used within this concentration range. The slope is $-58.6 \pm 0.9$ mV/dec, which is consistent with both the Nernst equation and the experimental data with ionic strength compensation. This confirms the differences seen in the original data can be explained by an activity effect.
Figure 4.23: Graph showing OCP against log to base 10 of $[\text{Cl}^-]$ on uncoated electrodes. OCP has been adjusted for activity using the Debye-Hückel (limiting) law (slope = $-58.6 \pm 0.9$, intercept = $21.9 \pm 1.2$, $R^2 = 0.9996$).

The experiment was then repeated using Nafion coated electrodes and ionic strength compensation (Figure 4.24). The slope from this experimental data was $-58.8 \pm 0.9$ mV/dec. This is again consistent with the Nernst equation and the previous experimental results for electrodes without Nafion which suggests there is no presence of a Donnan potential in these concentration ranges. It is possible that at very low chloride concentrations a Donnan potential may be measurable, but since these concentrations are not relevant in sweat this is not important for this work.

The intercept given by these graphs is offset from zero due to the potentials being recorded against a commercial Ag/AgCl electrode with a 3 M filling solution, rather than 1 M as defined in (4.3). In this case, the expected offset calculated by the Nernst equation would be 27.8 mV. The experimental data with NaNO$_3$ used for background ionic strength compensation (Figure 4.22) is consistent with this value for the uncoated electrode at $29.0 \pm 2.4$ mV. The offset from the Nafion coated electrode (Figure 4.24) is slightly below this value at $26.0 \pm 1.1$ mV,
however the difference is not significant and likely can be accounted for by other external factors such as small (mV) changes in the commercial electrode junction potential.

Figure 4.24: Graph showing OCP against log to base 10 of [Cl⁻] with background ionic strength compensation on Nafion coated electrodes (slope = −58.8 ± 0.9, intercept = 26.0 ± 1.1, R² = 0.9998).

The concentration of chloride in sweat varies by 30 mM (20 – 50 mM) and the response of the electrodes varies with chloride concentration following the Nernst equation, this leads to a variation in potential of 23.2 mV across this range. While there is some variation across the body in sweat chloride concentration, it is unclear how the concentration may vary over the target 24 hour measurement time. It is likely the values will be relatively stable over time since the healthy range of sweat chloride concentrations is quite narrow and is known not to overlap with the values used in cystic fibrosis testing [34].

There are alternative membrane coatings, such as polyvinyl butyral or hydrogel matrices, that can be used to maintain the chloride concentration at the
electrode [35] [36]. This means the potential would not vary with the chloride concentration of the external test solution, but could add complexity to the design. The potential at the reference electrode will affect the potential set at the working electrode in amperometric measurements, which is the type of measurement used for glucose sensing in this work. It was decided that the maximum potential variation across the relevant chloride concentration range was acceptable for amperometric glucose sensing without implementing any additional measures, due to the results and discussion regarding the choice of potential for glucose measurements in Chapter 5. Minimal changes in sensitivity of the glucose measurement were found in section 5.3.3. when the measurement potential was changed by 250 mV, therefore a variation of 23.2 mV at the reference electrode is acceptable.

4.7. Summary

In this chapter a fabrication method for a microscale on-chip Ag/AgCl reference electrode was developed. Electrochemical and chemical chlorination methods were compared by analysing the performance of the resulting electrodes. The electrode potential was stable over the duration of the electrodes lifetimes making them suitable for use as reference electrodes. The reference electrode potential was not consistent with the Nernst equation on chemically chlorinated electrodes indicating an issue with mixed potentials forming, however it was consistent on electrochemically chlorinated electrodes.

The effect of different chlorination percentages could be compared on the electrochemically chlorinated electrodes and it was found that a 45% chlorination of the silver layer (3 μC charge) on the 60 μm diameter electrodes was optimal. The higher level of control over the chlorination process provided by the electrochemical chlorination method led to less variability in the resulting reference electrodes. The lifetime at the optimum chlorination process was 25 ± 7 hours; the average lifetime was greater than the target of 24 hours however the standard deviation was high enough that it did not reliably meet the target. As dissolution of AgCl$_2$ was found to be the most likely failure mechanism on these electrodes, a Nafion membrane was used to mitigate dissolution of the electrode material. This increased the lifetime of the electrodes to 201 hours, 66 hours and 180 hours. The lifetime with the Nafion coating was reliably longer.
than the target lifetime, making these electrodes suitable for the target application.

Finally, the response of the Nafion coated and uncoated electrodes was compared to the Nernst equation, which gives −58.2 mV/dec of chloride concentration. It was found that both types of electrode followed the Nernst equation when the ionic strength of the solution was constant or activity correction was applied. Over the range of chloride in sweat, this would lead to a variation in potential of 23.2 mV, which was considered to be acceptable. It would be desirable to test a larger number of the electrodes, in particular the Nafion coated electrodes, to increase the accuracy of the reported results. However, the results presented are a reasonable proof-of-concept, which show the suitability of the fabricated reference electrodes for application to sweat sensing.

References


Chapter 4. Microfabricated On-Chip Reference Electrodes


Chapter 4. Microfabricated On-Chip Reference Electrodes


Chapter 5

Glucose Sensing with Nanoband Electrodes

5.1. Introduction
The working electrode is the electrode at which glucose is detected by the sensing system. This chapter addresses the development and characterisation of a nanoband working electrode for measuring glucose concentrations in sweat. Nanoscale electrodes have several advantages for glucose sensing, which were discussed in Chapter 2 and reiterated here. As well as their enhanced diffusion and signal to noise ratio (SNR), the very small active area means they deplete an analyte less than a macroelectrode during a measurement. This is beneficial in this work, because the very small volumes involved in sweat sensing means there is also a very small quantity of analyte available which could be depleted very quickly, changing the measured concentration. The concentration of glucose in sweat is lower than blood: 0.06 – 0.11 mM in sweat, compared to 4.9 – 6.9 mM in blood in healthy individuals [1]. Therefore, it is necessary to use an electrode with a sufficiently high sensitivity and low limit of detection (LOD) to measure in this range. This makes nanoelectrodes, such as nanoband electrodes, potential candidates for enhanced glucose sensing in sweat [2].

Nanoband electrodes have been used for glucose sensing previously [3], however those electrodes were functionalised using a thiol linker to attach the glucose oxidase enzyme (GOx) to the electrode surface. This makes the electrode response highly dependent on the GOx coverage. The electrodes used in this work were functionalised with a drop-cast, cross-linked film containing GOx, which should produce a more robust device. An indicative schematic of the operation of a nanoband electrode is shown in Figure 5.1. It includes a cross section of a single nanoband functionalised for glucose detection. However, as discussed in Chapter 3, the fabricated devices are designed to contain an array of these nanobands spaced evenly to produce an amplified, consistent current response.
Chapter 5. Glucose Sensing with Nanoband Electrodes

Figure 5.1: Diagram showing schematic of glucose sensing at a single nanoband cross-section (not to scale).

First the nanoband electrode array was designed and fabricated. The response of this electrode array was simulated and compared to the experimental results obtained using a simple redox reaction. The electrodes were then functionalised and characterised for glucose sensing. The reproducibility of these devices was determined, as well as the sensitivity/dependence of the functionalised electrode on the amount of (natural) oxygen mediator. Finally, the response to common interferents (ascorbic acid, uric acid and acetaminophen) was measured, both with and without a Nafion barrier membrane.

The overall sensing approach was for the glucose sensor to function under the scheme shown in Figure 5.2, assuming there is sufficient GOx to ensure the rate limiting step is not the enzyme kinetics. At time, \( t = 0 \) s, the entire solution will contain glucose at the spiked concentration. Immediately after this, GOx will begin to oxidise glucose and produce \( \text{H}_2\text{O}_2 \). Initially, the \( \text{H}_2\text{O}_2 \) will be localised to the enzyme, where it was produced. As time increases, \( \text{H}_2\text{O}_2 \) will diffuse further from the GOx into the bulk solution. At longer periods of time (e.g. by 30 s), \( \text{H}_2\text{O}_2 \) will have diffused throughout the enzyme film and formed a region of homogenous \( \text{H}_2\text{O}_2 \) concentration. Reproducible electrochemical measurements can then be performed within this homogenous region, assuming the measurement time is controlled to prevent the expansion of the diffusion profile outside of the region of
consistent $\text{H}_2\text{O}_2$ concentration. Within this region, the response of the device should be determined by the diffusional mass transport of $\text{H}_2\text{O}_2$ to the electrode surface.

Figure 5.2: Indicative schematic of the glucose sensing mechanism at the nanoband electrode. (a) Initially the enzyme film contains only glucose solution. (b) At short timescales GOx has started to oxidise glucose and produce $\text{H}_2\text{O}_2$, but it has not yet diffused far from the enzyme where it was generated. (c) At longer timescales (e.g. 30 s), the produced $\text{H}_2\text{O}_2$ has diffused throughout the enzyme film and can be considered a constant concentration within the area reached by the diffusion profile of the electrode during a measurement.
5.2. Fabrication
5.2.1. Design for Glucose Sensing

As discussed in Chapters 2 and 3, microfabrication is highly controllable meaning nanoband electrode arrays can be fabricated with tuneable dimensions such as the electrode thickness, the cavity size and the separation between cavities. Adapting the photomask allows a simple way for the electrodes to be tuned for different applications, such as the investigation of different analytes. The size and spacing of the nanoband cavities affects the electrode response due to the nature and degree of overlap of the diffusion profiles, which are established at each electrode. This is important because measurements taken when the electrodes are operating under steady-state conditions should give the most reproducible results. For nanoband electrode arrays this time will occur after the radial diffusion profile is established around the electrode cavity, but before the profile of adjacent electrodes begins to overlap. When the diffusion profile of adjacent electrodes overlaps the current will decrease. Further information and a diagram showing the diffusional profile growth at nanoband electrode arrays can be found in Chapter 3 and Figure 3.5.

Figure 5.3: Schematic showing one electrode from the mask design (produced in KLayout) for a single nanoband electrode chip. Dashed white box is not to scale and shows indicative area of magnification of individual nanoband cavities (shown in green).
The nanoband array dimensions (20 μm disc cavity diameter, 150 μm edge-to-edge spacing, 550 hexagonally spaced nanobands per electrode) were chosen to ensure the steady-state response occurred within a measurement time that can be easily evaluated using a simple potentiostat. The time for the diffusional profile to spread a specified distance can be calculated using (3.12). Taking H2O2 as the analyte of interest \( D = 1.43 \times 10^{-9} \text{ m}^2\text{s}^{-1} \) [4]) the ideal measurement region will occur between around 0.035 s and 1.97 s at this electrode geometry, this meets the measurement time requirements stated above. A diagram of one of the electrodes, taken from the photomask design, can be seen in Figure 5.3. These design requirements were determined to provide an easily accessible time region between the onset of a steady state current and the array overlap.

These mass transport considerations are valid for an analyte that is mixed evenly throughout the solution. Although this is the case for glucose, the H2O2 that is produced and measured at the electrode will not be spread evenly through the measurement solution. As shown in Figure 5.2, the H2O2 is generated locally near the electrode surface. Additionally, it will continue to be generated during the measurement meaning the effects of array overlap are likely to be less significant. The effect of localised H2O2 generation and the relationship between array overlap and nanoband spacing was not directly evaluated as part of this work. However, if the nanoband spacing can be decreased without array overlap occurring, it would increase the current response and therefore the sensitivity of the device.

5.2.2. Nanoband Electrode Fabrication Process

Electrodes were fabricated following the process provided by I. Schmüser [5], and runsheets for the nanoband electrode fabrication process can be found in Appendix B. Nanoband cavities can be defined using photolithography with tens of micron dimensions, and etching through the top insulating layer and through the metal. As shown in Figure 5.1 this will leave the nanoband electrode exposed with the nanoscale dimension defined by the thickness of the platinum layer, which is 50 nm thick in this work. Nanoband electrode arrays can be fabricated in this manner with high reproducibility of array elements since micro-scale lithography is a highly reproducible process. As stated in section 5.2.1, in this work a 20 μm diameter cavity was used with 150 μm edge-to-edge spacing of electrodes (hexagonally packed).
A diagram of the fabrication process for the nanoband electrodes is shown in Figure 5.4. A silicon wafer was insulated with 500 nm of thermally grown silicon dioxide. Platinum (50 nm) was deposited on the wafer on top of a titanium adhesion layer (10 nm) using electron-beam evaporation (Figure 5.4a), and wet etched with aqua regia to define the electrode outlines (Figure 5.4b). Platinum was chosen as the electrode material due to its catalytic ability to oxidise H$_2$O$_2$. It is also readily available in the cleanroom and has standard fabrication processes associated with it.

LPCVD silicon-rich nitride (SiRN) was then deposited on the platinum layer to form the top insulator (Figure 5.4c). A thickness of 300 nm was used as this was shown to provide a sufficient electrical and physical barrier layer [5]. This was then patterned and dry etched using RIE to expose the bond pads and dicing channels (Figure 5.4d). The cavities for the nanoband electrodes were defined using a 3 μm thick layer of photoresist. The SiRN was dry etched using RIE at a reduced power (75 W instead of the standard 150 W) to ensure a vertical sidewall profile. Following this, the Pt and Ti layers were etched using argon milling to expose the nanoband electrodes (Figure 5.4e). This vertical sidewall profile is vital since this ensures the electrode dimensions are reproducible and defined solely by the deposited metal thickness and cavity diameter. If the sidewall were not vertical

Figure 5.4: Cross-section showing key processing steps in fabrication of nanoband electrodes (not to scale). (a) Silicon wafer insulated with silicon oxide. 10 nm Ti and 50 nm Pt deposited. (b) Pt and Ti wet etched. (c) Pt layer insulated with silicon nitride. (d) Electrode bond pad exposed by etching through SiRN. (e) Nanoband cavities etched through SiRN and metal layers to expose Pt nanoband. Dotted line indicates location of cross-section in Figure 5.1. (f) Wafer diced and electrodes functionalised with enzyme layer for glucose sensing.
there would be a risk of a step at the metal layer, or a sloped metal etch both of which would change the exposed Pt thickness. Finally, the wafer was coated with protective photoresist and diced into individual chips. The photoresist layer was removed using acetone, IPA, and DI water, and microscope images of the completed electrodes can be seen in Figure 5.5. Finally, the electrodes were cleaned electrochemically before use; details of the electrochemical cleaning process can be found in Chapter 3.

Figure 5.5: Microscope images of a nanoband electrode chip with the top two images showing the nanoband array area and bond pad. Bottom images are magnifications of a section of the nanoband cavity array. Dotted boxes indicate approximate regions of magnification.

5.2.3. Functionalisation for Glucose Sensing

The nanoband electrodes were functionalised with a glucose oxidase layer (Figure 5.4f), which allows glucose to be quantified electrochemically. The glucose functionalisation recipe and procedure were provided by Nanoflex Ltd and results in a first generation glucose sensor. Solutions were prepared in either PBS 1X or DI water. The glucose functionalisation layer consists of glucose oxidase (GOx) from *Aspergillus Niger* with an activity of 224890 U g⁻¹ (1 U oxidises 1 μM glucose per second).
β-D-glucose per minute at pH 5.1 at 35°C), bovine serum albumin (BSA), glutaraldehyde (used for cross-linking the enzyme, BSA, and substrate), and lactitol.

Three solutions were prepared: GOx at 40 mg/ml in PBS 1X, BSA at 120 mg/ml and lactitol at 80 mg/ml in PBS 1X, and glutaraldehyde at 1.25% in DI water. The GOx solution, and the BSA and lactitol solution were combined together in a 1:1 ratio by volume. First, 0.9 μl of the glutaraldehyde solution was drop-cast onto the electrode, followed by an equal volume of the solution containing the remaining ingredients. The solutions were spread over the electrode surface using the pipette tip. The electrode was left to dry on the benchtop overnight before testing.

After some initial experiments were conducted, it was discovered that crystals were forming in the enzyme film (Figure 5.6b), which was affecting the results as a decreased current was measured from the electrodes with higher crystallisation in the films. The initial functionalisation recipe was investigated to identify any issues with the film composition. Since the crystallisation problem was visually identifiable (Figure 5.6), optical inspection was chosen as the method for comparing different process changes.

Figure 5.6: Example microscope images of (a) non-crystallised enzyme film, and (b) film with crystallisation.

The functionalisation solutions were tested using deionised water instead of PBS to ensure the crystallisation was not occurring due to the salt content. Lactitol is a type of sugar, and these have been shown to have a stabilising effect on proteins (such as BSA and GOx enzyme) [6]. Therefore, removing the lactitol from the system would likely have a negative effect. The microscope images in Figure
5.7 clearly show that the crystallisation problem was considerably worsened by removing the lactitol from the enzyme film, leaving a much denser and larger area of crystallisation in the film compared to Figure 5.6. Removing or changing the concentration of the other film components did not have a significant effect on the crystallisation, however the removal of the BSA did prevent the film from cross-linking sufficiently and it dissolved from the electrode when submerged in solution. This was not entirely unexpected since the role of the BSA is to help stabilise the enzyme, as well as help form a stable structure when cross-linked with the glutaraldehyde [7][8].

![Figure 5.7: Microscope images of enzyme film without lactitol at different magnifications, showing highest level of crystallisation of the tested functionalisation protocols.](image)

Since removing or modifying the ratios of the film components failed to solve the crystallisation problem, a different approach was needed. The initial functionalisation experiments were conducted in a chemistry lab with limited control over the ambient conditions such as humidity and temperature. This made the environment in the lab more reliant on external factors such as the temperature and humidity in the air outside. The humidity in Edinburgh is often high but also quite variable so this may be why there were variations in film crystallisation from experiments conducted at different times of year. The relative humidity can vary by more than 10% over a year and humidity changes can also occur over the course of an experiment (overnight).

Therefore, a humidity chamber consisting of a saturated sodium chloride solution was used to maintain a constant humidity level while the functionalisation layer was drying. Saturated sodium chloride at 25°C has a relative humidity of 75.29 ± 0.12% [9]. The relative humidity is also extremely stable with respective to temperature: between 15°C and 25°C the variation is only around 0.5%. This
provided a method to controllably set the humidity without relying on the ambient conditions. The use of the humidity chamber prevented the film from crystallising and increased the reproducibility of the functionalisation process.

5.3. Experimental Characterisation

The fabricated nanoband electrodes were characterised experimentally. First, the electrode operation was simulated to provide a theoretical comparison for experimental measurements conducted using ferrocenemethanol (FcMeOH) a standard redox agent. Once the behaviour of the electrode has been understood, the more complex glucose detection experiments could be undertaken.

Initially, the current response to additions of glucose was measured and compared to the theory and the response to FcMeOH. Following this, the measurement potential and time were adjusted to identify if there were optimal measurement parameters. The response of four functionalised electrodes were compared to investigate the reproducibility of the device. The glucose sensing results using these fabricated electrodes were also compared to relevant literature values from Chapter 2 for the application of sweat sensing.

5.3.1. Validation of Fabricated Electrodes

The nanoband electrode response was simulated in COMSOL and compared to the experimental response as a method of validation for the fabricated electrodes. The simulation used the model described in Chapter 3, using literature values of the diffusion coefficients of H₂O₂, glucose and FcMeOH. The diffusion coefficients (D) of H₂O₂ and glucose are 1.43 x 10⁻⁹ m²s⁻¹ and 0.7 x 10⁻⁹ m²s⁻¹ respectively in a 0.1 M sodium phosphate buffer at 25°C [4]. FcMeOH is a simple redox couple that can be used to validate the simulation results, its diffusion coefficient is 0.7 x 10⁻⁹ m²s⁻¹, which is the same as glucose [10].

The nanoband electrode was simulated with a bulk concentration of 100 μM with $D = 0.7 \times 10^{-9} \text{ m}^2\text{s}^{-1}$ (consistent with FcMeOH) both with and without adjacent electrodes to simulate the presence and absence of diffusional overlap. The results of this simulation are shown in Figure 5.8, from this it can be seen that the two simulations begin to deviate at around 4 s, this matches the calculations in section 5.2.1, which estimated overlap would occur at 4.02 s for this value of $D$. 
Chapter 5. Glucose Sensing with Nanoband Electrodes

Figure 5.8: COMSOL simulation results for arrays with (red dots) and without (blue crosses) array overlap. Simulated with a concentration of 100 μM and $D = 0.7 \times 10^{-9} \text{m}^2\text{s}^{-1}$ (consistent with FcMeOH). Inset is magnification between 0 and 8 s showing the divergence between the two simulations when array overlap begins to occur.

The current at a nanoband electrode can be calculated using the modified Saito equation (3.10). This equation requires the constant $B$ to be obtained for this electrode, which can be calculated from the simulation results. Taking $i_{\text{lim}}$ at 1 s for both values of $D$, gives 1.73 and 1.71 for $D = 0.7 \times 10^{-9} \text{m}^2\text{s}^{-1}$ and $D = 1.43 \times 10^{-9} \text{m}^2\text{s}^{-1}$ respectively. Taking into account that there is likely to be some uncertainty in the simulation results, these values are similar enough to be considered to be the same within experimental error, as expected. Additionally, in [11] a microsquare nanoband electrode was simulated and the constant $B$ was calculated as 0.956. This is a bit less than half the value calculated for the nanoband electrodes simulated in this chapter. The electrodes both have a 50 nm thick nanoband and 300 nm thick top insulator, however the nanoband cavities in this chapter were discs with a radius of 20 μm rather than square with an edge length of 10 μm. Considering these differences, the constant $B$ calculated here appears consistent with that reported in [11].
Chapter 5. Glucose Sensing with Nanoband Electrodes

Figure 5.9: CV of 100 μM ferrocenemethanol in PBS 1X at 100 mVs⁻¹ on a fabricated nanoband electrode. Electrical noise can be seen in this CV due to improper shielding during the measurement.

The simulated and experimental results were then compared to confirm the fabricated electrodes were behaving as predicted. The limiting current from the CV of 100 μM FcMeOH in PBS 1X (shown in Figure 5.9) is 64.8 nA taken at 0.4 V, which is consistent with the simulation value of 64.4 nA. This suggests the simulation is providing an accurate model of the electrode response and confirms that the electrode has been fabricated with the intended geometry and dimensions. The modified Saito equation (3.10) can be used with the value calculated for $B$ to compare the results from the glucose functionalised electrodes. Additionally, this means the COMSOL model could be used to simulate parameter tuning of the electrode geometry if future modifications were required.

5.3.2. Current Response to Glucose and Cleaning Procedure

To perform the experiments, the solution was stirred for 30 s using a magnetic stirrer, then left to settle for 30 s, followed by a 30 s CA measurement. Glucose was dissolved in PBS 1X at a concentration of 1 M to create a stock solution, which was refrigerated and left to mutarotate [12] overnight. CA
measurements were recorded at 0.5 V in PBS 1X spiked with glucose concentrations between 0 – 500 µM using functionalised nanoband electrodes. The concentrations used were 0, 10, 50, 100, 250, and 500 µM of glucose. The choice of potential and time point were further investigated in section 5.3.3. Other than in Figure 5.10, CA measurements were repeated 3 times at each glucose concentration using the same procedure stated above.

The data point at 30 s was used to plot the calibration curves shown in Figure 5.10. The initial current responses measured with the functionalised electrodes were in the single nA range, which was an order of magnitude lower than the current response measured in Figure 5.9 from FcMeOH. Although glucose detection is more complex due to the enzymatic reaction, this would not be expected to lead to such a large decrease in the current response.

The fabricated electrodes were cleaned electrochemically before functionalisation (as described in Chapter 3). However, it was thought that the reason for the current decrease may have been due to material from the functionalisation process coating the electrode surface and decreasing the active area. For this reason, a second cleaning procedure was added after functionalisation. The cleaning procedure used here was similar to the standard cleaning procedure, however the CVs were run to the positive potential limit leading to oxygen evolution and not the negative potential limit for hydrogen evolution.

Calibration curves were plotted before and after this second cleaning procedure to identify changes in the electrode response. It can be seen from Figure 5.10 that the initial currents were in the single nA range, whereas after the cleaning procedure was applied the currents have increased up to 100s nA at the higher concentrations measured. This has significantly increased the sensitivity of the glucose measurements by over ten times and led to a smaller error in the linear fit. Therefore, this post-functionalisation cleaning step was added to the electrode preparation procedure for all electrodes. It should also be noted that the linear range spans the concentration range of glucose in sweat of healthy individuals [1], this region is marked in grey in Figure 5.10b.
Figure 5.10: Glucose calibration curves in PBS 1X (a) before and (b) after the additional cleaning procedure was implemented (with (a) still shown on (b) in red). Line of best fit: (a) slope = 0.012 ± 0.001 nA μM⁻¹, $R^2 = 0.985$; (b) slope = 0.498 ± 0.008 nA μM⁻¹, $R^2 = 0.999$. Shaded grey region on (b) shows range of glucose concentrations in sweat for healthy individuals [1].

The chronoamperometry measurements used to plot the calibration curve in Figure 5.10b are shown in Figure 5.11. The current responses are stable and reach a limiting current after a short settling time, which is expected for a
nanoband electrode. The current response at the electrode should be a diffusion controlled process due to the high loading of enzyme on the chip and fast turnover rate of the enzyme. This was confirmed when the enzyme concentration in the film was doubled but the current response did not change significantly.

![Current vs. Time Graph](image)

**Figure 5.11:** CAs at 0.5 V for varying concentrations of glucose in PBS 1X. Each line is a CA measurement at a different glucose concentration (0, 10, 50, 100, 250, 500 mM) increasing in concentration in the direction of the arrow.

Since the process is under diffusion control, the limiting current should still be predictable using the modified Saito equation (3.11). Glucose will be converted into H$_2$O$_2$ within the enzyme film and diffuse to the electrode, therefore H$_2$O$_2$ was used as the analyte for the calculation of current. Using (3.11), with the $B$ value of 1.71 calculated previously and $n = 2$ for H$_2$O$_2$ oxidisation, gives an expected current of 259.5 nA. This agrees with the simulation result, which gave a current response of 259.2 nA at 1 s. The steady state current from 100 μM of glucose in PBS 1X at 1 s was 61.7 ± 0.7 nA from three repeated measurements. This is considerably lower than the calculated and simulated steady state current.

However, it has been shown previously that the current response from locally generated H$_2$O$_2$ can be around 60 times lower than the equivalent
concentration added to the bulk solution [3]. When \( \text{H}_2\text{O}_2 \) is generated locally, a large proportion of it will diffuse into the bulk solution where the \( \text{H}_2\text{O}_2 \) concentration is considerably lower. The currents recorded here were only 3.9 times lower than predicted from bulk \( \text{H}_2\text{O}_2 \) concentrations, which appears to be reasonable.

Additionally the reported \( \text{H}_2\text{O}_2 \) diffusion coefficient, which was used to calculate the current, was measured in a phosphate buffer solution at 25°C. However, the experiments were conducted at 21°C and diffusion occurred within a glutaraldehyde enzyme matrix, which will lead to smaller diffusion coefficient. 

Using the experimentally measured value for \( i_{\text{Lim}} \) \( (61.7 \pm 0.7 \text{ nA}) \), and a \( B \) value of 1.71 in (3.11), \( D \) can be calculated for the system as \( 0.340 \pm 0.004 \times 10^{-9} \text{ m}^2\text{s}^{-1} \). The diffusion coefficient for \( \text{H}_2\text{O}_2 \) has been reported in hydrogels cross-linked using glutaraldehyde as \( 0.27 \times 10^{-9} – 0.50 \times 10^{-9} \text{ m}^2\text{s}^{-1} \) [4]. The value for \( D \) calculated above is consistent with these literature values. This again supports the hypothesis that the measured current response at the functionalised electrodes is reasonable.

Next, the current response from four different electrodes with the same fabrication and functionalisation procedure were plotted as calibration curves in Figure 5.12(a). It can be seen from this spread of calibration curves in the graph and the summary from the line of best fit in TABLE 5.1, that the current responses show some variation. The calibration curves can be normalised using the current value at 500 \( \mu \text{M} \), as shown in Figure 5.12b. This leads to the lines of best fit falling on top of each other, removing the variation in the data. It is common to factory calibrate sensors, so this is a reasonable solution to account for the variability between devices.

\[ \text{TABLE 5.1} \]

Comparison of the linear fit at different electrodes from calibration curves in Figure 5.12(a).

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Sensitivity /nA ( \mu \text{M}^{-1} )</th>
<th>LOD /( \mu \text{M} )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (blue cross)</td>
<td>0.494 ± 0.007</td>
<td>2.22 ± 0.01</td>
<td>0.999</td>
</tr>
<tr>
<td>2 (red square)</td>
<td>0.506 ± 0.008</td>
<td>2.52 ± 0.02</td>
<td>0.999</td>
</tr>
<tr>
<td>3 (purple diamond)</td>
<td>0.639 ± 0.013</td>
<td>1.96 ± 0.02</td>
<td>0.998</td>
</tr>
<tr>
<td>4 (green circle)</td>
<td>0.574 ± 0.016</td>
<td>1.13 ± 0.03</td>
<td>0.997</td>
</tr>
</tbody>
</table>
Figure 5.12: (a) Calibration curves plotted from CAs at 0.5 V with data points taken at 1 s, from four fabricated electrodes with the same functionalisation procedure. Error bars show STD of three repeated measurements on the same electrode. (b) The same calibration curves normalised using 500 μM data point. Different line colours and marker symbols indicate different electrodes.
The LODs given in TABLE 5.1 are considerably lower than the 60 μM minimum in healthy individuals as well as the 10 μM minimum in people with diabetes. The sensitivities found in literature tend to vary from single to tens of nA/μM which is higher than the sensitivities measured here. However, the electrodes used here do have advantages in their simplicity of fabrication and compatibility with measuring in flow, which is discussed further in Chapter 6. Additionally, the enzyme functionalisation layer was not extensively optimised in this work. Further experiments could be performed to optimise the layer both in terms of sensitivity, reproducibility, and lifetime.

5.3.3. Measurement Optimisation

The calibration curves in section 5.3.2 showed that the fabricated electrodes can be used to measure at the relevant concentrations of glucose in sweat. In this section, the measurement protocol is discussed in greater detail to optimise the sensor performance and further characterise the response of the electrode.

![Calibration curve for glucose in PBS 1X plotted using CA measurements at 0.5 V with data points taken at 1 s. Concentration measurement range was from 0 to 1 mM. Error bars show STD of three repeated measurements on the same electrode.](image)
The calibration curves presented in section 5.3.2 were recorded up to a concentration of 500 μM and were linear within this range. The linear measurement range can be identified by plotting a calibration curve over a wider range of glucose concentrations between 0 and 1 mM in PBS 1X. The calibration curve was plotted using CA measurements at 0.5 V with data points taken at 1 s. From the calibration curve shown in Figure 5.13, it is clear that the current begins to reach a plateau at around 600 – 700 μM of glucose added.

The glucose oxidase enzyme requires dissolved oxygen in the solution to regenerate after oxidising glucose (2.1). This is a well-known drawback of first-generation glucose sensors [13] and was discussed in Chapter 2. Therefore, a concentration will be reached where the enzyme is unable to oxidise additional glucose and this will occur due to the limited concentration of oxygen rather than the kinetics of the enzyme. This leads to a plateau in the current recorded at the electrode despite the additional glucose added to the measurement solution. The concentration of dissolved oxygen in PBS at 25°C is around 1 mM, although it varies slightly with glucose concentration [14]. Additional oxygen will also be generated by the oxidation of H₂O₂ at the electrode during a measurement so this concentration is an estimate. At 1 mM of glucose added to the solution the recorded current has begun to saturate, as this is a similar value to the concentration of dissolved oxygen, it is reasonable to attribute the current plateau to the limited availability of oxygen at the enzyme.

As discussed in Chapter 2, the concentration range of glucose in sweat is 0.06 – 0.11 mM in healthy individuals and 0.01 – 1 mM in people with diabetes [1]. The linear region of the calibration curve extends over the concentration range of glucose in healthy individuals, it is only at the higher end of the concentration range of glucose in people with diabetes that the curve begins to saturate. The upper concentration limit in diabetics is considerably higher than the upper limit in healthy individuals so it was not thought to be necessary to be able to measure a linear relationship up to 1 mM at this stage of development. The aim of a wearable sweat sensor is to provide continuous monitoring, meaning a user would be notified once the upper healthy concentration limit was reached. This should allow appropriate action to be taken before the sensor response begins to saturate. In future, the linear region could be extended by coating the electrode
with a membrane, such as polyvinyl chloride, to inhibit the diffusion of glucose compared to oxygen [13].

Therefore, a concentration of 500 μM was chosen as an appropriate upper concentration limit for this work, which is over four times higher than the upper healthy concentration limit. There are other devices in the literature discussed in Chapter 2, which measured to a maximum of only 100 μM or 200 μM glucose. The upper concentration range used in this work is consistent with the literature and extends higher than the upper concentration in healthy individuals.

![CVs at 100 mVs⁻¹ in PBS 1X (blue) and PBS 1X with 1 mM glucose (red). Dotted lines indicate the potentials chosen for CA measurements.](image)

Figure 5.14: CVs at 100 mVs⁻¹ in PBS 1X (blue) and PBS 1X with 1 mM glucose (red). Dotted lines indicate the potentials chosen for CA measurements.

The choice of measurement potential is also important as it controls the electrochemical reaction taking place and using an appropriate potential can maximise the sensitivity of the device. The calibration curves shown previously were plotted using CAs measured at 0.5 V. CVs can be used to select the potential to apply during the CA measurements. Figure 5.14 shows a CV recorded in a solution containing 1 mM of glucose in PBS 1X and in a solution containing no glucose. The CV in the solution containing glucose shows an oxidation wave.
characteristic of the response of \( \text{H}_2\text{O}_2 \) on platinum beginning around 0.35 V. At negative potentials oxygen reduction occurs, the current measured from this reaction decreases when glucose is added to the solution as oxygen is consumed to regenerate the enzyme after oxidising glucose (2.1).

![Figure 5.15: Calibration curve of glucose plotted using CAs measured at 0.5 V (blue crosses) and 0.75 V (red squares), data points taken at 1 s. Error bars show STD of three repeated measurements on the same electrode.](image)

Generally, the potential corresponding to the peak/limiting current (or beyond) would be chosen to maximise the electrode response. Nanoelectrodes usually show a characteristic sigmoidal response and reach a limiting current, rather than the peaks that are typically seen for macroelectrodes. However, in a solution containing \( \text{H}_2\text{O}_2 \) passivation of the platinum electrode occurs as the potential is increased [15], which is the reason a peak current is seen in Figure 5.14 rather than a limiting current. Additionally, common interferents can be oxidised in the same potential window as \( \text{H}_2\text{O}_2 \) (these are discussed in greater detail in section 5.4.2). Therefore, selecting a lower potential can avoid electrode
passivation and minimise any unwanted additional currents measured from these interferents.

The measurement potentials were chosen as 0.5 V and 0.75 V from the CV as they are both within the oxidation window with 0.75 V close to the peak oxidation potential and 0.5 V closer to the onset of the oxidation wave. The calibration curves shown in Figure 5.15 were produced from the CAs recorded at these potentials. The measurements were taken using the same electrode and were recorded sequentially at each concentration point to ensure the conditions were as consistent as possible. The results are summarised in TABLE 5.2, which shows there is a relatively small difference in sensitivity between the calibration curves measured at 0.5 V and 0.75 V, additionally the LOD was slightly larger at 0.75 V. This is beneficial as it allows a comparable sensitivity to be achieved while keeping the measurement potential low.

**TABLE 5.2**

<table>
<thead>
<tr>
<th>Potential</th>
<th>Sensitivity /nA μM⁻¹</th>
<th>LOD /μM</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 V</td>
<td>0.294 ± 0.003</td>
<td>0.249 ± 0.011</td>
<td>0.99952</td>
</tr>
<tr>
<td>0.75 V</td>
<td>0.319 ± 0.001</td>
<td>0.525 ± 0.002</td>
<td>0.99999</td>
</tr>
</tbody>
</table>

Different time points can be selected from the CAs to plot the calibration curves. From Section 5.2.1 for a solution with H₂O₂ added throughout it was expected that the optimal results would be obtained before 1.97 s, corresponding to the region where the device is operating at a steady-state current prior to array overlap. However, the effect of overlap appears not to be significant since the data in Figure 5.16b shows the sensitivity increasing as the time is increased. LOD was not plotted, but it is inversely proportional to the sensitivity, therefore the LOD will reduce as the sensitivity increases. This implies the current is increasing over time, looking at the CA results in Figure 5.16a this does seem to be the case however the change in current is very small. Additionally, the difference in sensitivity is less than 10% between the lowest and highest sensitivities calculated. This is reasonable since H₂O₂ is being generated throughout the
duration of the measurement and this will disrupt the diffusional mass transport profiles normally seen in a homogenous solution.

Figure 5.16: (a) Calibration curves plotted at: 0.1s (orange plus), 1s (green circle), 5s (purple diamond), 10s (red square), and 30s (blue cross). Error bars show STD of three repeated measurements on the same electrode. (b) Sensitivities extracted from these calibration curves.
Chapter 5. Glucose Sensing with Nanoband Electrodes

The nanoband electrodes have a small active area and there is a high amount of glucose oxidase functionalised on the devices. This makes it likely that the enzyme is turning over glucose at a much faster rate than H$_2$O$_2$ can be measured, leading to an accumulation of H$_2$O$_2$. The measurement solution is stirred between repeated CA measurements at each concentration and these do not show any systematic increase in current. This supports the increase in sensitivity and current being due to accumulation of H$_2$O$_2$ and also confirms the stirring process is sufficient to redistribute the analytes throughout the solution, as expected.

Accumulating H$_2$O$_2$ inside the enzyme film is also likely to lead to faster degradation of the film over time. However, it is worth noting these experiments were conducted with a large (50 ml) and stationary measurement solution. The aim is to ultimately measure in flow since sweat is not stationary, and this should reduce the accumulation of H$_2$O$_2$ but it is unclear to what extent this will occur. The current increase occurred after 1 s and the reason for this is not well understood. Therefore, it was decided that the CA measurements would be recorded up to 30 s and the data point at 1 s would be used to produce the calibration curves. This could be re-evaluated when flow measurements are undertaken to identify any changes caused by flow.

Overall, the functionalised nanoband electrodes are promising for measuring glucose in sweat due to their ability to measure linearly over the relevant concentration range without encountering issues from oxygen dependence. The calculated LODs are lower than the minimum expected value in healthy people and people with diabetes, making them suitable for this application. Additionally, the functionalised electrodes only showed small changes in the current response when different time points and potentials were selected. This demonstrates the robustness of the devices to variations in these parameters.

5.4. Additional Considerations

There are additional parameters that should be considered when designing a glucose sensor. This includes parameters that affect the enzyme kinetics, such as temperature, and interfering species that can be measured in the same potential window, such as ascorbic acid, acetaminophen and uric acid. This is not an exhaustive list of parameters and a more extensive study could be undertaken.
in future. The effect of pH was not investigated as part of this work, but is also known to have an effect on the glucose oxidase enzyme. Additionally, it would be beneficial to analyse the lifetime of the glucose sensor by running longer term measurements and identifying the decrease in current over time. These experiments were not performed in this work due to time constraints but would be necessary to fully characterise the device. This section briefly discusses some selected key parameters and their effect on the glucose sensor.

5.4.1. Temperature

Temperature is known to affect the activity of the glucose oxidase enzyme. A calibration of current against temperature was performed for a constant concentration of 100 μM glucose in PBS 1X and is shown in Figure 5.17. The current was measured as before using CA at 0.5 V and the temperature was controlled with a water bath. There were issues with the temperature stability provided by the water bath, therefore the errors recorded on the resulting calibration graph are higher than would be expected and there are unreported errors in the x axis.

The reaction rate constant is related to temperature following the Arrhenius equation:

\[ k = A e^{-\frac{E_a}{RT}} \]  

(5.1)

where \( k \) is the rate constant, \( A \) is a constant for the chemical reaction, \( E_a \) is the activation energy, \( R \) is the universal gas constant, and \( T \) is the absolute temperature.

In the system detailed in this chapter, the current is related to the turnover rate of the enzyme and can be used in place of \( k \). The Arrhenius equation can then be used to extract a linear trendline by plotting the natural log of current against the inverse of temperature between 31°C and 40°C, which is shown in Figure 5.17. This relationship gives a variation of 14.3 nA between 21°C (room temperature, at which the experiments in this chapter were measured) and 31°C. This is a relatively large variation in skin temperature, but would have a noticeable effect on the results since this would represent a 21.3% change in current from 21°C (using the average of the results in Figure 5.12a at 100 μM glucose).
Chapter 5. Glucose Sensing with Nanoband Electrodes

Figure 5.17: Calibration plot of natural logarithm of current against inverse of temperature. Line of best fit: slope = $-29.6 \pm 0.9$, intercept = $-16.10 \pm 0.02$, $R^2 = 0.997$.

An integrated temperature sensor could be used to measure any changes in temperature and the known relationship between temperature and current response could then be used to compensate for these changes. Temperature sensors are generally simple to fabricate since they do not require the biological elements which add complexity to systems like glucose sensors. These types of sensors have been multiplexed with glucose sensors in the literature to provide temperature compensation [16][17][18].

5.4.2. Interfering Species

Ascorbic acid, acetaminophen, and uric acid are common interferents to first generation glucose sensors, which were discussed in Chapter 2. The concentrations of the interferents used in this work were 10 μM, 100 μM, and 50 μM respectively, as these are their relevant concentrations in sweat [19][20]. The concentrations of the interfering species are within the concentration range of glucose in sweat, which is problematic as it means they are likely to result in unwanted currents of similar orders of magnitude. Although acetaminophen is an important interferent for in-vivo measurements, it is not clear how important it is
as an interferent in sweat [21]. However, it has been included in the measurements for comparison as it is a potential interferent and it provides an example of an interferent which has a neutral charge, whereas ascorbic and uric acid are both negatively charged.

Initial experiments were performed on bare platinum to provide a clearer overview of the reactions which were taking place. CVs between $-0.35 \text{ V}$ and $0.8 \text{ V}$ were used in this section instead of CAs to allow the interferent oxidation reaction to be visualised. The data was analysed using the mean current at $0.5 \text{ V}$ from 5 consecutive CVs. The potential should also help to minimise the current response occurring from the oxidation of the interfering species. Additionally, a Nafion membrane was drop-cast onto the electrodes using the method described in Chapter 4 and its effectiveness at reducing the response from interferents was evaluated. A 10 μl drop was spread across the electrode area. The background current changes with the addition of the Nafion layer so the recorded currents for the interferents are each compared to the PBS background on electrodes with and without Nafion coatings. A summary of the experimental results can be seen in Figure 5.18 and TABLE 5.3.

**TABLE 5.3**

Comparison of current responses to 10 μM ascorbic acid, 100 μM acetaminophen and 50 μM uric acid in PBS 1X at 0.5 V on bare and Nafion coated electrodes.

<table>
<thead>
<tr>
<th>Current Response /nA</th>
<th>Bare Electrode</th>
<th>Nafion Coated Electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{PBS}$</td>
<td>25.2 ± 0.05</td>
<td>15.7 ± 0.08</td>
</tr>
<tr>
<td>$I_{Ascorbic}$</td>
<td>32.3 ± 0.07</td>
<td>16.1 ± 0.06</td>
</tr>
<tr>
<td>$I_{Difference}$</td>
<td>7.1 ± 0.11</td>
<td>0.4 ± 0.14</td>
</tr>
<tr>
<td>$I_{PBS}$</td>
<td>23.2 ± 0.06</td>
<td>16.6 ± 0.22</td>
</tr>
<tr>
<td>$I_{Acetaminophen}$</td>
<td>50.8 ± 0.06</td>
<td>22.4 ± 0.11</td>
</tr>
<tr>
<td>$I_{Difference}$</td>
<td>27.7 ± 0.12</td>
<td>5.8 ± 0.33</td>
</tr>
<tr>
<td>$I_{PBS}$</td>
<td>20.2 ± 0.07</td>
<td>7.1 ± 0.02</td>
</tr>
<tr>
<td>$I_{Uric}$</td>
<td>37.4 ± 0.05</td>
<td>6.8 ± 0.04</td>
</tr>
<tr>
<td>$I_{Difference}$</td>
<td>17.2 ± 0.12</td>
<td>-0.3 ± 0.06</td>
</tr>
</tbody>
</table>
Figure 5.18: CVs at 100 mVs$^{-1}$ of PBS 1X (blue) and PBS 1X with interferent (red) on a (a, b, c) bare platinum nanoband electrode and (d, e, f) Nafion coated platinum nanoband electrode. Where the interferent is (a, d) 10 𝜇M ascorbic acid, (b, e) 100 𝜇M acetaminophen, and (c, f) 50 𝜇M uric acid.

Although the ascorbic acid is oxidised in the relevant potential region, the resulting oxidation current from the 10 𝜇M concentration is small. This oxidation current was still significantly decreased with the addition of the Nafion layer. The results show a 20.1 times decrease in the current measured from ascorbic acid when the Nafion layer is added to the electrode, equivalent to 5.0% of the uncoated current difference. The decrease in current response with the Nafion
layer was expected to be high since both the Nafion membrane and the ascorbic acid are negatively charged meaning there should be both a diffusion barrier as well as screening due to repelled charges.

The solution spiked with acetaminophen resulted in a larger current response than those with ascorbic or uric acid. Acetaminophen was added at a much higher concentration of 100 μM so it was expected to produce a higher current. Acetaminophen is uncharged so it was also expected that the Nafion layer would have a smaller effect on the oxidation current from acetaminophen than the other negatively charged interferents. However, there was still a large decrease in the current. The current resulting from the oxidation of acetaminophen after the addition of the Nafion layer is 4.8 times less without Nafion, equivalent to 21.0% of the uncoated current difference. The interference is still noticeable, however it is a significant reduction from the original results.

Since uric acid is also negatively charged, it was expected that Nafion would be quite effective at screening it from the electrode. The results do show that this is somewhat the case, as the current response from uric acid oxidation was decreased when the Nafion membrane was applied. However, the response from the uric acid is different on the Nafion coated electrode. The measured current with uric acid added to the solution was lower than the background current. This result was repeated on another electrode and the same behaviour was recorded.

A similar result has also been measured in the literature with glucose oxidase functionalised electrodes where the interfering effect of uric acid lead to a decrease in current rather than the expected increase [22]. The authors hypothesized that this unexpected current decrease was due to accumulation of allantoin, which is a product of uric acid oxidation. This product accumulation could also be occurring in the Nafion membrane and leading to the reduced current response. It is also possible there was an issue with the film deposition leading to a decrease in the background current when the uric acid was added to the solution. Since the current difference on the Nafion coated electrode was negative, the magnitudes of the current differences can be compared instead. The current difference was decreased by 59.8 times on the Nafion coated electrode, this is equivalent to 1.7% of the current difference on the uncoated electrode. Despite this unexpected behaviour, the Nafion layer still provided significant protection.
from uric acid oxidation but more work could be undertaken to fully understand the cause of the unexpected current response on Nafion coated electrodes.

The presence of the Nafion membrane adds an additional diffusional barrier which should have an effect on all the species diffusing to the electrode, including glucose and H$_2$O$_2$. Glucose is quite large and slow to diffuse, however H$_2$O$_2$ is small and diffuses around twice as fast so it should be affected less by the Nafion membrane. Therefore, it was important to ensure the addition of the Nafion layer would not eliminate the response of the electrode to glucose. Figure 5.19 shows a comparison of CVs of 100 μM of glucose with and without the Nafion membrane and the results are summarised in TABLE 5.4. The measurements showed a decrease in current response of 1.24 times, with 80.3% of the current remaining. This was a greater retention of current response than recorded during the interferents measurements likely because H$_2$O$_2$ is uncharged and diffuses quickly. However, this would still lead to a small drop in sensitivity of the sensor.

Figure 5.19: CVs at 100mVs$^{-1}$ of PBS 1X (blue) and PBS 1X with 100 μM glucose (red) on a (a) bare platinum nanoband electrode and (b) Nafion coated platinum nanoband electrode.

Nafion is a negatively charged membrane, therefore it was expected that Nafion would have a greater screening effect on ascorbic acid and uric acid, which are negatively charged, and a smaller effect on acetaminophen and glucose, which are neutral. It is difficult to directly compare the effect between the interferents due to the different concentrations at which they have been tested, however the decrease in current did appear to be greater for the negatively charged
interferents. Overall, measuring at a lower potential and the addition of a Nafion layer provides the electrodes with a significant decrease in unwanted oxidation current from the three common interferents tested. The current response from glucose was also decreased, but less significantly than the interferents meaning Nafion could be used as a membrane for glucose sensing.

An alternative approach would be to measure the current response from the interferents in-situ on a bare platinum electrode and compare this to the current recorded at the functionalised glucose electrode. This electrode would need to be located far enough away from the glucose sensing electrode to prevent cross-talk from H$_2$O$_2$ but could provide a relatively simple method of background correction. There are also alternative methods of glucose detection, discussed in Chapter 2, which offer benefits for avoiding interferents by using an enzyme mediator or a H$_2$O$_2$ electrocatalyst (such as Prussian blue). Both of these methods can be used to lower the operating potential outside the oxidation window of the interferents discussed in this chapter. However, these methods add complexity and expense to the fabrication of the device and are not covered in this work.

\textit{TABLE 5.4}

\textit{Comparison of current responses to 100 μM glucose in PBS 1X at 0.5 V on bare and Nafion coated electrodes.}

<table>
<thead>
<tr>
<th>Current Response /nA</th>
<th>Bare Electrode</th>
<th>Nafion Coated Electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{PBS}$</td>
<td>64.9 ± 0.08</td>
<td>50.7 ± 0.07</td>
</tr>
<tr>
<td>$I_{Glucose}$</td>
<td>120.7 ± 0.10</td>
<td>95.6 ± 1.3</td>
</tr>
<tr>
<td>$I_{Difference}$</td>
<td>55.8 ± 0.18</td>
<td>44.9 ± 1.4</td>
</tr>
</tbody>
</table>

\section*{5.5. Summary}

In this chapter, platinum nanoband electrodes arrays were designed and fabricated. COMSOL was used to simulate the current response of the microfabricated electrodes and the response was consistent with the experimental results. An electrode functionalisation protocol was developed for glucose sensing and it was found that environmental conditions, such as humidity, were vital to achieve a reproducible response. The results from glucose measurements showed that the addition of a second cleaning procedure was key to ensuring the optimum current response was achieved. The current response from glucose measurements
with the electrodes was matched to calculated values and deemed to be consistent.

The concentration range, time and potential used to produce the CAs to plot calibration curves was optimised, and the reproducibility of the sensor was compared between devices. Overall, the fabricated nanoband electrodes were capable of measuring at the relevant concentrations of glucose in the sweat of healthy individuals (0.06 – 0.11 mM [1]). The calibration curves had linear relationships to concentrations of at least 500 μM. This could be extended further through the addition of a membrane, such as polyvinyl chloride [13], if the 1 mM upper concentration limit of glucose in people with diabetes was determined to be required. The LOD was below the 10 μM minimum concentration of glucose in the sweat of people with diabetes [1]. The sensitivity was slightly lower than other devices recorded in literature, but the nanoband electrodes can be produced using standard microfabrication techniques and offer advantages for measuring in flow (discussed in Chapter 6).

The effect of temperature and temperature compensation was also discussed, along with the response of the electrode to ascorbic acid, acetaminophen and uric acid interferents. A Nafion membrane was used to reduce the response from these interferents and the response to glucose was measured with the Nafion coated electrode to ensure the sensitivity of the device would not be decreased beyond a working limit. To fully characterise the sensor further experiments should be performed to investigate the effects of pH and also the lifetime of the sensor by characterising the response over longer periods.

References


Chapter 5. Glucose Sensing with Nanoband Electrodes

Chapter 6

Development of a Through-Flow Nanoband Electrode

6.1. Introduction

In Chapter 2 the importance of the method used for the collection and sampling of sweat was discussed. Effective sweat sampling prevents the sweat pores from becoming blocked and minimises the contamination from the skin surface and the mixing of old and new sweat. Nanoband electrodes were shown in Chapter 5 to be capable of measuring at the relevant concentrations of glucose in sweat. This type of electrode could potentially be modified for through-flow operation if the nanoband cavity were etched entirely through the wafer to produce channels. Additionally, it was expected that due to the enhanced radial diffusion likely to be produced by the combination of the nanoelectrode and the microscale pore diameter, flow insensitivity could exist at these electrodes across a range of flow rates. Flow insensitivity has been reported in the literature at microband electrodes in a microchannel [1] [2]. This chapter therefore presents novel development and research expanding on the previous work on nanoband electrodes (Chapter 5), through the fabrication and characterisation of nanoelectrode arrays inside microscale pores in a thin (0.5 μm) membrane supported by a silicon substrate for through-flow sensing. A diagram summarising the system developed in this chapter is shown in Figure 6.1.

There is some previous work relating to nanopore cavities in the literature, for example [3][4], however these are not through-flow devices. There are examples of micro- and nanobands in micro- and nanochannels, which use electro-osmosis to drive the flow [5][6]. These were used to identify geometries for total conversion of analytes flowing past the electrode and have a much higher ratio of electrode band thickness to channel radius than used in this chapter.
Previous work has established the electric current due to flow in a pipe as a function of the geometry of the electrode and type of flow. The Levich equation can be used to calculate the current at a band macroelectrode in a pipe under laminar flow conditions (Figure 6.2) [7]:

\[ I_{lim} = 5.43nF \frac{cD \nu_f^2 \rho \gamma}{x_E^2} \]  

(6.1)

where:

- \( n \) is the number of electrons transferred,
- \( F \) is the Faraday constant,
- \( c \) is the concentration,
- \( D \) is the diffusion coefficient,
- \( \nu_f \) is the volumetric flow rate, and
- \( x_E \) is the electrode thickness.
Figure 6.2: Diagram showing a band electrode in a pipe, which can be evaluated using the Levich equation (6.1).

The Levich equation (6.1) applies to a macroelectrode in a macroscale pipe whereas this work relates to a nanoelectrode in a microchannel, which may lead to deviations from the response described by (6.1). For this reason, it is important to consider some assumptions in the Levich equation. First, the velocity profile is assumed to be laminar and can then be approximated, using the Levêque approximation, as linear at the electrode surface. This means concentration changes are confined close to electrode. Next, the diffusion in the direction of the flow is assumed to be negligible (i.e. no axial diffusion). Additionally it is assumed that curvature effects of the tube can be ignored. Finally, radial diffusion is assumed to be insignificant and is neglected.

Deviations from this Levich response have been seen in the literature at low flow rates or smaller than macroscale geometries (both in the radius of pipe and electrode thickness) [1]. Thompson et al. performed simulations of the effect of axial diffusion, radial diffusion and without Levêque approximation, which showed the current response deviated from Levich at lower flow rates [8]. The current response could be lower or higher than Levich depending on geometry, for example for a high ratio of channel radius to electrode thickness axial diffusion was increasingly important. Other work states that the axial diffusion is significant for microbands of ≤ 10 μm thickness, except at very high flow rates [9]. Newman and Aoki [10] have both made analytical approximations for current enhancement, which appears to occur due to the enhanced contribution from the electrode edges, a well-known feature of micro- and nanoelectrodes.

The deviations from the Levich equation (6.1) seen in the literature are likely to occur at the through-flow nanoband electrodes developed in this chapter. A flow insensitive response would be useful, since in-situ sweat flow rates are
challenging to measure so it is not desirable for the electrode response to vary with flow rate as well as analyte concentration.

6.2. Fabrication

The electrodes developed in this section are based on the standard nanoband electrode design used for glucose sensing described in Chapter 5. The cavity diameter (20 μm), spacing between electrodes (150 μm), and number of nanobands in the array (550) are all unchanged, but the cavity is replaced with a channel that allows liquid to flow through the electrode. The fabrication of these through-flow electrodes required processing of the back side of the wafers. To facilitate this, wafers were either thinned using a backside grinding tool or purchased as double side polished (DSP) to ensure there was no native oxide on the back side. The final process used DSP wafers. The fabrication process described in this chapter was aimed towards developing a robust and reproducible electrode which can be tested and characterised, rather than optimising for yield or mass manufacturing. Future work would most likely move to using cheap, flexible substrates, which would involve considerable adaptation of the production process. The runsheets for the fabrication of the through-hole nanoband electrodes discussed here can be found in Appendix B.

In order to fabricate through-hole electrodes, the bulk silicon substrate must be etched or removed to open up the nanoband cavities. Deep reactive ion etching (DRIE) and anisotropic wet etching using potassium hydroxide (KOH) were two techniques that were investigated to etch through the silicon substrate. DRIE was used to remove silicon in deep channels of the same diameter as the nanoband cavities, while KOH etching removed material over a large area greater than the size of the entire nanoband array footprint.
6.2.1. Deep Reactive Ion Etching (DRIE)

Figure 6.3: Cross-section (not to scale) showing proposed key processing steps in fabrication of nanoband electrodes using DRIE. (a) Silicon wafer insulated with silicon oxide. 10 nm Ti and 50 nm Pt deposited, patterned, wet etched and insulated with silicon nitride. Si substrate patterned and etched using DRIE from back side. (b) Electrode bond pad exposed by etching through SiRN. (c) Nanoband cavities etched through SiRN and metal layers to expose Pt nanoband. This etch meets with channels from the substrate etch in (a) to create channels through the entire wafer.

The approach taken with the DRIE process can be seen in Figure 6.3. The wafers were first thinned by backside grinding to reduce the wafer thickness from 525 μm to 250 μm. This significantly reduced the etch time, but there was still a significant depth of silicon (250 μm) to etch and a thick mask was required to protect the surrounding areas. This was achieved using 4.5 μm of positive photoresist (SPR™ 220-4.5) on top of a hard mask comprising 0.5 μm of PECVD silicon oxide.

Figure 6.4: SEM of cross section of DRIE showing diameter at different etch depths.
The initial samples using DRIE of silicon showed mixed results. The process was time-consuming and as the etch time and resulting etch depth increased, the fragility of the wafer also increased, making the subsequent processing steps more challenging. The sidewall profile from the etch was also less consistent than expected; in some cases the diameter at the midpoint of the channels was almost 50% wider than at the entrance as can be seen in Figure 6.4. It is not essential that the entire length of the channel retains the patterned diameter, since its main purpose is to direct liquid past the electrode. However, the entrance diameter is a key parameter, and irreproducibility of this parameter is a concern. Undercuts or sloping in the sidewalls surrounding the nanoband electrodes can lead to the exposure of more of the electrode material, resulting in a microband rather than a nanoband and therefore inconsistent results. However, DRIE is a well-known etching method and it could be optimised to produce more accurate results.

![Diagram (not to scale) showing (a) intended etching and alignment for the through-nanoband channels, and (b) etching of nanoband channels with poor alignment.](image)

The biggest drawbacks of this method are the wafer fragility and the difficulty of integration with the nanoband fabrication process presented in Chapter 5. The channels in the silicon wafer are too deep to continue etching through the insulators and metal layers from the back side of the wafer. However, aligning the 20 μm diameter cavities from the front side of the wafer would be extremely difficult to do perfectly (Figure 6.5) which would then lead to inconsistencies in the way the liquid flows through the electrodes and affect the symmetry of the diffusion profiles during electrochemical measurements.
6.2.2. Potassium Hydroxide (KOH) Etching

Figure 6.6: Cross-section (not to scale) showing key processing steps in fabrication of nanoband electrodes using KOH etching. (a) Silicon wafer insulated with silicon dioxide. 10 nm Ti and 50 nm Pt deposited, wet etched and insulated with silicon nitride. Si substrate etched using KOH from back side. (b) Electrode bond pad exposed by etching through SiRN. (c) Nanoband cavities etched through SiRN and metal layers to expose Pt nanoband. This etch connects to the etched substrate area in (a) to create channels through the entire wafer.

An alternative approach to DRIE is to use an anisotropic wet etching process, such as that achieved with KOH, to etch through the silicon wafer. The etch rate of 30% KOH in DI water at 80°C is 1100 nm/min, the concentration used here was 33% and it was found that the KOH etch took around 10 hours to complete (the process time was not tightly controlled as over-etching was not a concern due to minor excess silicon etching in the horizontal plane not affecting the geometry of the active electrode area). In KOH etching of silicon, the etch progresses preferentially in the <100> plane of the silicon crystal structure. For the standard wafers which were used in this work, the etch is directed at a 54.7° angle into the wafer. Therefore, the entire area of silicon under the footprint of the nanoband array was etched to leave a thin membrane (consisting of platinum sandwiched between insulation layers) as shown in Figure 6.6a, rather than channels for each individual nanoband cavity. This makes the lithography of the nanoband cavities simpler since the membrane can be subsequently etched through from one side with a large alignment tolerance.

A large area etch could also have been achieved using DRIE, however there are advantages to using KOH etching. It is a less aggressive process, which is more favourable to the survival of the thin membrane that remains after the etch has been completed. KOH etching is also an inexpensive microfabrication process and although the etch time is long, entire cassettes of wafers can be etched simultaneously meaning the throughput is high. Optimisation of the yield is not a key concern at this time, since it is more important to ensure this fabrication process produces reproducible prototype electrodes which can be tested and

characterised. Backside grinding or the use of thinner than standard wafers would reduce the etch time, however the nominal 525 μm thickness of 100 mm diameter wafers was found to increase the stability of the wafers (compared to thinned wafers and measured by decreased wafer breakages); therefore, wafers were used without additional thinning for KOH etching. Figure 6.7 shows an example of successful silicon nitride membrane test structures after the KOH etching was completed.

In order to ensure that the membranes were robust enough to survive further processing, test samples were etched with 20 μm diameter circles. The lithography and etch were performed on the front side of the wafer and backside alignment was used on the virtual mask aligner tool. In Figure 6.7 it can be seen that the holes have been successfully etched through the entire membrane (which appear black in the figure) compared to those on the areas with underlying bulk silicon (which appear white/silver). Fabrication development for the nanoband electrode etch will be discussed in Section 6.2.3, but these initial tests confirmed that the membranes could be further processed and should allow through-hole nanoband electrodes to be fabricated.

Figure 6.7: Example of nanoband cavity etch through membrane and bulk wafer (no metal layers, only insulators) (right). Dashed outline showing the location of the membrane.
The membrane etch was then combined with the rest of the nanoband fabrication process. Since the membranes were relatively fragile, it was preferable to minimise any processing steps after the bulk silicon was etched, and so the membrane layers were deposited prior to KOH etching. LPCVD silicon-rich nitride (~300 nm) was used as the top insulating layer as it is resistant to KOH etching [11], and therefore protects the underlying Ti/Pt layers. A layer of 0.5 μm silicon dioxide was grown on the silicon wafers to act as an insulator between the silicon substrate and the deposited Ti/Pt layers, and this formed the bottom layer of the membrane after the KOH etch. The combination of silicon dioxide and silicon nitride layers balance each other in terms of stress in the layer, which should increase the robustness of the devices. However, silicon dioxide is slowly etched by KOH, which makes it a non-ideal etch stop [11]. The KOH bath is a shared resource and its concentration can vary over time which would affect the etch duration and this could lead to inconsistency in the thickness of the bottom insulation layer across devices. To prevent this, a thin layer of silicon nitride (130 nm) was also added on top of the silicon dioxide (beneath the Ti/Pt layers) and the wafers were over etched in the KOH solution to remove the oxide layer at the membrane and ensure a reproducible insulation layer thickness. This allowed a level of variability in the KOH etch time while still maintaining consistency in the resulting devices.

Since the insulating layers were deposited using LPCVD and thermal oxidation, they covered both the front and back of the wafer (this symmetry also helped reduce stress across the wafer). The back side of the wafer was patterned and the insulating layers were removed with a RIE and the remainder used as a hard mask for the KOH etch. Although a band of silicon dioxide on the back side of the wafer is exposed to the KOH, the etch rate is extremely slow (7.7 nm/min [11]) so even after several hours in KOH the undercut depth was found to be relatively insignificant. Additionally, the presence of the SiRN etch stop meant the active area of the electrode was unaffected. This meant the deposition of all the insulator and metal layers could be performed while the wafer was most robust, prior to etching the bulk silicon substrate. Figure 6.8 contains a photograph of the back side of a wafer after KOH etching, which shows the etched membranes consisting of SiRN – Ti/Pt – SiRN. Electrodes were staggered across the wafer to
maximise the distance between adjacent membranes, increasing the overall stability of the wafer.

Figure 6.8: Photograph of the backside of a 100 mm wafer showing membranes (consisting of Pt sandwiched between SiRN) after the KOH etching step. Dashed outline shows a membrane that broke during the etching step.

6.2.3. Nanoband Etch Process

In the next major stage to fabricate the through-hole nanoband electrodes, the bond pads were exposed using RIE, and the nanoband electrode channels through the membrane were patterned and etched. There were additional processing issues due to the fragility of the wafer after the membrane was etched. One of the main difficulties is that the membranes could be damaged by the vacuum chucks, which are used in many of the cleanroom tools. There were a few possible ways around this including making the membrane etch the final step, reducing or removing the vacuum strength where possible, increasing the membrane thickness or decreasing the membrane area, or mounting the wafer on a carrier. It was decided that mounting the wafer was the most promising option as it would require the least changes to the fabrication process and therefore require the least development time.

Initially the wafer was mounted on a second wafer using photoresist, however it was challenging to unmount the wafer before etching the nanoband arrays. A better option was to use dicing tape as a wafer mount, and a low adhesive tape was chosen which was easier to separate from the wafer. As long as the tape was carefully applied to the back of the wafer (e.g. without creating air bubbles), this was sufficient to protect the membranes from damage from vacuum chucks. The low adhesive tape was then manually peeled from the wafer after the photoresist was exposed.

![Diagram (not to scale) showing (a) intended nanoband electrode etch, (b) sloped insulator etch leading to a sloped nanoband electrode etch, and (c) sloped insulator etch leading to excess exposed Pt electrode during nanoband etch.](image)

Figure 6.9: Diagram (not to scale) showing (a) intended nanoband electrode etch, (b) sloped insulator etch leading to a sloped nanoband electrode etch, and (c) sloped insulator etch leading to excess exposed Pt electrode during nanoband etch.

The sidewall profile of the nanoband electrodes is a critical parameter to ensure the electrodes retain a nanoscale dimension. If the sidewalls were sloped the band may also become sloped, or may overhang the insulator leading to microscale dimensions (or uncontrolled and irreproducible dimensions) as shown in Figure 6.9. For this reason, nanoband resist patterning was a key process step since this defines the area for the nanoband etch. Lithography using a virtual mask aligner tool was more compatible with these fragile wafers than the mask aligner since it does not use a vacuum chuck, and it also allowed more flexibility with the mask design since the design could be adjusted electronically without purchasing a physical mask. The disadvantage is that the resist sidewall profile was less vertical when exposed using the virtual mask aligner compared with the mask aligner because the light source is less directional. Dose tests were carried out using a positive photoresist to see if this process could be optimised, however these were ultimately unsatisfactory. An alternative approach was to use negative
resist rather than positive as the cross-linking process is controlled in a different way.

Cross-linking of negative photoresists (including the nLOF 2035 resist used here) was initiated during exposure, and enhanced with a post exposure bake (PEB), which caused the resist to become insoluble in developer. A higher temperature PEB could increase the stability of the patterned resist in developer but it could also cross-link areas of resist which were not exposed. The optimum temperature of the PEB does not depend on the resist thickness, but on the type of resist and desired resist profile. The nLOF resist that was used is intended for lift-off processes so the optimal use parameters intend to leave a resist profile with an undercut. When nLOF resist is exposed according to the datasheet instructions, the exposure dose does not transmit through the entire depth of the resist (in fact, nLOF quite significantly attenuates the exposure through its depth). The subsequent PEB will then cross-link the resist that was exposed, which will be towards the surface of the resist rather than the surface of the wafer. When the resist is developed, an undercut will form where the developer preferentially removes the non-cross-linked resist at the wafer surface while the insoluble resist at the top remains.

To design a process using this resist for RIE, it is necessary to adapt the parameters. To achieve a more vertical sidewall, it is necessary to apply a higher exposure, a hotter PEB or a shorter development time. The datasheet and additional information report [12][13] contain examples showing the effects of different parameters, this states that a PEB of 115°C will give a more vertical sidewall profile than the standard 110°C. Increasing the exposure dose will have a smaller effect but will also increase the vertical profile at the sidewalls. Since the exposure will be performed using a virtual mask aligner, which uses a different wavelength of light, the doses used in the datasheet do not perfectly match. This means that a dose test was required to find the optimum value for the process. The datasheet also states that a high temperature hard bake could additionally reduce the resist undercut as well as provide extra stability for dry etching, so a hard bake at 130°C was also added to the process.

A simple mask design was created that consisted of a mixture of 20 μm and 40 μm diameter circles spaced 150 μm apart. This pattern was repeated across

an approximately 4 mm by 3 mm rectangular area, which could be repeated across the wafer in the virtual mask aligner software for dose testing. The dose test parameters were set to estimate the correct dose for the pattern block at the centre of the wafer, then the dose was set to change by ±10% in the horizontal axis respective to the centre block. Figure 6.10 shows the difference in the size of the exposed platinum nanoband after RIE due to different resist profiles. The left image is using 350 mJcm⁻² and the right image shows a much higher dose 525 mJcm⁻² which resulted in significant excess platinum exposed after the dry etch (Figure 6.10b). From the dose test, 350 mJcm⁻² was chosen as the optimum dose for the nanoband etch.

Figure 6.10: SEM images of test cavities for through-flow nanoband electrodes etched using RIE patterned with negative resist, which were exposed using 350 mJcm⁻² (left) and 525 mJcm⁻² (right) using the virtual mask aligner.

The resist was then stripped in ACT at 60°C. The resist was difficult to remove and in some cases could not be completely removed from areas such as the dicing channels. Resist stripping with an alternative resist stripper such as AZ 400T or AZ Remover 770 (Microchemicals), which are the recommended resist strippers, may have led to improved photoresist removal. The high exposure dose and high temperature bake during the resist processing, and the inability to use sonication during resist stripping were the most likely cause of the difficulties in fully removing the photoresist from some devices. Finally, the wafer was spray coated with a protective layer of resist and diced using a dicing saw with reduced water flow. The resist could then be removed and the electrodes were ready for cleaning and testing. Figure 6.11 shows an SEM image of a completed electrode.
from the backside which shows the sloped sidewalls of the membrane. Individual nanobands could be seen at higher magnifications inside the membrane area.

Figure 6.11: SEM image of backside of a diced electrode showing etched membrane area.

This process did lead to consistent and reproducible electrodes (electrodes were functional and gave reproducible electrochemical responses), however there are some fabrication steps which could be improved in the future. Once the electrode design has been finalised, it would be preferable to process the wafers using a physical mask with positive photoresist rather than negative photoresist on the virtual mask aligner. It may be possible to overcome the issues with fragility by using a low vacuum and non-contact (proximity) mode on the mask aligner to reduce membrane breakages. Alternatively, performing the final dry etches for the contact pads and nanoband electrodes before the KOH etch would further simplify the fabrication process. This was not undertaken in this work due to time constraints and concerns about damaging the nanoband electrodes or contaminating the KOH bath, which was shared by other cleanroom users and does not allow wafers with exposed metal layers to be processed. From the data provided by Williams, platinum should not be etched by the KOH bath, however the titanium adhesion layer may be affected [11]. It may be possible to adjust the parameters of the KOH etch to prevent titanium etching or to replace the titanium with a different adhesion layer [14].

6.2.4. Cell for Electrode Testing

In order to test and characterise the electrodes a polydimethylsiloxane (PDMS) cell was designed to hold the fabricated working electrode along with
counter and reference electrodes. PDMS is commonly used for microfluidic devices and was therefore chosen to form the cell used in this work [15]. The cell allows electrolyte to be controllably flowed across the counter, reference and through-flow nanoband working electrodes at a known flow rate. This cell was used to collect the experimental results from the fabricated through-hole electrodes.

![Figure 6.12: Photograph of 3D printed mould design after smoothing with acetone, dimensions indicated as input to the 3D printer.](image)

PDMS was mixed in a 10:1 ratio of elastomer to curing agent and placed in a vacuum chamber for at least 1 hour to ensure all bubbles were removed. Moulds for PDMS (Figure 6.12) were designed in OpenSCAD and 3D printed with acrylonitrile butadiene styrene (ABS), which is a type of thermoplastic polymer commonly used for 3D printing. The moulds were smoothed with acetone or acetone vapour to ensure a good release from the PDMS. The moulds were placed in aluminium foil and the PDMS mixture was carefully poured over them and left to fully cure at room temperature. The cured PDMS was cut into blocks and the 3D printed mould removed to leave the cell chamber with dimensions 4 mm x 10.5 mm x 5 mm.
A platinum wire counter electrode (CE), a luggin capillary connected to a commercial Ag/AgCl reference electrode (RE), and a needle syringe connected to a syringe pump were pushed through the PDMS cell walls. The through-hole nanoband electrode was placed front-side down at the top of the PDMS cell to fully enclose the chamber. The electrodes and needles were sealed using Araldite or silicone to ensure the cell was completely watertight. A diagram and photographs of the setup can be seen in Figure 6.13. The syringe pump was used to control the flow of solution through the cell and out through the working electrodes. The

OCP between the working electrode (WE) and RE was typically checked to ensure there was electrical contact, with no bubbles trapped in the system; when this was confirmed electrochemistry was then performed using the cell.

6.3. Experimental Results
6.3.1. Initial Experimental Results

The experimental results in this chapter were performed in the Pyrochemical Research Lab in the School of Chemistry. Initial electrochemical measurements were performed using the fabricated through-flow electrode in the PDMS cell at some exemplar flow rates. Ferrocenemethanol (FcMeOH) at a concentration of 100 μM dissolved in PBS 1X was used as the simple redox couple (as in Chapter 5) for characterisation of the device and system.

CVs were measured and show the expected shape of the FcMeOH oxidation wave at a nanoband electrode (Figure 6.14). As expected, the current increases with flow rate over the range tested, and the waveform appeared to stay the same.

Figure 6.14: CVs of 100 μM ferrocenemethanol in PBS 1X recorded at a scan rate of 100 mVs⁻¹ at 0 mlh⁻¹ (0 m³s⁻¹, blue), 0.5 mlh⁻¹ (1.39 x 10⁻¹⁰ m³s⁻¹, red), 1 mlh⁻¹ (2.78 x 10⁻¹⁰ m³s⁻¹, purple), and 5 mlh⁻¹ (1.39 x 10⁻⁹ m³s⁻¹, green). CVs show oxidation current increasing with increasing flow rate.

with increasing flow rates. In this chapter, the syringe pump used to control the flow rates into the cell was set using mlh$^{-1}$, this unit has been converted to the SI unit of m$^3$s$^{-1}$ in the text for comparison with other data, and two x axes have been used to display the flow rates using both units where appropriate.

Analysis using a modified Tafel plot provides a simple method to determine whether the observed waveform has a shape consistent with an electrochemically reversible reaction. Modified Tafel analysis can be undertaken by rearranging the Nernst equation (3.1) using $\frac{i_{\text{Lim}}}{i} - 1 = \frac{c_{\text{ox}}}{c_{\text{red}}}$ to give:

$$E = E' + \frac{RT}{nF} \ln \left( \frac{i_{\text{Lim}}}{i} - 1 \right)$$

(6.2)

where:

$i_{\text{Lim}}$ is the limiting current.

This equation can then be used to plot $\ln \left( \frac{i_{\text{Lim}}}{i} - 1 \right)$ against potential ($E$) in a plot called a modified Tafel plot. This plot results in a straight line in the region of $x = E'$ with the slope equal to $-\frac{nF}{RT}$.

Modified Tafel plots for the fabricated through-flow electrodes at 0 mlh$^{-1}$ (0 m$^3$s$^{-1}$) and 5 mlh$^{-1}$ (1.39 x 10$^{-9}$ m$^3$s$^{-1}$) can be seen in Figure 6.15. As expected, both the forward and backward scans are linear in the region surrounding $E'$, and only slightly offset. The backwards scans begin to curve before the forward scans away from $E'$. This occurs to a greater extent in the data recorded at the 0 mlh$^{-1}$ (0 m$^3$s$^{-1}$) flow rate. The curvature is likely caused by either overlap of the diffusion profiles between adjacent electrodes or product formation, both of which would be decreased by flow, consistent with Figure 6.15.

For the experimental conditions used $\frac{nF}{RT}$ is 38.9 V$^{-1}$, which is the same within experimental error as the slopes presented in TABLE 6.1. The backwards scan at 0 mlh$^{-1}$ (0 m$^3$s$^{-1}$) deviated furthest from this theoretical value, however it was also the plot most affected by the curvature and therefore has the highest error.
Figure 6.15: Modified Tafel plots from CVs recorded at a scan rate of 100 mVs\(^{-1}\) of 100 \(\mu\)M FcMeOH in PBS 1X at flow rates of (a) 0 mlh\(^{-1}\) (0 m\(^3\)s\(^{-1}\)) and (b) 5 mlh\(^{-1}\) (1.39 \(\times\) 10\(^{-9}\) m\(^3\)s\(^{-1}\)). Both graphs show the forward scan (blue dots) and the backwards scan (red squares). Trendlines are fit between 0.20 and 0.25 V and the linear fit values are given in TABLE 6.1.
The results presented in the CVs therefore indicate that the fabricated through-flow nanoband electrodes give the characteristic electrochemical responses expected for this electrochemically reversible reaction as seen with standard nanoband electrodes (Chapter 5). Additionally, changing the flow rate did not fundamentally change the shape of the oxidation curve as the modified Tafel plots remain consistent with (6.2).

**TABLE 6.1**

Linear fit to the data presented in Figure 6.15 between 0.20 and 0.25 V.

<table>
<thead>
<tr>
<th>Flow rate, scan direction</th>
<th>Slope/V(^{-1})</th>
<th>Intercept</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mlh(^{-1}) (0 m(^3)s(^{-1})), forwards</td>
<td>-40.9 ± 0.1</td>
<td>9.16 ± 0.03</td>
<td>0.9994</td>
</tr>
<tr>
<td>0 mlh(^{-1}) (0 m(^3)s(^{-1})), backwards</td>
<td>-42.4 ± 0.3</td>
<td>9.83 ± 0.06</td>
<td>0.998</td>
</tr>
<tr>
<td>5 mlh(^{-1}) (1.39 x 10(^{-9}) m(^3)s(^{-1})), forwards</td>
<td>-37.9 ± 0.1</td>
<td>8.45 ± 0.02</td>
<td>0.9997</td>
</tr>
<tr>
<td>5 mlh(^{-1}) (1.39 x 10(^{-9}) m(^3)s(^{-1})), backwards</td>
<td>-37.2 ± 0.1</td>
<td>8.41 ± 0.03</td>
<td>0.9993</td>
</tr>
</tbody>
</table>

6.3.2. Simulation with Ferrocenemethanol (FcMeOH)

In Section 6.1, it was discussed that the effect of flow on nanoband electrodes may be small due to their enhanced diffusional mass transport. However, it is clear from Figure 6.14 that the addition of flow did have some effect on the current response, therefore it was important for the magnitude of this effect to be characterised. It was decided that simulations should first be performed as a method to understand the interaction between mass transport due to diffusion and convection and allow the effect of parameter modification to be easily characterised. The parameters include using a range of flow rates (0 – 100 mlh\(^{-1}\), or 0 – 2.78 x 10\(^{-8}\) m\(^3\)s\(^{-1}\)), two different characteristic values for diffusion coefficients for FcMeOH (D = 0.7 x 10\(^{-9}\) m\(^2\)s\(^{-1}\)) and hydrogen peroxide (D = 1.43 x 10\(^{-9}\) m\(^2\)s\(^{-1}\)) to enable comparison with experimental work, and different values for electrode band thickness (\(x_E\) = 10, 25, 50 and 100 nm). The results of these simulations are presented and discussed in Section 6.3.3 and Section 6.3.5. Simulations were performed in COMSOL, using the model described in Chapter 3 where a single nanoband pore is simulated and the response of the full array extrapolated. It should be noted that the laminar flow module was used for the simulations of the velocity field, so the flow pattern in the simulation models was
exclusively laminar (an assumption of the Levich equation (6.1) – the applicability of this assumption is tested by comparison to experimental measurement).

![Image](image_url)

Figure 6.16: COMSOL simulation ($x_E = 50$ nm, in 20 μm diameter pore with 150 μm edge-to-edge pore separation) for ferrocenemethanol ($D = 0.7 \times 10^{-9}$ m²s⁻¹) at 100 μM bulk concentration for arrays with (red dots) and without (blue crosses) overlap from the diffusion profiles of adjacent electrodes. Dashed vertical line shows the approximate calculated overlap time. The insets are the resulting simulated concentration profiles at 1s (left – before overlap) and at 30.2s (right – after overlap).

In Chapter 5 the standard nanoband array electrodes were simulated and the effect of array overlap on the current response was shown. The through-flow electrodes presented here were based on this electrode design (array of 550 nanoband electrodes, $x_E = 50$ nm, in a 20 μm diameter pore, with 150 μm edge-to-edge electrode separation) so it is expected that there will be a similar effect of array overlap. Figure 6.16 shows the results from simulations of the through-flow nanoband array electrodes with and without the effect of array overlap (first by using symmetry to produce an infinite array of neighbouring pores at the correct placement, and second by having a suitably large simulation box with no neighbouring pores). The time at which the currents deviate and overlap was seen to occur, is shown by the dashed line. It is comforting that this is
consistent with that shown previously in Chapter 5, which confirms this is due to diffusional overlap from neighbouring pores. The insets in Figure 6.16 show concentration profiles from the simulation, which confirms the spread and overlap of the diffusion profiles graphically.

Using the modified Saito equation (3.11), the constant $B$ can be calculated for this through-flow electrode as $B = 3.23$. This is slightly less than double the value calculated for the standard nanoband electrode (1.73 from Chapter 5). This is considered to be reasonable, since the diffusional profile extends from the through-flow electrode in both directions rather than only one, so an approximate doubling of current response is expected.

### 6.3.3. Relationship between Current Response and Flow Rate

In Section 6.1, it was established that tubular electrodes follow the relationship described by (6.1) under flow conditions. Relevant literature was also discussed, which showed deviations in the response of electrodes that do not meet the assumptions of (6.1). This section aims to characterise the simulated response of the fabricated through-flow electrodes and identify conditions where the mass transfer limited current response follows or deviates from the flow dependency characteristic of (6.1). To achieve this, simulations were performed for FcMeOH at 100 µM concentration for flow rates from $0 - 100 \text{ mlh}^{-1}$ ($0 - 2.78 \times 10^{-8} \text{ m}^3\text{s}^{-1}$). As in section 6.3.2, electrodes were simulated both with and without adjacent electrodes to simulate the presence and absence of diffusional overlap, since the current response over time should then give insights into the interaction between mass transport due to diffusion and convection and array overlap.

The results of this simulation are shown in Figure 6.17. The current response is plotted against $V_f^{1/3}$ since this would result in a linear relationship were the data to follow the same form as the Levich equation (6.1). The current response is plotted using points taken at different times (0.1, 1, 10, 30.2, and 100 s), chosen to be both before and after array overlap in section 6.3.2.

It can be seen from these simulation results that there are two regions that appear to follow different relationships, one at low flow rates (diffusion dominates flow) and one at high flow rates (flow dominates diffusion) with a transition region between them. At high flow rates all the time points produce the same current
and show a linear dependence on $V_f^{1/3}$, which is indicative of a flow relationship similar to that described by (6.1). Those at the low (and zero) flow rates show a time-dependent response, which is indicative of a diffusion-controlled process. The response with and without adjacent electrodes to simulate diffusional overlap are represented by dots and circles respectively in Figure 6.17.

Figure 6.17: Simulation results of mass transfer limiting current ($i_{lim}$) against the cube root of volumetric flow rate for an array of electrodes with overlap (dots) and without overlap (circles) for data points taken at 0.1 (blue), 1 (red), 10 (orange), 30.2 (purple), and 100 s (green). Arrow shows the direction of data points taken at increasing times. Dashed line shows the theoretical current required to convert all the analyte passing the electrode flowing through the pore at steady-state (transport by flow alone). The dot-dash line shows the calculated flow value from (6.3) where the mass transport due to diffusion and convection are equal.

At high flow rates both simulations give the same results (dots and circles are coincident) showing no array overlap and no changes over the selected time points, consistent with flow dominating transport. However, at low flow rates current response from the simulation with adjacent electrodes continues to decrease over time whereas the other simulation initially decreases but reaches a
time-independent current response between 1 and 10 s. This is consistent with the simulation results presented in Figure 6.16, which showed deviation of the simulated current response due to diffusional overlap from adjacent electrodes; this indicates diffusion is dominating here. Between these regions there is a zone with a mixed mass transport process, which is a combination of mass transport due to both diffusion and flow. It is harder to quantify the current and flow relationship within this zone (e.g. the assumption which led to the Levich equation (6.1) break down) and it could be preferable to avoid operating within it.

It is worth noting from comparing this response to the dashed line in Figure 6.17, given by:

\[ i = nFcV_f \]  (6.3)

which is the current required to convert all the analyte flowing through the pores, that these diffusion controlled and flow controlled regions have a relatively high and low analyte conversion efficiency (proportion converted when flowing through the pores) respectively. This could allow through pore conversion efficiency to be tuned by adjusting flow. However, this will be more complicated in the case of glucose sensing, where the measured analyte is produced locally.

It is worth noting that, although Levich-like behaviour is observed at high flow, there is a significant non-zero intercept, which is comparable to the diffusion-limited current at zero flow. In Section 6.141 the assumptions for the Levich equation were discussed. One of these assumptions is that axial diffusion is negligible (diffusion along the axis of flow). Due to the device dimensions and flow rates in which the device is operating, it therefore seems that this contribution from diffusion is essentially additive and cannot be ignored.

It is possible to estimate the flow rate at which the mass transport due to diffusion and that of flow (convection) will be comparable. The ratio of convection to diffusion is known as the Péclet number \((Pe)\), and when it is greater than 1 convection starts to become the dominant mass transport mechanism. The Péclet number can be calculated as follows for this system:

\[ Pe = \frac{\text{transport due to convection}}{\text{transport due to diffusion}} = \frac{\nu}{\frac{T}{2D}} = \frac{\nu}{\frac{T}{L^2}} \]  (6.4)

where:
\( v \) is the flow velocity,

and \( L \) is the characteristic length, in this case the pore radius \( (r) \).

This can be rearranged to calculate the velocity at which \( Pe = 1 \):

\[
v = \frac{2D}{r} \tag{6.5}
\]

calculating the velocity and converting it to a flow rate gives a value of 0.087 ml h\(^{-1}\) (2.42 \times 10^{-11} m^3 s\(^{-1}\)) for these electrodes (shown by the dark grey dot-dash line in Figure 6.17). Although this is just an indication of which transport method is dominant, it is interesting that it seems to be consistent with the transition in Figure 6.17 between the diffusion controlled (flow independent) region, which occurs at low flow rates, and flow controlled region at high flow rates.

The final consideration from the simulation results shown in Figure 6.17 is the deviation in gradient from that expected from the Levich equation; the gradient of this linear relationship is higher in the simulation than that predicted by Levich. This difference is discussed further in Section 6.3.5.

6.3.4. Validation of Theory with Experiment

Before further analysing the simulation data and further investigating the deviations in the response from the Levich equation, it was important to ensure the simulations were consistent with the experimental data and that any deviations were not an artefact of the simulation model. CA measurements over 30 s were therefore recorded for 100 \( \mu \)M FcMeOH in PBS 1X at 0 – 100 ml h\(^{-1}\) (0 – 2.78 \times 10^{-8} m^3 s\(^{-1}\)) at 0.4 V (mass transfer controlled oxidation). The CA measurements were taken after the flow had been established within the PDMS cell and 5 repeated measurements were taken and averaged to give the current response (Figure 6.18).

These experimental data were initially offset from the simulation data, however it was believed this could be due to a difference in the value of \( D \) (most likely caused by a difference in temperature) used in the simulation and the actual value under experimental conditions. The constant \( B \) calculated previously was used in the modified Saito equation (3.11) with the experimental results to determine the best fit experimental value of \( D \) as 5.07 \times 10^{-10} m^2 s\(^{-1}\). This value was then used in the COMSOL model to simulate the current response. The results of this simulation were compared to the experimental results and are also shown in Figure 6.18. The
datapoints collected from the experimental and simulated response below a flow rate of 5 mlh\(^{-1}\) (1.39 \(\times\) \(10^{-9}\) m\(^3\)s\(^{-1}\)) appeared consistent, confirming both the applicability of the COMSOL simulations and the fidelity of the electrode fabrication. A deviation between the experimental and simulated response was seen at flow rates above 5 mlh\(^{-1}\) (1.39 \(\times\) \(10^{-9}\) m\(^3\)s\(^{-1}\)), this may be an artefact of the simulation but it would be desirable to understand if there could be a physical reason for this.

![Graph showing current against the cube root of volumetric flow for simulated results (circles) with data points taken at 1 s with \(D = 5.07 \times 10^{-10}\) m\(^2\)s\(^{-1}\), and experimental results (dots) with data points taken at 0.1, 1, 10, and 30 s for 100 \(\mu\)M ferrocenemethanol (arrow shows direction of increasing time). Shaded region indicates the relevant range of flow rates of sweat for the footprint area of the through-flow electrode.](image)

**Figure 6.18:** Current against the cube root of volumetric flow for simulated results (circles) with data points taken at 1 s with \(D = 5.07 \times 10^{-10}\) m\(^2\)s\(^{-1}\), and experimental results (dots) with data points taken at 0.1, 1, 10, and 30 s for 100 \(\mu\)M ferrocenemethanol (arrow shows direction of increasing time). Shaded region indicates the relevant range of flow rates of sweat for the footprint area of the through-flow electrode.

Fluid flow is considered to be laminar when the Reynold’s number \((Re)\) is less than around 2000 [16]. The Reynold’s number can be calculated using:

\[
Re = \frac{u_0 h}{v}
\]  

where:
\( u_0 \) is the solution velocity at the centre of the channel,

\( h \) is the channel height (in this case diameter), and

\( \nu \) is the kinematic viscosity of the solution.

\( Re \) was calculated for the system and its maximum value is 1.55 at the highest experimental flow rate of 50 mlh\(^{-1}\)\( \times 1.39 \times 10^{-8} \text{ m}^3\text{s}^{-1} \), which suggests the flow pattern is laminar in the channel.

However, in addition to \( Re \), the distance taken for the establishment of the laminar flow profile upon entry to the pipe is important. Prior to the establishment of this profile, the flow cannot be considered to be laminar. The distance for this to occur is known as the entry length, and is calculated by [17]:

\[
l_e = 0.1hRe
\]  

(6.7)

The entry length was calculated for the through-flow nanoband electrode system, and below 5 mlh\(^{-1}\)\( \times 1.39 \times 10^{-9} \text{ m}^3\text{s}^{-1} \) the entry length is less than the height of the insulating layer (which corresponds to the distance from the pore entrance to the electrode). Above this flow rate, a laminar flow profile may not have been established which could explain the deviation seen between the experimental and simulation results shown at the highest flow rates in Figure 6.18. These flow rates are significantly higher than the flow rates expected from sweat, so this should not be an issue for the intended application. These higher flow rates were investigated since understanding the response of the fabricated electrodes to flow could be used for other high flow applications, or allow the development of a flow rate sensor in the future.

6.3.5. Characterising Deviations from the Levich Equation

As these simulations closely matched the experimental results within the laminar flow range, the simulation model was shown to provide a good response consistent with through-flow electrodes. Therefore further simulations were performed to investigate changes in the device geometry and the effect on deviations in gradient from the Levich equation (6.1) at high flow.

Figure 6.19: COMSOL simulation results of current response at a single nanoband (not an array) against the cube root of volumetric flow over the linear region with trend line plotted between data points and extrapolated to zero flow. Simulations are for 100 µM H₂O₂ \((D = 1.43 \times 10^{-9} \text{ m}^2\text{s}^{-1})\) (blue squares), 100 µM ferrocenemethanol \((D = 0.7 \times 10^{-9} \text{ m}^2\text{s}^{-1})\) (red crosses) at \(x_E = 50\) nm (solid line), and \(x_E\) equal to 10 nm (dot-dash line), 25 nm (dotted line), and 100 nm (dashed line) for 100 µM ferrocenemethanol \((D = 0.7 \times 10^{-9} \text{ m}^2\text{s}^{-1})\).

Simulations were performed at two different values of the diffusion coefficient for FcMeOH \((D = 0.7 \times 10^{-9} \text{ m}^2\text{s}^{-1})\) and H₂O₂ \((D = 1.43 \times 10^{-9} \text{ m}^2\text{s}^{-1})\) with an electrode thickness of 50 nm, and different values for electrode thickness \((x_E = 10, 25, 50\) and 100 nm) with \(D\) set to \(0.7 \times 10^{-9} \text{ m}^2\text{s}^{-1}\).

The results from these simulations can be seen in Figure 6.19, and the gradient and offset from the fitted trend line are summarised in TABLE 6.2. The simulation results were plotted and analysed without multiplying the current by the number of electrodes in the array to simplify the analysis. If the results follow the \(D^{2/3}\) relationship from Levich equation (6.1), the difference in the gradients for the two simulations should vary by a ratio of \(\frac{1.43 \times 10^{-9}}{0.7 \times 10^{-9}}\)^{2/3}, which is equal to 1.61.
The ratio from the simulation was calculated as 1.71, which suggests the $D^{2/3}$ relationship still applies.

This leaves $x_E$ as the final parameter to investigate, and the results from the simulation do not appear to follow the $x_E^{2/3}$ relationship. There was not an obvious simple relationship, which could be used to replace $x_E^{2/3}$. In TABLE 6.2 a constant $G$ is presented, which is a multiplication factor for the gradient of the Levich equation (6.1), and could be considered as similar to the constant $B$ in the modified Saito equation (3.11) where each electrode geometry would result in a constant value of $G$. This would modify the Levich equation as follows:

$$I_{lim} = 5.43 G n F c D^{2/3} \chi^{1/3} x_E^{2/3}$$

The constant $G$ can then be used to determine any corrective factor in the Levich equation to account for the difference in gradient from Levich for the through-flow electrodes presented in this chapter. As shown in TABLE 6.2, the value of $G$ decreased towards 1 as the size of the electrode increased, suggesting progression towards Levich behaviour as the nanoband electrode increased in size.

This is reasonable as the Levich equation (6.1) applies to macroelectrodes. Additionally, the results in [1] also showed that microelectrodes experience a different relationship to flow than that predicated by Levich. This paper presented a Levich-like regime with an increased current response at the transition region between diffusion control and Levich behaviour. In the work presented in this chapter, this Levich-like region results in a response that can be predicted by the Levich equation (6.1) with the addition of a multiplication factor $G$, which depends on the electrode dimension.

In Section 6.3.3, it was proposed that the intercept from the trend line in the flow dependent region is comparable to the diffusion limited, steady-state nanoelectrode current observed at zero flow. Overall, the intercept current was observed to be only weakly dependent on the electrode thickness, with 92.1% of the intercept value recorded for a halving of the nanoband thickness (from 50 nm to 25 nm). It has been reported that the limiting current at nanoband electrodes is quite insensitive to their thickness due to the predominance of edge diffusion

[18], therefore these results are consistent with this previous work using similar electrodes.

**TABLE 6.2**

Table of parameters from Figure 6.19 and calculated constants. $G$ is a multiplication factor accounting for the difference in the gradient recorded compared to the Levich equation (6.1). $B$ is the constant in the modified Saito equation (3.11), which was calculated using the value of the intercept.

<table>
<thead>
<tr>
<th>$D$ /x $10^{-9}$ m$^2$s$^{-1}$</th>
<th>$x_E$ /nm</th>
<th><strong>Intercept</strong> /$10^{-10}$ A</th>
<th><strong>Gradient</strong> /nA(m$^3$s$^{-1}$)$^{-1/3}$</th>
<th>$R^2$</th>
<th>$G$</th>
<th>$B$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>10</td>
<td>1.82 ± 0.04</td>
<td>362 ± 2</td>
<td>0.995</td>
<td>1.89</td>
<td>2.69</td>
</tr>
<tr>
<td>0.7</td>
<td>25</td>
<td>2.10 ± 0.02</td>
<td>504 ± 1</td>
<td>0.998</td>
<td>1.43</td>
<td>3.11</td>
</tr>
<tr>
<td>0.7</td>
<td>50</td>
<td>2.28 ± 0.03</td>
<td>714 ± 1</td>
<td>0.998</td>
<td>1.27</td>
<td>3.38</td>
</tr>
<tr>
<td>0.7</td>
<td>100</td>
<td>2.45 ± 0.04</td>
<td>1100 ± 2</td>
<td>0.9994</td>
<td>1.24</td>
<td>3.63</td>
</tr>
<tr>
<td>1.43</td>
<td>50</td>
<td>4.47 ± 0.11</td>
<td>1220 ± 4</td>
<td>0.996</td>
<td>1.35</td>
<td>3.24</td>
</tr>
</tbody>
</table>

To further investigate the origin of the intercept, the observed values were used as the limiting current in the modified Saito equation (3.11), and values for the constant $B$ were calculated. The results in TABLE 6.2 show that as the thickness of the nanoband is increased, the value of $B$ increases, which is as expected. Additionally, the value of $B$ at both values for the diffusion coefficient are similar: 3.38 and 3.24. These values are also consistent with the value of $B$ calculated in Section 6.3.2 as 3.23, using the steady-state response at a through-flow electrode without flow. Overall, this indicates that the intercept of the trend line from the higher flow region is indicative that the additional diffusion controlled current response is effectively added to the flow controlled mass transport.

This appears consistent with the Nernst-Planck equation (3.5), which shows an additive relationship between flux due to diffusion and convection and [9], which states that axial diffusion (in the direction of flow) is significant for the current response at microbands, at all but very high flow rates. This means that even once the electrode response begins to become affected by flow, the diffusion response is still significant; the results presented here suggests it is additive in this case. This can be seen in the simulation results for the concentration profiles in Figure 6.20. These profiles show that even at 50 mlh$^{-1}$ (1.39 x 10$^{-8}$ m$^3$s$^{-1}$) flow, there is still significant radial diffusion occurring at the electrode, meaning the response will not be only due to transport of material by flow. Due to time constraints, glucose measurements were not performed as part of this work as it was important to first acquire a greater understanding of the system behaviour.

6.4. Summary

Reproducible processes were developed to fabricate through-flow nanoband electrodes, based on the nanoband electrodes presented in Chapter 5. There were significant fabrication challenges to overcome to achieve through-wafer etching of these electrodes, protecting the fragile membrane, and ensuring the reproducibility of the etch to define the nanoband cavities through the wafer. The
developed procedure uses KOH etching to remove the bulk silicon material resulting in a membrane of SiO$_2$ and SiRN. Low adhesive dicing tape was used to mount the wafer for further processing steps to ensure compatibility with the vacuum chucks, which are common on cleanroom tools. Finally a process for dry etching using negative resist was developed to etch through the remaining membrane, which resulted in reproducible Pt nanoband dimensions for the through-flow electrodes.

These electrodes were tested and simulated and were found to have almost double the current response of the standard nanoband electrodes in Chapter 5 ($B = 3.23$, compared to 1.73). The current response of the electrodes changed with flow, but appeared to still follow the Nernst equation from modified Tafel plots.

The electrode response was simulated to further investigate the relationship to flow. A flow insensitive, flow sensitive and transition region were identified through these simulations. The flow insensitive region occurred over the relevant range of sweat flow rates for a device of this footprint area. The flow rate for the onset of the transition region could be estimated using the Péclet number. Additionally, the flow sensitive region deviated from the Levich equation (6.1), with both the offset and gradient of the linear region differing from that predicted.

The experimental results across a range of flow rates were consistent with the equivalent simulation results, and deviations could be accounted for by an adjustment of $D$ and considering the entry length for establishment of laminar flow in the channel. Therefore, further simulations were performed to characterise the deviations from the Levich equation (6.1). It was found that the deviations in the offset current could be accounted for by added diffusion (calculated using modified Saito (3.11)), and the deviations in gradient could be accounted for by an effect from the small electrode size (calculated with a $G$ value, which can be used to modify the Levich equation (6.8)).

This work could be used to design a system to optimise the response at given volumetric flow rates. As the diameter of the pore increases, the velocity will decrease (assuming the same footprint area, the same number of pores in the array and therefore the same volumetric flow). As pore diameter ($L$) decreases (6.4), the flow part of $Pe$ will increase with $L^{-3}$, and the diffusion part will increase.
with $L^{-2}$. This means that as $L$ (or pore diameter) decreases, the flow insensitive region at low flow rates (corresponding to the flow rate of sweat) will shrink and begin to become flow sensitive. This may seem counterintuitive, but without changing any other parameters the velocity through each pore (and therefore past each electrode) increases as the radius decreases. If an electrode were designed to behave in this way (e.g. sensitive to flow in the relevant range of flow rates for sweat), then it could be multiplexed with the device presented here and both concentration and flow rate could be extracted from the combined system. It remains to be evaluated whether such an electrode is realistic to fabricate, since it is likely to require significant redevelopment to the design presented here.

References


Chapter 7

Wafer-Scale Processing and Optimisation

7.1. Introduction
The final chapter of work in this thesis relates to optimisation of the fabrication or characterisation processes that occur at chip-level. Several of the fabrication processes used in this work require processing of individual chips, for example reference electrode chlorination and Nafion drop-casting. Additionally, the ability to characterise chips while they are still at wafer-scale rather than diced would allow measurements to be performed faster. Characterising or processing individual chips is time consuming and costly, and can introduce a higher variability between devices compared to a standardised wafer-scale process.

Electrochemical sensors are typically characterised and tested after the wafer has already been diced into chips, and often after the chips have been packaged. Packaging chips can be expensive so it is preferable for any failed devices to be identified before reaching this step. However, electrochemical testing of each individual chip is time-consuming and expensive. Therefore, it would be desirable to be able to test and characterise electrochemical devices at the wafer-scale to avoid packaging or further processing of failed devices.

The final process steps for the fabrication of the electrochemical devices presented in Chapters 4 and 5, require an electrochemical processing step to chlorinate the reference electrode and a Nafion deposition step. The wafer-scale electrochemical testing setup described in section 7.2 was used to perform electrochemical processing and Nafion drop-casting as well as device characterisation.
Nafion can be processed using standard microfabrication techniques, this is discussed in more detail in section 7.3. Since the electrodes are chlorinated before Nafion deposition, dicing the wafer into chips for chlorination prevents wafer-scale processing of Nafion. Therefore, wafer-scale electrochemical chlorination would allow microfabrication compatible Nafion processing to be possible. Wafer-scale electrochemical processing and characterisation would streamline the fabrication process saving both time and costs. The work reported in section 7.2 presents an extended system from [1] and looks at the feasibility of fully-automated wafer-scale testing, enables measurement in microlitre liquid samples and can perform localised fabrication. This work was published in [2] (Copyright © 2020, IEEE).

Nafion has been used as a biocompatible membrane to reduce interferents, extend electrode lifetimes and as an encapsulating layer to reduce biofouling. The final sensor system will have components with limited lifetimes, such as the enzyme layer in Chapter 5 and reference electrode in Chapter 4. The Nafion coating is used both as a protective layer and to increase the lifetime of components such as the reference electrode. Therefore, if the Nafion fails (e.g. by tearing or delamination) then this will compromise other components in the system, which are relying on the layer for protection or lifetime extension. This makes it important to characterise and to increase the adhesion and lifetime of Nafion films, this is discussed in section 7.3. The Nafion layer is applied as the final step of the fabrication procedure for the electrodes, which is advantageous, as it is a delicate material which can be easily damaged by standard fabrication processes.

Manufacturability of Nafion coated devices is also important as mass manufacturing requires a wafer-scale approach. Although the system in section 7.2 was used for wafer-scale processing of drop-cast Nafion, there are disadvantages to the drop-casting approach such as timing constraints due to processing chips one-by-one on the wafer and concerns over reproducibility of drop shape and volume. A wafer-scale approach using a
spin-coating technique is also discussed in section 7.3. This technique is highly compatible with microfabrication processes, would allow the throughput to be increased as well as ensuring the Nafion structures are uniform [3].

Limited work has been reported about wafer-scale microfabrication of Nafion membranes, therefore the development and optimisation of the microfabrication process for Nafion is an important goal. The work in this section is a continuation of previous work by Marland et al. [4] to characterise and improve the adhesion of Nafion when applied as an electrode coating layer. A refined version of the test structures is presented, and expanded results including further process optimisation and structure testing provided. Adaptations which may be required when integrating the Nafion membrane with the other components of the sensor are also discussed. Parts of the work presented in section 7.3 were published in [5] (Copyright © 2020, IEEE).

7.2. Wafer-Scale Electrochemical Processing and Characterisation

7.2.1. System Design and Wafer Fabrication

In order to facilitate wafer-scale processing and testing, the following components were required:

- An automated syringe pump with single microliter volume resolution containing a syringe with the required measurement solution;
- A customized jig (Figure 7.1) with probe tips for electrical connections, silica tubing (250 μm internal diameter) to dispense the solutions, an Ag/AgCl wire reference electrode, and an endoscope camera for precision optical tracking of the probe tip, tubing and solution locations;
- A test wafer comprising the devices to be characterized;
- A computer controlled 3D stage that controls the position of the test wafer relative to the custom probe jig;
- A potentiostat for the electrochemical measurements of the on-chip electrodes.

The system described here was used to chlorinate an on-chip reference electrodes, drop-cast a Nafion membrane, and to perform electrochemical test measurements. The controller and x-y-z stage of a standard 3D printer (Wanhao I3 Duplicator) were used as proof of concept in this work, the three position channels (with 10 μm resolution) retained the same function. The fourth channel, which would normally control the print head, was used to control the syringe pump (with single microlitre resolution) for dispensing the Nafion and measurement solutions. The modifications to the 3D printer were primarily completed by the other authors of [2], the wafer fabrication and experimental characterisation of the system form part of this thesis.

Figure 7.1: Diagram (not to scale) (adapted from [2], Copyright © 2020, IEEE) showing the jig designed for the 3D printer print head, which was used to hold the probe tips, tubing and camera over a single chip.
The 3D printer could be programmed to automatically move between chips on the wafer and dispense solution. A custom jig (shown in Figure 7.1) was designed for the 3D printer print head in collaboration with I. Schmueser, C. L. Mackay, and J. P. Elliott [2], which held the probes to connect to the electrode contact pads, a silica dispensing tube (250 μm inner diameter) positioned above the electrode working area, a camera to allow observation of the solution dispensing, and a Ag/AgCl wire to be used as a stable reference potential. The Ag/AgCl wire was used here for chlorinating the on-chip reference electrode, but could also have been used as the reference electrode for chip characterisation if no on-chip reference electrode was available.

Figure 7.2: (a) Schematic (adapted from [2], Copyright © 2020, IEEE) of mask used to fabricate the wafers (produced in KLayout). Magnification of the microdisc working electrode, reference and counter electrodes (WE, RE, CE). (b) Cross-section (not to scale) showing cross section from the dotted line in (a) showing the layers of the final device.
Figure 7.2a shows a diagram of the wafer design that was used to aid the development of the wafer-scale testing system. The wafer contains 14 chips with simple three electrode cells each consisting of a Pt microdisc working electrode, Ag/AgCl reference electrode, and Pt macroscale counter electrode. The devices were fabricated following a similar process to that reported in previous chapters, for which the runsheet can be found in Appendix B. This complete procedure integrates the individual fabrication of the working and reference electrodes into one complete flow. A cross section of the fabricated layers on the electrodes can also be seen in Figure 7.2b.

In order to corral the solution restricting it only to desired areas, alternating hydrophobic and hydrophilic regions are defined around the device. This is achieved using a photoresist layer (Figure 7.3), as the material is simple to deposit and pattern, and can be easily removed at the end of the testing process. Solutions could then be drop-cast using the
automated set-up, inside the area defined by the photoresist. In this work the wafer was aligned manually, by eye, on the 3D printer stage.

7.2.2. Wafer-Scale Processing

Before the electrodes were characterised, the final processing steps were completed using the wafer-scale system. The on-chip silver electrode was chlorinated using the Ag/AgCl wire, which was attached to the 3D printer system as a reference electrode. The silver on-chip electrode was connected as the WE and the Ag/AgCl wire connected as the RE. It would have been possible to use one of the on-chip Pt electrodes as a pseudo-reference electrode but it would not necessarily maintain a stable or reproducible potential during the measurement. Further information on the importance of controlling the chlorination process can be found in Chapter 4. The additional benefit of using the Ag/AgCl wire is that the OCP can be immediately measured between the wire and newly chlorinated on-chip reference electrode. This potential should be zero after the chlorination process has been completed. Therefore, the on-chip electrode can be both chlorinated and then tested in the same step.

A measurement solution of 0.1 M KCl in deionised water was placed in the syringe and the deposition over the electrodes was controlled by the print head channel of the 3D printer. The electrodes were chlorinated at a constant potential of +40 mV vs the Ag/AgCl wire. The potential was measured between the wire and the resulting on-chip reference electrodes, a measured potential of approximately 0 V confirmed the successful fabrication. The potential used in this section to control the chlorination is different to that used in Chapter 4. This is due to the different reference electrodes used for the chlorination processes. In the rest of this work, this was a commercial Ag/AgCl electrode with a 3 M filling solution, whereas the reference electrode used in this section was a chlorinated silver wire with no filling solution (therefore the potential was set by the 0.1 M KCl measurement solution).
Figure 7.4: (a) CV of chloride formation (oxidation peak above 0 V) and subsequent removal (reduction peak below -0.05 V) on the silver electrode vs. an Ag/AgCl wire at wafer-scale in 0.1 M KCl at 50 mVs$^{-1}$. (b) Chlorination of a silver electrode (until fully chlorinated) with chronoamperometry at 0.04 V using the same setup. Plotted using data presented in [2].

Figure 7.4a shows five consecutive CVs of the chlorination (oxidation) and de-chlorination (reduction) of the on-chip silver electrode. This shows the stability of the setup and electrodes since the process was reproducible.
over multiple cycles of chlorination. Figure 7.4b shows an example of the current and charge against time when an electrode was chlorinated by applying a constant potential of +40 mV between the on-chip silver electrode and the Ag/AgCl wire. The on-chip reference electrodes were chlorinated and confirmed to be successful by measuring their potential against the Ag/AgCl wire.

Once the electrodes were chlorinated, the Nafion membrane could be drop-cast on the wafer (either before or after the electrodes were characterised). Nafion was diluted 1:4 with deionised water to increase the viscosity, which helped to control the spread of the drops [6]. The solution in the syringe was replaced by the Nafion solution and its deposition on the wafer was controlled in the same way as previously. The movement of the stage of the 3D printer and the print head extruder could be programmed to follow an automated sequence. This allowed the Nafion membrane to be drop-cast on each chip without manual intervention if the wafer was initially aligned on the 3D printer stage.

7.2.3. Electrochemical Testing

The electrochemical performance of the full electrode cell was then tested. Dissolved oxygen was used as the example redox reaction, which meant the same 0.1 M KCl solution could be used to both chlorinate the Ag electrode and for simple electrochemical testing. Due to the small volume, evaporation becomes significant leading to an increase in the concentration of analyte in solution, an effect which was seen in [1]. However, dissolved oxygen maintains a constant concentration even if the water evaporates during the testing process. This allows a very small volume of liquid to be used as there is no need to compensate for evaporation loss. Figure 7.5 shows a linear sweep voltammogram of one of the devices, the linear sweep displays the characteristic wave-shaped response of a microelectrode. The system can therefore be used to inform decisions about further processing by identifying non-functional chips during the fabrication stage as a screening step prior to dicing and packaging. A video of one of the
structures being measured with the system described in this section can be found under: doi.org/10.7488/ds/2759. Further work was completed by I. Schmueser to integrate the system with a probe station and generate a wafer map of the current response from the oxygen reduction reaction [2].

Figure 7.5: Linear sweep voltammogram showing the reduction of atmospheric dissolved oxygen in 0.1 M KCl at 50 mVs$^{-1}$ on a 50 µm Pt microdisc using the fabricated electrodes at wafer-scale (plotted using data presented in [2]).

7.3. Nafion Fabrication and Optimisation

As stated in the Introduction, parts of the work in this section were published in [5] and were based on a continuation of the work presented in [4]. The wafers used in this section were fabricated jointly with J.R.K. Marland to ensure process consistency between these publications, and initial analysis of the set of microscope images from square test structures on one wafer were jointly analysed. The remaining sets of microscope images of the square test structures were split in half and analysed separately. The circle test structures were only analysed as part of this thesis, along with the drop-cast Nafion shown in Figure 7.8b.
7.3.1. Test Structure Design

As stated in the introduction, there are some disadvantages to drop-casting and it would be desirable to deposit the Nafion membranes using standard microfabrication processes. The ability to perform electrochemical processing (section 7.2.2), such as reference electrode chlorination, without dicing means wafers could be further processed. This provides the opportunity to pattern Nafion using standard microfabrication techniques rather than drop-casting. Wafers containing Nafion test structures were used in this section to quantitatively explore and allow optimisation and characterisation of the design and patterning of Nafion membranes.

The test structure design consisted of a layout of chips containing square or circular test structures ranging in width (or diameter for the circles) from 1.6 mm down to 12.5 μm. Each block comprised sixteen instances of the squares or circles at each of eight widths, and the overall layout consisted of eighteen instances of both blocks distributed evenly across the surface of a 100 mm diameter silicon wafer. These were used to optimise the fabrication process of the Nafion layer and to investigate membrane dimensions, and adhesion to the underlying base layer materials.

The previous work by Marland et al. [4] used square and grid test structures. The conclusions from this work suggested an increasing trend in Nafion survival rates as structure size decreased, as well as identifying that the structure corners could potentially act as initial failure points. Therefore, it was decided to adapt the previous test structure design. The grid structures were removed since they did not seem to show any additional data trends beyond those from the squares, and they were also a less practical design when it came to encapsulating electrodes.

The grid structures were replaced with circles to remove the potential corner failure points mentioned above. The minimum feature size was also decreased from 200 μm to 12.5 μm. A selection of the widths (200, 400, 800 and 1600 μm) were retained ensuring that a comparison could be
made with the previous results. A Leica microscope with an automated microscope stage was used to speed up image acquisition. The final test structure layout was adjusted to match the view field on the microscope to improve imaging efficiency. This new test structure design can be seen in Figure 7.6 and was used to test additional fabrication parameters as well as investigate the effect of a circular design and reduced feature size.

![Figure 7.6: Mask design for Nafion test structures on 100 mm wafer and magnified view of the 2 square and circle test structure blocks.](image)

### 7.3.2. Fabrication

The underlying insulating layers and platinum electrode layer used for the test structures were chosen as they are likely materials for commercial electrode device production. The insulating layers were deposited on 100 mm diameter silicon wafers using PECVD at low or high frequency to produce silicon nitride (LFSIN/HFSIN) and silicon oxide (LFSIO/HFSIO). The platinum and an underlying titanium seed layer were both deposited using electron beam evaporation.
The full process run used for the Nafion device fabrication can be found on the runsheet in Appendix B. Initially the wafers were cleaned using an oxygen plasma to ensure a smooth surface, free of particles. To promote the adhesion of the Nafion, the wafers were treated for 5 minutes in a Silane A-174 solution diluted at a 1:50 ratio with ethanol [7], and dried at 60°C in a Tennay oven for 30 minutes [8].

Spin-coating was used to deposit the Nafion because it can form part of a standard mass-manufacturing fabrication process and allows a highly reproducible thin-film to be obtained. Spin-coating parameters such as spin speed and solution viscosity can be changed in order to control the thickness of the resulting film [3], however a standard set of conditions was used here to allow comparisons between different membrane designs and underlying layers. The Nafion solution was spin-coated onto the wafers at 500 rpm, a low spin speed was used to obtain a thicker layer of Nafion. As the Nafion is a suspension, it was agitated before application on the wafer to ensure a more even distribution. In section 7.2.2 Nafion was diluted in water to change the viscosity for drop-casting, an additional advantage of spin-coating is that no dilution is required and the Nafion can be used directly as sourced. The layer was dried in air at room temperature until the solvent had visually evaporated, and then thermally annealed for 5 minutes at 120°C [9]. Annealing promotes robustness and increases solvent resistance of the Nafion film [10], but annealing at too high a temperature or for too long can make the film brittle [9].

Following application of the Nafion, wafers were primed in HMDS and spin-coated with SPR350 positive photoresist at 4000 rpm. The high spin speed was used to ensure a thin layer (~1 µm) of photoresist. The test structure pattern was transferred into the photoresist by photolithography (Karl Suss MA8), Nafion is sensitive to solvents including photoresist developer and acetone, which was used to strip the photoresist after processing. The thinner resist layer meant that the film spent less time in these potentially damaging solutions. The thickness of photoresist was still
sufficient to protect the covered areas of Nafion during the subsequent reactive ion etch process.

Previous work [4] compared MF-26A and AZ726 developers, which were used for 10 second periods before rinsing with deionised water to avoid long periods of submersion that could damage the Nafion. This was repeated until fully developed which typically took 3 – 4 cycles. This process is less compatible with standard microfabrication process flows due to the cycling between developer and deionised water. Therefore, a new developer option was tested which used a mixture of MF-26A diluted at 2:1 with deionised water. This development method was used for 90 s without any cycling, which makes it more compatible with manufacturing. It was also hypothesised that the diluted developer could additionally lead to improved adhesion or lifetime since it was less aggressive on the Nafion.

The thinner film of photoresist was particularly important when using the diluted developer as the dilution increases the development time. The timing is critical because it is extremely difficult to visually observe when the resist layer has been fully developed due to the problem of distinguishing the presence or absence of Nafion on the wafer (when inspected by eye while submerged in developer). Additionally, when using the diluted developer, if the wafer is removed, dried and then re-submerged, the development process doesn’t give reproducible results, so it is preferable to fully develop the wafer in one attempt.

Following the development of the photoresist, Nafion was etched using RIE with an oxygen plasma to remove the unwanted areas. Finally, the photoresist was stripped from the wafers by submersion in two baths of acetone for 30 seconds each. This was followed by submersion in water for 5 minutes to remove the acetone. Normally an isopropanol rinse would be used to remove acetone residue but this was replaced with an extended soak in deionised water, since Nafion is soluble in lower aliphatic alcohols (which includes isopropanol). Microscope images of the final fabricated Nafion test structures can be seen in Figure 7.7.
Profilometry measurements of the wafers developed with diluted MF-26A gave a mean thickness of 340 ± 15 nm across the wafers (n = 10 wafers). Figure 7.8a shows an example profilometry measurement using 1 mg stylus force, in which it can be seen that the Nafion structure has good uniformity across its diameter. The most likely cause of the initial small spike in height (at around x = 100 μm) is due to a small physical deformation of the Nafion structure caused by the Dektak stylus. Despite the increased rigidity resulting from annealing, the structures were only partially annealed to prevent them becoming too brittle so they still retain some deformability. The profilometry also appears to show steep vertical sidewalls on the Nafion suggesting the structures retain their patterned shape after processing (rather than reflowing or deforming at the edges).

Figure 7.8b shows an example profilometry measurement of a Nafion drop for comparison. It can be seen that the drop-cast Nafion has a very large edge bead – up to almost 10 times thicker than the thinnest point (around x = 1.5 mm). The drop-cast Nafion, at almost 5 mm in diameter, is considerably wider than the spin-coated structures where the largest diameter was 1.6 mm. However, it is more challenging to pipette small drops of Nafion since they must be aligned to the underlying electrodes by eye so a larger volume of liquid is necessary to ensure full coverage of the electrode. Patterning the spin-coated Nafion using photolithography allows more precise alignment and therefore smaller structures.
Figure 7.8: Example profilometry measurements across (a) spin-coated (400 μm diameter square), and (b) drop-cast (2 μl) Nafion membranes.
Profilometry measurements showed that the drop-cast structures were much less symmetrical than those of the spin-coated structures, which may be due to the drop-cast Nafion deforming to a greater extent with the Dektak stylus since it has not been annealed (which increases the rigidity). It could also occur as a result of the pipette not being perfectly vertical during drop-casting which could possibly lead to an asymmetrical Nafion drop. The thickness at the centre is around 100 nm in height, which is considerably lower than the spin-coated Nafion. Overall, the structure of the spin-coated Nafion is significantly more uniform and reproducible across the wafer. The uniformity in thickness is likely to affect the mass-transport of species to the electrode since the region covered by the membrane with respect to distance from the electrode will be consistent. This is likely to lead to better reproducibility between electrode responses.

7.3.3. Nafion Structure Testing

To investigate whether any of the design parameters affected Nafion durability, wafers with test structures fabricated as described above were immersed in an aqueous phosphate buffered saline (PBS) solution. Wafers were left in the solution for 6 weeks. Survival of the test structures was evaluated by comparison of optical microscope images before and after immersion. A minimum of 6 instances of each test structure layout were tested for each combination of developer and underlying material. Structures were classified as having survived if they were undamaged at the end of the test, or failed if they were damaged or absent (Figure 7.9). Previously there was a clear dependence on the developer used during processing, with wafers processed using MF-26A showing higher overall survival, compared to those processed using AZ726 [4]. The difference in failure rate corresponds with the finding that damage to the Nafion edges was far higher in wafers processed in AZ726. This suggests that the two effects may be linked, with damage during processing causing partial edge delamination that ultimately leads to failure of the structure during testing. Due to these results, it was expected that the wafers processed using the
diluted developer will show at least equal adhesion results to the undiluted MF-26A since they both show complete survival after development.

![Image](image_url)

**Figure 7.9:** Microscope image showing examples of survival and failure of (a) square and (b) circular Nafion test structures on HFSIN following immersion in PBS (left: survived, right: failed). Visible outline of structure remains after failure due to over-etching or roughening of wafer substrate during Nafion RIE step.

There was previously a slight trend towards survival based on base layer material, particularly when using the AZ developer. The Pt base layer had the best Nafion survival rate and the two silicon nitride base layers had the highest failure rates. The results from these developers also showed a weak trend between structure size and survivability, with the smallest size structure potentially showing a slightly increased survival rate compared to the others. Nafion is known to swell in liquids [11], which could generate stress within the film that would likely be increased in larger structures. This was the reason the smaller structures were added, however the additional tests using the new mask design were processed with diluted MF-26A and didn’t show any trend in survival rate for base layer/material or structure size (Figure 7.10). This may be partially due to an overall increase in survival with the diluted developer.
Figure 7.10: Durability of (a) square (plotted using data presented in [5]), and (b) circle Nafion test structures in PBS. Mean survival rates are shown for wafers processed in diluted MF-26A after immersion in PBS for 6 weeks. Error bars indicate the standard deviation.
The test structures developed using diluted MF-26A showed a survival rate of $98.6 \pm 5.9\%$ to $100.0 \pm 0.0\%$ (mean ± STD) across all the wafers tested ($n = 18$ blocks of square and circle test structures for each substrate). Although the wafers developed using diluted and undiluted MF-26A both showed 100% survival during processing there is a noticeable improvement in the durability results, particularly on the HFSIN substrate. Therefore it is possible that there was also damage caused by the undiluted MF-26A, which was not visible during optical inspection, but could have weakened the structure and made it more susceptible to failure. It is possible that if the structures were tested for an increased duration or more aggressively, then some additional trends might start to appear in this dataset. However, the results achieved for 6 weeks in PBS show excellent survival rates, and this time period is far greater than that which might be expected for other sensor components.

Overall, these results show the diluted MF-26A developer is the best developer choice out of those tested in this work and in [4] for the Nafion fabrication process. The initial results showed that HFSIO was the optimal base layer choice for Nafion adhesion, however the use of diluted MF-26A as the developer means that any of the tested base layers could be chosen without reducing the device lifetime over the 6 week test period. Similarly, there was no trend in the size of the Nafion structure or shape (square or circle). Overall, this provides greater flexibility in the sensor fabrication process as there are less limitations on material layers and Nafion geometry.

Testing the adhesion strength through immersion in PBS is not the most realistic method of investigating the durability of the Nafion structures. A more realistic test was performed through immersion in PBS and additional agitation of the solution with an orbital shaking table at 125 rpm. The added motion should better simulate the movement and disturbance a sensor may experience when worn. The optimised fabrication process with the diluted developer method was used and Nafion structures
were evaluated in the same way as before through optical microscopy (with the same classification of survival and failure).

Figure 7.11: Durability of (a) square (plotted using data presented in [5]), and (b) circle Nafion test structures in PBS. Mean survival rates are shown for wafers processed in diluted MF-26A after immersion in PBS and agitated by an orbital shaking table at 125 rpm for 6 weeks. Error bars indicate the standard deviation.
The structures all showed near-complete survival after 6 weeks of agitation (Figure 7.11), with a survival rate ranging from 98.3 ± 6.0% to 100.0 ± 0.0% (mean ± standard error of the mean) across all the base layer materials and structure sizes (1600 – 12.5 µm) that were tested (n = 18 blocks of square and circle test structures for each substrate). There were no trends for the durability on different underlying materials or structure sizes. Again, it is possible that over longer time periods the survival results may still follow the trend established previously with the less optimal developers. These survival results match well with the survival results from the durability measurements using the diluted MF-26A. They show that the Nafion structures can withstand prolonged immersion and mechanical agitation, which suggests membranes fabricated in this way should survive when used as a protective membrane on a sensor.

7.4. Summary

A system for wafer-scale electrochemical processing and testing was developed and demonstrated using a modified 3D printer. The system is capable of indexing to chips within a wafer and dispensing electrolyte or Nafion solutions as well as performing electrochemical processing and characterisation. This includes chlorination of a silver electrode to form the on-chip Ag/AgCl reference electrode and linear sweep voltammetry of dissolved oxygen to test the functionality of individual chips on the wafer. The system can therefore be used to inform decisions about further processing by identifying non-functional chips during the fabrication stage as a screening step prior to dicing and packaging. This work was also integrated with a probe station and a wafer map was generated using the current response from the oxygen reduction reaction [2].

Although it was possible to drop-cast Nafion using this wafer-scale processing system, spin-coating offers a higher throughput and compatibility with microfabrication techniques. Nafion test structures from 12.5 µm to 1600 µm were fabricated on wafers with various base layers (Pt and PECVD insulators) to optimise durability and identify any trends in
survival. The process described in this chapter with the diluted MF-26A development method was compatible with standard microfabrication techniques and resulted in highly uniform and reproducible Nafion structures. The survival rate of the Nafion structures was $98.6 \pm 5.9\%$ to $100.0 \pm 0.0\%$ after immersion in PBS for 6 weeks. The survival rate was additionally measured as $98.3 \pm 6.0\%$ to $100.0 \pm 0.0\%$ during enhanced testing, when immersed in PBS and agitated with an orbital shaker table at 125 rpm. Six weeks is a considerably longer duration than the lifetime of other device components so this means the Nafion layer is highly unlikely to be the point of failure of the sensor. The near complete Nafion survival for all base layer materials and structure sizes over the test period, as well as the reproducibility and compatibility with microfabrication suggest are very promising for future use as part of a wearable (or implantable) sensor.

References


Chapter 8

Conclusions

8.1. Summary

The aim of this work has been to develop a novel through-flow nanoband electrode array for use in sweat sensing, with glucose demonstrated as a proof-of-concept analyte. To achieve this goal, the essential components of the system that have been designed, fabricated and characterised are an on-chip Ag/AgCl reference electrode, and a Pt nanoband electrode array working electrode.

In Chapter 4, micro-disc on-chip Ag/AgCl reference electrodes were successfully fabricated using chlorination of deposited thin-film silver (250 nm). Optimisation of the chlorination process resulted in electrodes with a stable potential, consistent with the Nernst equation. The variation in potential across the relevant concentrations of Cl⁻ in sweat was calculated and found to be low enough to not lead to significant variation in the current measured at the working electrode. The failure mechanism of the electrodes was identified, and the addition of a Nafion membrane resulted in reference electrodes with measured lifetimes significantly higher than the application target of 24 hours.

Since the chlorination process for these reference electrodes was electrochemical, this potentially decreases the compatibility of the devices with mass manufacturing wafer-scale processing techniques. A method was presented in Chapter 7 to chlorinate the electrodes at the wafer-level using a modified 3D printer, which increased the throughput of the chlorination step. This system allowed electrodes to be chlorinated electrochemically without dicing the wafer into chips, providing the additional benefit that the wafers could be further processed after electrochemical chlorination. This system was also used to demonstrate electrochemical testing of fabricated electrodes at the wafer-level, which increased the throughput compared to dicing and testing individual chips.
Chapter 8. Conclusions and Future Work

Additionally, this system was used to demonstrate an automated method of drop-casting Nafion membranes onto the electrodes.

A simple first-generation glucose sensor was developed on Pt nanoband electrodes, which are a type of nanoelectrode that can be fabricated using standard microfabrication techniques. The electrode was simulated using COMSOL and the response was found to match to theory and experimental results. A reliable functionalisation procedure was developed to allow glucose detection. The working electrode was shown in Chapter 5 to be a high-sensitivity device, with a measurable, linear relationship to greater than 500 μM glucose and a limit of detection below 10 μM. The (natural) oxygen mediator was found to be sufficient for the device response to operate linearly across this range of concentrations, meaning no artificial mediator was required. This makes nanoband electrodes a promising technology for sweat sensing applications. Additionally, a Nafion membrane was applied to the nanoband electrodes and was shown to decrease the electrode response to interferents including ascorbic acid, acetaminophen, and uric acid. The electrode response to glucose was decreased less significantly than the response to the interferents.

The advantages of using a Nafion membrane across the whole device were both to decrease the effect of interferents on the working electrode (Chapter 5) and the cross-functionality of extending the lifetime of the reference electrode (Chapter 4). The ability to deposit a single membrane across the entire set of electrodes also increases the manufacturability and lowers the cost of the resulting device. The Nafion membrane was an important part of the device design, therefore developing a suitable Nafion deposition and patterning process was necessary. A wafer-scale fabrication process was presented in Chapter 7, which led to survival of close to 100% of the Nafion membrane test structures over the course of six weeks of testing. This was the case even under more realistic sweat testing conditions, with the test solution constantly agitated. These results were recorded on a range of wafer substrates, which demonstrated the robustness of the fabrication process.

Finally, the nanoband electrodes were further developed to allow through-flow operation. Significant challenges were overcome to develop a fabrication procedure, which resulted in reproducible devices. This involved
determining an appropriate through-wafer etching process using KOH, establishing a wafer mounting system to process wafers while minimising damage to the fragile membranes, and developing the nanoband cavity etch to ensure a vertical sidewall profile resulting in reproducible exposure of the Pt nanobands.

The response of these electrodes to flow was then characterised across a wide range of flow rates, through both simulations and experiments. The use of COMSOL simulations in this work allowed changes to be made to the electrode thickness and diffusion coefficient without the need for multiple fabrication runs. This allowed a better understanding of the relationship between current at the electrode and flow rate to be developed. The results of the simulations and experiments showed a reproducible Levich-like region at higher flow rates, with an enhanced sensitivity to flow compared to that predicted by the Levich equation, and a flow insensitive region across the relevant range of low flow rates for sweat measurement. These regions of flow insensitivity and sensitivity open up the possibility of multiplexing two appropriately designed electrodes to extract both flow and concentration results, which are key challenges in sweat sensing.

These through-flow electrodes were not functionalised and tested for glucose sensing as part of this work. However, the through-flow nanoband electrodes are likely to have an increased sensitivity due to their almost doubled current response to ferrocenemethanol (FcMeOH) compared to the standard nanoband electrodes. A reproducible functionalisation procedure was presented in Chapter 5, which could be used, but further characterisation of the electrodes for glucose sensing would be required to ensure the behaviour was not significantly changed. However, the results presented in this work did not give any reason to expect that either the flow insensitive region or the linear region for glucose detection would be significantly different from that demonstrated individually.

8.2. Applicability and Future Challenges for Sweat Sensing

The work in this thesis was focused on the development of electrodes and fabrication processes as proof-of-concept work towards a wearable sweat sensing device. The results of the work show good promise for use in sweat sensing. This section is not intended as an exhaustive analysis of the remaining challenges in
developing a wearable sweat sensor. However, some potential directions for future work are presented and discussed.

There remains a requirement for some fundamental characterisation experiments to be undertaken on the electrodes presented in this work. These are a pH study, to measure the behaviour of both the working and reference electrodes with respect to changes in pH, and also a lifetime study of the functionalised nanoband electrode. This additional work would fill the main gaps in knowledge regarding the behaviour and functionality of the microfabricated devices to better characterise their performance under the changing environmental conditions at the skin surface.

It would be desirable for the system components to be integrated onto a single chip. It should be trivial to integrate the counter and working electrodes, since the nanoband electrode fabrication process already separates the process steps needed to define and etch the bond pad, from those that produce the nanoband cavities. It would be simple to adapt the photomask to include the counter electrode patterning and etching within the steps to form the bond pads. The reference electrode should also be relatively simple to integrate with the standard nanoband electrode fabrication process. The photoresist used in the silver lift-off step is sufficiently thick that it would fill the entire depth of the nanoband cavities, which should mitigate any risk of unintentional silver deposition at the working electrode. The main integration challenge would be the through-flow nanoband electrodes due to their fragile membranes, which would be challenging to combine with the silver lift-off process. To combine these electrodes on the silicon substrates used for prototyping, the silver lift-off process would likely need to be performed without any sonication, which would add to the duration of this process step and may negatively impact the quality of the silver surface.

The silicon technology used in this work was intended as a prototyping substrate for the development and characterisation of the fabricated electrodes for proof-of-concept. Silicon wafers are a good choice of substrate for this purpose due to the standardised processing techniques that have been developed, which can be used to fabricate large quantities of reproducible chips. However, in order to be used as a wearable sensor, the device would need to be transferred to a
flexible substrate to increase the ability of the device to conform to the skin surface. As well as improved functionality as a wearable sensor, this should additionally increase the robustness of the through-flow electrodes since the fragile membrane would no longer be required. Flexible substrates are commonly available, and have been used previously for wearable sensors [1][2]. A new substrate would require the fabrication flow to be adapted to use different techniques. However, this would allow the electrodes to be combined on one chip and result in a robust device, which could conform to the skin and therefore be more suitable for use as a wearable sweat sensor. Redeveloping the system onto a flexible substrate would allow on-body tests to be performed to characterise the response of the sensor for the intended application.

Glucose detection was demonstrated as the proof-of-concept analyte in this work. However, many other analytes were discussed in Chapter 2 that would be valuable to detect and measure in sweat. The system presented in this work was developed for amperometric detection at an enzyme-functionalised electrode. This would make analytes such as lactate, cortisol, or ethanol suitable for further investigation. Multiplexed detection could improve the measurement accuracy, and resulting analysis, by providing additional data about the user’s health. In addition, electrodes to measure other health signals such as skin temperature and pH could be separately developed and would again lead to enhanced accuracy and extended system capabilities [3].

References


Appendix A

Materials

- Edge connectors: Mouser Electronics, SAMTEC PEC-03-01-T-S.
- Nafion solution: Sigma Aldrich, Perfluorinated resin solution containing Nafion™ 1100W 5 wt. % in lower aliphatic alcohols and water, contains 15-20% water.
- Phosphate buffered saline, 10X concentrate: Sigma Aldrich, BioPerformance Certified, suitable for cell culture.
- Glutaraldehyde (Grade II, 25% in H₂O; Sigma Aldrich) diluted to 1.25% in DI water.
- Glucose oxidase (GOx) from *Aspergillus Niger* (type VII, Sigma Aldrich) with an activity of 224890 Ug⁻¹ (1 U oxidises 1 μM β-D-glucose per minute at pH 5.1 at 35°C).
- Bovine serum albumin (BSA) (≥98%, Sigma Aldrich).
- Lactitol (~99%, Sigma Aldrich).
- 1,1,1,3,3,3 hexamethyldisilazane (HMDS) (97%, Sigma Aldrich)
- Photoresists (Microchemicals):
  - Megaposit SPR350.
  - Megaposit SPR 220-3.0.
  - Megaposit SPR 220-4.5.
  - AZ nLOF 2035.
- Developers: Megaposit MF-26A and AZ 726 MIF.
- Resist strippers:
  - ACT CMI-S (Merck), consisting of: N,N-Dimethylacetamide (90 % - 100 %), 2-Methylaminoethanol (5 % - 10 %), Quinolin-8-ol (1 % - 5 %)
  - MICROPOSIT Remover 1165 (Microchemicals)
- Aqua Regia, consisting of 3:2:1 HCl:H₂O:HNO₃, at 30°C.

Fabrication Tools

- Furnaces: Tempress 8” Oxide Furnace (HITEC) and 8” Nitride Furnace (HITEC).
- Mask aligner: Karl Suss MA.
- Virtual mask aligner: MicroWriter ML3, Durham Magneto Optics
- Dry etcher: JLS RIE80.

**Silicon Wafers**
- **SSP:**
  
<table>
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</tr>
<tr>
<td></td>
<td>Type/Dopant: N/Phos</td>
</tr>
<tr>
<td></td>
<td>Orientation: &lt;100&gt;</td>
</tr>
<tr>
<td></td>
<td>Resistivity: 1-10 ohmcm</td>
</tr>
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<td></td>
<td>Thickness: 525 +/- 25 µm</td>
</tr>
<tr>
<td></td>
<td>Finish: SS Polish</td>
</tr>
<tr>
<td></td>
<td>TTV: &lt;5 µm</td>
</tr>
<tr>
<td></td>
<td>Bow-Warp: &lt;30 µm</td>
</tr>
<tr>
<td></td>
<td>Particles: &lt;10 @ 0.3 µm</td>
</tr>
<tr>
<td></td>
<td>Flats SEMI</td>
</tr>
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</table>

- **DSP:**
  
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<tr>
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<td>Diameter: 100 mm</td>
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<tr>
<td></td>
<td>Type/Dopant: N/Phos</td>
</tr>
<tr>
<td></td>
<td>Orientation: &lt;100&gt;</td>
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<tr>
<td></td>
<td>Resistivity: 1-20 ohmcm</td>
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<tr>
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<td>Thickness: 500 +/- 25 µm</td>
</tr>
<tr>
<td></td>
<td>Finish: DS Polish</td>
</tr>
<tr>
<td></td>
<td>TTV: &lt; 5 µm</td>
</tr>
<tr>
<td></td>
<td>Bow-Warp: &lt; 30 µm</td>
</tr>
<tr>
<td></td>
<td>Particles: &lt; 10 @ 0.3 µm</td>
</tr>
<tr>
<td></td>
<td>1 SEMI Flat</td>
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Appendix B – Runsheets
Original runsheet for reference electrode fabrication provided by Ilka/Cami.

PROJECT DETAILS

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<th>Batch No:</th>
<th>No. of Wafers</th>
</tr>
</thead>
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<td>Process flow:</td>
<td>Wafer spec: 4” Si</td>
<td>Mask Set:</td>
<td>Mask Set Rev:</td>
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</tbody>
</table>

**Lower insulator**

<table>
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<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Thermal oxidation</td>
<td>Furnace 1</td>
<td>WETOX14, 40 min (~0.5µm)</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Measure oxide thickness</td>
<td>Nanospec</td>
<td>Oxide on silicon</td>
<td></td>
</tr>
</tbody>
</table>

**Electrode metal 1: Ti/Pt deposition and patterning**

<table>
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<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Deposit TiPt</td>
<td>ANS</td>
<td>10nm Ti, 50nm Pt</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>IPA, DIW, N2 clean</td>
<td>Solvent wet deck</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Dry</td>
<td>Hotplate</td>
<td>90s, 110°C (parameters not critical)</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>SPR220-3.0, 500RPM 5 sec, 2500RPM 60 sec</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 90 sec</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Expose</td>
<td>Karl Suss</td>
<td>Low vac / spacer, Exp 15 sec</td>
<td>Clean masks before and after</td>
</tr>
<tr>
<td>8.</td>
<td>Develop</td>
<td>Dish</td>
<td>MT26A, 60-90s; rinse dH2O; dry N2</td>
<td>Track by eye</td>
</tr>
<tr>
<td>9.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check development</td>
<td>Do not continue unless developed right!</td>
</tr>
<tr>
<td>10.</td>
<td>Aqua regia</td>
<td>Acid wet deck</td>
<td>3:2:1 HCl:H2O:HNO3, 30degC, agitate, etch rate ~1nm/min</td>
<td>Track by eye</td>
</tr>
<tr>
<td>11.</td>
<td>Measure oxide conductivity</td>
<td>Multimeter</td>
<td>Should be open circuit</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Measure oxide thickness</td>
<td>Nanospec</td>
<td>Oxide on silicon</td>
<td>Should be = thickness of thermal oxide grown</td>
</tr>
<tr>
<td>13.</td>
<td>Wet strip resist</td>
<td>Solvent wet deck</td>
<td>ACT, 50°C, with sonication 2x15mins; 10min IPA then IPA wash, DIW wash, N2 dry</td>
<td>Careful, ACT is nasty! Should come off more or less right away when in ACT; if it doesn’t, move wafer to fresh ACT after 15min and check again after extra 15min; IPA is to wash off ACT, DIW to wash off IPA</td>
</tr>
<tr>
<td>14.</td>
<td>Inspect wafer</td>
<td>Microscope</td>
<td>Check resist fully removed</td>
<td></td>
</tr>
</tbody>
</table>

**Top insulator deposition and patterning**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>O2 clean</td>
<td>Barrel asher</td>
<td>30 min</td>
<td></td>
</tr>
</tbody>
</table>
2. Deposit bi-layer
   Equipment: PECVD
   Process: HFSiOSiN
   Comments: Use sequencer

3. IPA, DIW, N2 clean
   Equipment: Solvent wet deck
   Process Parameters: 90s, 110°C (parameters not critical)

4. Dry
   Equipment: Hotplate
   Process Parameters: 110°C, 90 sec

5. Prime
   Equipment: Box
   Process Parameters: HMDS, 10 min

6. Coat resist
   Equipment: Polos
   Process Parameters: SPR220-3.0, 500RPM 5 sec, 2500RPM 60 sec

7. Soft bake
   Equipment: Hotplate
   Process Parameters: 110°C, 90 sec

8. Expose
   Equipment: Karl Suss
   Process Parameters: Low vac / spacer, Exp 15 sec

9. Develop
   Equipment: Dish
   Process Parameters: MF26A, 60-90s; rinse dH₂O; dry N₂
   Comments: Track by eye

10. Inspect resist
    Equipment: Microscope
    Process Parameters: Check development
    Comments: Do not continue unless developed right!

11. Etch oxide
    Equipment: JLS
    Process Parameters: Prog 21 (CHF₃ 17.7scm, Ar 20.1scm, 20mT, 200W), 10 min
    Comments: Make sure machine is clean (wipe with IPA)

12. Etch SiN
    Equipment: JLS
    Process Parameters: Prog 4 (Nitride), 6 min

13. Measure oxide on silicon in dicing channels
    Equipment: Nanospec
    Process Parameters: Oxide on silicon
    Comments: Expect just under thermal oxide thickness

14. Wet strip resist
    Equipment: Solvent wet deck
    Process Parameters: ACT, 50°C, with sonication; then IPA wash, DIW wash, N₂ dry
    Comments: Careful, ACT is nasty! Should come off more or less right away when in ACT; if it doesn’t, move wafer to fresh ACT after 15min and check again after extra 15min; IPA is to wash off ACT, DIW to wash off IPA

15. Inspect wafer
    Equipment: Microscope
    Process Parameters: Check resist removed

---

**Electrode metal 2: Lift off patterning of Ag**

<table>
<thead>
<tr>
<th>Step</th>
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<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>O₂ clean</td>
<td>Barrel asher</td>
<td>30 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>nLOF AZ 2035</td>
<td>Approx. 3µm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>700RPM 10 sec, 3000RPM 45 sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 1min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Expose</td>
<td>Karl Suss</td>
<td>Low vac / spacer, 15s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Post-exposure bake</td>
<td>Hotplate</td>
<td>110°C, 1 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Develop</td>
<td>Dish</td>
<td>AZ726, 2 min; rinse dH₂O; dry N₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check developed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>O₂ clean</td>
<td>Barrel asher</td>
<td>5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Deposit Ag</td>
<td>ANS</td>
<td>500nm silver</td>
<td>See Stewart Ramsey for ANS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>This mask is for capping both the electrode and bond pad, if you do not want metal on the bond pad, just put a strip of tape on if when it goes into the deposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Resist strip</td>
<td>Dish</td>
<td>ACT 60°C until gone, short (~60s) bursts of US</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step</td>
<td>Description</td>
<td>Equipment</td>
<td>Process Parameters</td>
<td>Comments</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td>-----------</td>
<td>-------------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Rinse</td>
<td>Dish</td>
<td>Rinse IPA, rinse DIW, dry N₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Chlorination</td>
<td>Dish</td>
<td>50mM FeCl₃ in water, 60 sec</td>
<td>Duration can be shortened to few seconds, kind of self-limiting reaction but leaving it too long will chlorinate all the Ag and kill the RE</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Rinse</td>
<td>Dish</td>
<td>dH₂O, dry N₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Inspect wafer</td>
<td>Microscope</td>
<td>Check resist fully removed</td>
<td>WAFERS NEED TO BE KEPT FROM LIGHT NOW! The Ag/AgCl is photosensitive! Do not stop at this step if at all avoidable</td>
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</tbody>
</table>

**Completion**

<table>
<thead>
<tr>
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<th>Process Parameters</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dice wafer into chips</td>
<td>Disco</td>
<td>3.75 / 35</td>
<td>Talk to Ewan</td>
</tr>
<tr>
<td>2.</td>
<td>Washing resist off</td>
<td>Solvent wet deck</td>
<td>30s in acetone bath, move to another acetone bath for 30s without drying in between, move to big water dish for 5min, gently dry with N₂</td>
<td>Be very gently with them, e.g. no pointing squeezy bottles or N₂ guns at them, or the Nafion will fall off</td>
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</table>
Adapted runsheet for reference electrode fabrication.

**PROJECT DETAILS**

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<td>Wafer spec: 4” Si</td>
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Runsheet for adapted process for reference electrodes.

### Lower insulator

<table>
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<th>Process Parameters</th>
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<tr>
<td>1</td>
<td>Thermal oxidation</td>
<td>Furnace 1</td>
<td>WETOX14, 40 min (~0.5µm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Measure oxide thickness</td>
<td>Nanospec</td>
<td>Oxide on silicon</td>
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### Electrode metal 1: Ti/Pt deposition and patterning

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<th>Process Parameters</th>
<th>Comments</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Deposit TiPt</td>
<td>ANS</td>
<td>10nm Ti, 50nm Pt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Inspect</td>
<td>Nanospec</td>
<td>Measure reflectance</td>
<td>480nm line ~170% is good, &lt;160% is bad</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>IPA, DIW, N2 clean</td>
<td>Solvent wet deck</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Dry</td>
<td>Hotplate</td>
<td>90s, 110°C (parameters not critical)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Coat resist</td>
<td>Polos</td>
<td>SPR22D-3.0, 500RPM 5 sec, 2500RPM 60 sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 90 sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Expose</td>
<td>Karl Suss</td>
<td>Low vac / spacer, Exp 15 sec</td>
<td>Clean masks before and after</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Develop</td>
<td>Dish</td>
<td>MF26A, 60-90s, rinse dH2O, dry N2</td>
<td>Track by eye</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check development</td>
<td>Do not continue unless developed right!</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Aqua regia</td>
<td>Acid wet deck</td>
<td>3:2:1 HCl:H2O:HNO3, 30degC, agitate, etch rate ~1nm/min</td>
<td>Track by eye. Water first</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Measure oxide conductivity</td>
<td>Multimeter</td>
<td>Should be open circuit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Measure oxide thickness</td>
<td>Nanospec</td>
<td>Oxide on silicon</td>
<td>Should be = thickness of thermal oxide grown</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Wet strip resist</td>
<td>Solvent wet deck</td>
<td>ACT, 50°C, with sonication 2x15mins; 10min IPA then IPA wash, DIW wash, N2 dry</td>
<td>Careful, ACT is nasty! Should come off more or less right away when in ACT; if it doesn’t, move wafer to fresh ACT after 15min and check again after extra 15min; IPA is to wash off ACT, DIW to wash off IPA</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Inspect wafer</td>
<td>Microscope</td>
<td>Check resist fully removed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Top insulator deposition and patterning

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 O₂ clean</td>
<td>Barrel asher</td>
<td>30 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Deposit bi-layer</td>
<td>Furnace 3</td>
<td>300 nm si-rich nitride</td>
<td>Measure using Nanospec</td>
<td></td>
</tr>
</tbody>
</table>
### Electrode metal 2: Lift off patterning of Ag

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>O₂ clean</td>
<td>Barrel asher</td>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>nLOF AZ 2035 700RPM 10 sec, 3000RPM 45 sec</td>
<td>Approx. 3µm</td>
</tr>
<tr>
<td>4.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 1min</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Expose</td>
<td>Karl Suss</td>
<td>Low vac / spacer, 15s</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Post-exposure bake</td>
<td>Hotplate</td>
<td>110°C, 1 min</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Develop</td>
<td>Dish</td>
<td>AZ726, 2 min; rinse dH₂O; dry N₂</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check developed</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>O₂ clean</td>
<td>Barrel asher</td>
<td>5 min</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Deposit Ag</td>
<td>ANS</td>
<td>10 nm Ti, 250 nm silver</td>
<td>See Stewart Ramsey for ANS This mask is for capping both the electrode and bond pad, if you do not want metal on the bond pad, just put a strip of tape on if when it goes into the deposition</td>
</tr>
<tr>
<td>11.</td>
<td>Resist strip</td>
<td>Dish</td>
<td>ACT 60°C until gone, short (~60s) bursts of US</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Rinse</td>
<td>Dish</td>
<td>Rinse IPA, rinse DIW, dry N₂</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Inspect wafer</td>
<td>Microscope</td>
<td>Check resist fully removed</td>
<td>WAFERS NEED TO BE KEPT FROM LIGHT NOW!</td>
</tr>
</tbody>
</table>
The Ag/AgCl is photosensitive! Do not stop at this step if at all avoidable

<table>
<thead>
<tr>
<th>Completion</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dice wafer into chips</td>
<td>Disco</td>
<td>3.75 / 35</td>
<td>Talk to Ewan. Use protective resist</td>
</tr>
<tr>
<td>2.</td>
<td>Washing resist off</td>
<td>Solvent wet</td>
<td>30s in acetone bath, move to another acetone bath for 30s without drying in between, rinse with IPA, rinse thoroughly in water, gently dry with N2</td>
<td>Be very gently with them, e.g. no pointing squeezy bottles or N2 guns at them, or the Nafion will fall off</td>
</tr>
</tbody>
</table>
Runsheet for nanoband electrodes provided by Ilka.

|-----------------|-------------------------|---------------------------------|-----------------------------------|

### WAFER CLEAN- If required

**Grow insulator**

<table>
<thead>
<tr>
<th>step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500nm Thermal SiO₂</td>
<td>Furnace 1</td>
<td>WETOX14, 0:40 mins</td>
</tr>
<tr>
<td>2</td>
<td>Inspect</td>
<td>Nanospec</td>
<td>Measure thickness, 5 pt</td>
</tr>
</tbody>
</table>

**Deposit electrode metal**

<table>
<thead>
<tr>
<th>step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Deposit Titanium</td>
<td>ANS</td>
<td>See SR</td>
</tr>
<tr>
<td>2</td>
<td>Deposit Platinum</td>
<td>ANS</td>
<td>See SR</td>
</tr>
<tr>
<td>3</td>
<td>Inspect</td>
<td>Microscope</td>
<td>Look for particulates/defects</td>
</tr>
<tr>
<td>4</td>
<td>Inspect</td>
<td>Nanospec</td>
<td>Measure reflectance (480nm line ~170% is good, anything &lt;160% is bad)</td>
</tr>
<tr>
<td>5</td>
<td>Inspect</td>
<td>4 point probe</td>
<td>Measure sheet resistance / resistivity</td>
</tr>
</tbody>
</table>

### Lithography metallisation

<table>
<thead>
<tr>
<th>step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IPA clean</td>
<td>Solvent wet deck</td>
<td>IPA, DIW, N2 dry</td>
</tr>
<tr>
<td>2</td>
<td>Bake</td>
<td>Hot plate</td>
<td>90degC, 1 min, this is for drying of the wafer, exact T,t not critical</td>
</tr>
<tr>
<td>3</td>
<td>Box Prime Wafers</td>
<td>Wet Bench, HMDS box</td>
<td>10mins, HMDS Primer, check if there is liquid in little vials in the back</td>
</tr>
<tr>
<td>4</td>
<td>Spin on Photoresist</td>
<td>Spin coater</td>
<td>SPR 220 – 3/ 500rpm, 5s then 2500rpm, 60s</td>
</tr>
<tr>
<td>5</td>
<td>Activate photoresist</td>
<td>Hot plate</td>
<td>110°C, 90 s</td>
</tr>
<tr>
<td>6</td>
<td>Clean mask</td>
<td>Solvent wet deck</td>
<td>Acetone clean</td>
</tr>
<tr>
<td>7</td>
<td>Inspect</td>
<td>Microscope</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Expose wafer</td>
<td>Karl Suss mask aligner</td>
<td>Low vac contact, 12s., METALLISATION</td>
</tr>
<tr>
<td>9</td>
<td>Develop</td>
<td>Solvent wet deck</td>
<td>MF26A developer, ~1-1.5min (visual track)</td>
</tr>
<tr>
<td>10</td>
<td>Rinse</td>
<td>DI gun</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Dry</td>
<td>N2 gun</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Inspect</td>
<td>Microscope</td>
<td>Check if has developed under a microscope; if in doubt, redo the entire lithography, take photos of lithography test lines</td>
</tr>
</tbody>
</table>

### Etch metallisation

<table>
<thead>
<tr>
<th>step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqua regia</td>
<td>Acid wet deck</td>
<td>3:2:1 HCl:H₂O₃:HNO₃, 30degC, agitate. etch rate ~1nm/min (Pt) and 0.1nm/min (Ti), DO NOT ADD WATER TO ACID EVER!</td>
</tr>
<tr>
<td>2</td>
<td>Rinse</td>
<td>Acid wet deck</td>
<td>DIW rinse</td>
</tr>
<tr>
<td>3</td>
<td>Inspect</td>
<td>Microscope</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Measure step height</td>
<td>Dektak</td>
<td>Confirm step height of Ti/Pt deposit</td>
</tr>
</tbody>
</table>

### Resist strip metallisation

<table>
<thead>
<tr>
<th>step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ACT bath 1</td>
<td>Ultrasonic bath</td>
<td>2 x 15 mins. ACT. Water Bath, 50°C, US on, this is overkill for most resist layers, a few minutes in room temperature ACT should do the trick, use US bath</td>
</tr>
<tr>
<td>3</td>
<td>IPA Rinse</td>
<td>Solvent wet deck</td>
<td>To wash off ACT residue</td>
</tr>
</tbody>
</table>
### Deposit insulator

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Clean</td>
<td>Barrel Asher</td>
<td>30 mins</td>
</tr>
<tr>
<td>2</td>
<td>300nm Si$_3$N$_4$</td>
<td>Furnace 3</td>
<td>Use test wafer (to measure thickness)</td>
</tr>
<tr>
<td>3</td>
<td>Inspect</td>
<td>Nanospec</td>
<td>Measure thickness, 5 pt</td>
</tr>
</tbody>
</table>

### Lithography insulator 1

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IPA clean</td>
<td>Solvent wet deck</td>
<td>IPA, DIW, N2 dry</td>
</tr>
<tr>
<td>2</td>
<td>Bake</td>
<td>Hot plate</td>
<td>90degC, 1 min, this is for drying of the wafer, exact T,t not critical</td>
</tr>
<tr>
<td>3</td>
<td>Box Prime Wafers</td>
<td>Wet Bench, HMDS box</td>
<td>10mins, HMDS Primer, check if there is liquid in little vials in the back</td>
</tr>
<tr>
<td>4</td>
<td>Spin on Photoresist</td>
<td>Spin coater</td>
<td>SPR 220 – 3/ 500rpm, 5s then 2500rpm, 60s</td>
</tr>
<tr>
<td>5</td>
<td>Activate photoresist</td>
<td>Hot plate</td>
<td>110°C, 90 s</td>
</tr>
<tr>
<td>6</td>
<td>Clean mask</td>
<td>Solvent wet deck</td>
<td>Acetone clean</td>
</tr>
<tr>
<td>7</td>
<td>Inspect</td>
<td>Microscope</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Expose wafer</td>
<td>Karl Suss mask aligner</td>
<td>Low vac contact, 12s., CONTACTS</td>
</tr>
<tr>
<td>9</td>
<td>Develop</td>
<td>Solvent wet deck</td>
<td>MF26A developer, ~1-1.5min (visual track)</td>
</tr>
<tr>
<td>10</td>
<td>Rinse</td>
<td>DI gun</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Dry</td>
<td>N2 gun</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Inspect</td>
<td>Microscope</td>
<td>Check if has developed under a microscope; if in doubt, redo the entire lithography, take photos of lithography test lines</td>
</tr>
</tbody>
</table>

### Etch insulator 1

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoride RIE</td>
<td>JLS RIE 80</td>
<td>#25, CF, 60sccm + Ar 4sccm, 60mT, 75W, DC bias voltage needs to be &gt;100V (else abort), etch rate ~20nm/min</td>
</tr>
<tr>
<td>2</td>
<td>Inspect</td>
<td>Microscope</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Measure track oxide thickness</td>
<td>Nanospec</td>
<td>Should read as max initial oxide thickness once nitride fully removed</td>
</tr>
<tr>
<td>4</td>
<td>Measure contact conductivity</td>
<td>Multimeter</td>
<td>Should read as resistance of Pt only once nitride fully removed</td>
</tr>
</tbody>
</table>

### Resist strip insulator 1

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ACT bath 1</td>
<td>Ultrasonic bath</td>
<td>2 x 15 mins, ACT, Water Bath, 50°C, US on, this is overkill for most resist layers, a few minutes in room temperature ACT should do the trick, use US</td>
</tr>
<tr>
<td>3</td>
<td>IPA Rinse</td>
<td>Solvent wet deck</td>
<td>To wash off ACT residue</td>
</tr>
<tr>
<td>4</td>
<td>DI water rinse</td>
<td>Solvent wet deck</td>
<td>To wash off IP A residue</td>
</tr>
<tr>
<td>5</td>
<td>Dry</td>
<td>Solvent wet deck</td>
<td>N2 gun</td>
</tr>
<tr>
<td>6</td>
<td>Inspect</td>
<td>Microscope</td>
<td></td>
</tr>
</tbody>
</table>

### Lithography insulator 2

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IPA clean</td>
<td>Solvent wet deck</td>
<td>IPA, DIW, N2 dry</td>
</tr>
<tr>
<td>2</td>
<td>Bake</td>
<td>Hot plate</td>
<td>90degC, 1 min, this is for drying of the wafer, exact T,t not critical</td>
</tr>
</tbody>
</table>

---

213
| 3 | Box Prime Wafers | Wet Bench, HMDS box | 10mins, HMDS Primer, check if there is liquid in little vials in the back |
| 4 | Spin on Photoresist | Spin coater | SPR 220 – 3/500rpm, 5s then 2500rpm, 60s |
| 5 | Activate photoresist | Hot plate | 110°C, 90s |
| 6 | Clean mask | Solvent wet deck | Acetone clean |
| 7 | Inspect mask | Microscope | |
| 8 | Expose wafer | Karl Suss mask aligner | Low vac contact, 12s., CAVITIES |
| 9 | Develop | Solvent wet deck | MF26A developer, ~1-1.5min (visual track) |
| 10 | Rinse | DI gun | |
| 11 | Dry | N2 gun | |
| 12 | Inspect | Microscope | Check if has developed under a microscope; if in doubt, redo the entire lithography, take photos of lithography test lines |

**Etch insulator 2**

<table>
<thead>
<tr>
<th>step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoride RIE</td>
<td>JLS RIE 80</td>
<td>#25, CF₄ 60sccm + Ar 4sccm, 60mT, 75W, DC bias voltage needs to be &gt;100V (else abort), etch rate ~20nm/min, run for the time required for the contacts etch to complete</td>
</tr>
<tr>
<td>2</td>
<td>Ar mill</td>
<td>JLS RIE 80</td>
<td>#8, Ar 25sccm, 30mT, 200W, DC bias voltage needs to be &gt;200V (else abort), etch rate ~7.8nm/min, can drill Pt into underlying insulator and make it conductive, if that happens top ~100nm of that layer needs to be removed</td>
</tr>
<tr>
<td>3</td>
<td>Inspect</td>
<td>Microscope</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Measure oxide thickness in cavities</td>
<td>Nanospec</td>
<td>Should read as max initial oxide thickness once nitride fully removed, only works right for cavities with at least 30um diameter, if all cavities are smaller, measurement will be off, in that case compare colours with tracks after stripping the resist</td>
</tr>
</tbody>
</table>

**Resist strip insulator 2**

<table>
<thead>
<tr>
<th>step</th>
<th>Description</th>
<th>Equipment</th>
<th>Recipe/Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ACT bath 1</td>
<td>Ultrasonic bath</td>
<td>2 x 15 mins, ACT, Water Bath, 50°C, US on, this is overkill for most resist layers, a few minutes in room temperature ACT should do the trick, use US</td>
</tr>
<tr>
<td>2</td>
<td>IPA Rinse</td>
<td>Solvent wet deck</td>
<td>To wash off ACT residue</td>
</tr>
<tr>
<td>3</td>
<td>DI water rinse</td>
<td>Solvent wet deck</td>
<td>To wash off IPA residue</td>
</tr>
<tr>
<td>4</td>
<td>Dry</td>
<td>Solvent wet deck</td>
<td>N2 gun</td>
</tr>
<tr>
<td>5</td>
<td>Inspect</td>
<td>Microscope</td>
<td></td>
</tr>
</tbody>
</table>

**REVISION HISTORY**

<table>
<thead>
<tr>
<th>REVISION</th>
<th>AUTHOR</th>
<th>DATE</th>
<th>REASON FOR CHANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>original</td>
<td>IS</td>
<td>15/02/2019</td>
<td>/</td>
</tr>
</tbody>
</table>
# Runsheets for test wafers for through-wafer etching using DRIE.

## PROJECT DETAILS

<table>
<thead>
<tr>
<th>Customer: Fiona</th>
<th>Project: Through-holes</th>
<th>Batch No:</th>
<th>No. of Wafers:</th>
</tr>
</thead>
</table>

### Process flow:
- **Wafer spec:** 4” Si
- **Mask Set:**
- **Mask Set Rev:**

## Process Flow

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Grind wafer</td>
<td>Wafer grinder</td>
<td>250 um wafer</td>
<td>Wafer is ~525um so need to remove ~275um to end up with 250um wafer. Ask Karina/Ewan.</td>
</tr>
<tr>
<td>2.</td>
<td>O₂ clean</td>
<td>Barrel asher</td>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Deposit bi-layer</td>
<td>PECVD</td>
<td>HFSIO, 500nm</td>
<td>Check times</td>
</tr>
<tr>
<td>4.</td>
<td>Deposit insulator on carrier</td>
<td>PECVD</td>
<td>HFSIO, 2um</td>
<td>Lots of oxide</td>
</tr>
<tr>
<td>5.</td>
<td>IPA, DIW, N₂ clean</td>
<td>Solvent wet deck</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Dry</td>
<td>Hotplate</td>
<td>90s, 115°C (parameters not critical)</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>SPR220-4.5, 700RPM 10 sec, 3000RPM 60 sec</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>115°C, 90 sec</td>
<td>Ramp up from room temp</td>
</tr>
<tr>
<td>10.</td>
<td>Measure resist</td>
<td>Nanospec</td>
<td></td>
<td>Measure thickness</td>
</tr>
<tr>
<td>11.</td>
<td>Expose</td>
<td>Karl Suss</td>
<td>contact / spacer, Exp 30 sec</td>
<td>Clean masks before and after. May need to lower the pins (wafer is thinned)</td>
</tr>
<tr>
<td>12.</td>
<td>Hold</td>
<td></td>
<td></td>
<td>Hold for 35 mins</td>
</tr>
<tr>
<td>13.</td>
<td>Post exposure bake</td>
<td>Hotplate</td>
<td>115°C, 90 sec</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Develop</td>
<td>Dish</td>
<td>MF26A, 60s; rinse dH₂O; dry N₂</td>
<td>Track by eye</td>
</tr>
<tr>
<td>15.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td></td>
<td>Check development</td>
</tr>
<tr>
<td>16.</td>
<td>Hard bake</td>
<td>Hotplate</td>
<td>115°C, 5 mins</td>
<td>Do not continue unless developed right!</td>
</tr>
<tr>
<td>17.</td>
<td>Etch oxide</td>
<td>ILS</td>
<td>Prog 21 (CHF₃ 17.7sccm, Ar 20.1sccm, 20mT, 200W), min</td>
<td>Make sure machine is clean (wipe with IPA). Check etch time.</td>
</tr>
<tr>
<td>18.</td>
<td>Etch Si</td>
<td>Plasmatherm</td>
<td>Standard bias recipe, 650 cycles, 1 hour</td>
<td>Etch all the way through, use a carrier wafer. Ask Peter.</td>
</tr>
<tr>
<td>19.</td>
<td>Wet strip resist</td>
<td>Solvent wet deck</td>
<td>1165, 60°C, with sonication 2x15mins; 10min IPA then IPA wash, DIW wash, N₂ dry</td>
<td>Careful, 1165 is nasty! If it doesn’t come off, move wafer to fresh 1165 after 15min and check again after extra 15min; IPA is to wash off ACT, DIW to wash off IPA</td>
</tr>
<tr>
<td>20.</td>
<td>Inspect wafer</td>
<td>Microscope</td>
<td></td>
<td>Check resist fully removed</td>
</tr>
<tr>
<td>21.</td>
<td>Thermal oxidation</td>
<td>Furnace 1</td>
<td>WETOX14, 40 min (~0.5µm)</td>
<td>Need to ramp up slowly because the wafer is thinned. Only do this if liquid won’t pass through already.</td>
</tr>
</tbody>
</table>
Runsheet for fabrication of through-flow nanoband electrodes.

PROJECT DETAILS

<table>
<thead>
<tr>
<th>Customer: Fiona</th>
<th>Project: Through-Hole Electrodes</th>
<th>Batch No:</th>
<th>No. of Wafers:</th>
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<tbody>
<tr>
<td>Process flow:</td>
<td>Wafer spec: 4&quot; Si</td>
<td>Mask Set:</td>
<td>Mask Set Rev:</td>
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</table>

### Lower insulator

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Deposit insulator/membrane layer</td>
<td>Furnace 1</td>
<td>500 nm oxide</td>
<td>Use DSP wafers Measure using Nanospec</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Deposit insulator/membrane layer</td>
<td>Furnace 3</td>
<td>130 nm Si-rich nitride</td>
<td>Aim on the high side. Measure using Nanospec</td>
<td></td>
</tr>
</tbody>
</table>

### Electrode metal 1: Ti/Pt deposition and patterning

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Deposit Ti/Pt</td>
<td>ANS</td>
<td>10nm Ti, 50nm Pt</td>
<td>On front side</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Inspect</td>
<td>Nanospec</td>
<td>Measure reflectance</td>
<td>480nm line ~170% is good, &lt;160% is bad</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>IPA, DIW, N2 clean</td>
<td>Solvent wet deck</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Dry</td>
<td>Hotplate</td>
<td>90s, 110°C (parameters not critical)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>SPR220-3.0, 500RPM 5 sec, 2500RPM 60 sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 90 sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Expose</td>
<td>DMO</td>
<td>Check dose (~135)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>PEB</td>
<td>Hotplate</td>
<td>110°C, 90 sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Develop</td>
<td>Dish</td>
<td>MF26A, 60-90s; rinse dH2O; dry N2</td>
<td>Track by eye</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check development</td>
<td>Do not continue unless developed right!</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Measure oxide conductivity</td>
<td>Multimeter</td>
<td>Should be open circuit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Measure oxide thickness</td>
<td>Nanospec</td>
<td>Oxide on silicon</td>
<td>Should be = thickness of thermal oxide grown</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Wet strip resist</td>
<td>Solvent wet deck</td>
<td>1165, 65°C, with sonication 2x15mins; 10min IPA then IPA wash, DIW wash, N2 dry</td>
<td>Careful, 1165 is nasty! Should come off more or less right away when in 1165; if it doesn't, move wafer to fresh 1165 after 15min and check again after extra 15min; IPA is to wash off 1165, DIW to wash off IPA</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>Inspect wafer</td>
<td>Microscope</td>
<td>Check resist fully removed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td></td>
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</table>

### Top insulator deposition and patterning

<table>
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<tr>
<th>Step</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>O2 clean</td>
<td>Barrel asher</td>
<td>60 min</td>
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</tr>
<tr>
<td>Step</td>
<td>Description</td>
<td>Equipment</td>
<td>Process Parameters</td>
<td>Comments</td>
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<td></td>
</tr>
<tr>
<td>1.</td>
<td>O₂ clean</td>
<td>Barrel asher</td>
<td>30 min</td>
<td></td>
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</tr>
<tr>
<td>2.</td>
<td>Etch silicon</td>
<td>KOH bath (80°C)</td>
<td>Etch all the way through silicon (around 10 hours+)</td>
<td>Make sure resist fully removed. Ask Cami/Richard for help (bath takes 1 hour to heat up). Check by eye. BE CAREFUL, do not touch KOH, use PPE. Leave in the water bath overnight/when you need to stop. Possible metal exposure in bath.</td>
<td></td>
</tr>
</tbody>
</table>

**Membrane silicon etch**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>SPR220-4.5, 700RPM 10s, 3000RPM 45s</td>
<td>Put blue dicing tape on back of wafer, be very careful not to leave air bubbles.</td>
</tr>
<tr>
<td>3.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 90 sec</td>
<td>DO NOT USE VACUUM, REMOVE TAPE</td>
</tr>
</tbody>
</table>

**Contact pad, RE and CE etch**

<table>
<thead>
<tr>
<th>Step</th>
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<th>Process Parameters</th>
<th>Comments</th>
</tr>
</thead>
</table>

---

2. Deposit bi-layer Furnace 3 300 nm si-rich nitride Measure using Nanospec

3. IPA, DIW, N₂ clean Solvent wet deck

4. Dry Hotplate 90s, 110°C (parameters not critical)

5. Prime Box HMDS, 10 min

6. Coat resist Polos SPR220-4.5, 500RPM 5 sec, 2500RPM 60 sec On backside, try to protect edges so the resist isn’t removed

7. Soft bake Hotplate 115°C, 90 sec

8. Expose DMO Check dose (~300) Backside alignment

9. PEB Hotplate 115°C, 90 sec

10. Develop Dish MF26A, 60-90s; rinse dH₂O; dry N₂ Track by eye

11. Inspect resist Microscope Check development Do not continue unless developed right!

12. Etch SiN JLS Prog 4 (Nitride), 8 min Sloped walls, check time. Measuring using Nanospec, should just be oxide thickness (check fit).

13. Etch SiO JLS Prog 21 (Oxide), 20 min Check time.

14. Measure oxide in etched areas Nanospec Oxide on silicon Should be around zero, ensure insulators have been etched to the silicon (check fit).

15. Wet strip resist Solvent wet deck 1165, 65°C, with sonication 2x15mins; 10min IPA then IPA wash, DIW wash, N₂ dry Careful, 1165 is nasty! Should come off more or less right away when in 1165; if it doesn’t, move wafer to fresh 1165 after 15min and check again after extra 15min; IPA is to wash off 1165, DIW to wash off IPA

16. Inspect wafer Microscope Check resist removed
<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td>Put blue dicing tape on back of wafer, be very careful not to leave air bubbles</td>
</tr>
<tr>
<td>2.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>nlof2035, 700RPM 10s, 3000RPM 45s</td>
<td>DO NOT USE VACUUM, REMOVE TAPE</td>
</tr>
<tr>
<td>3.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 60 sec</td>
<td>Ask Stewart to help (should be able to see so don’t need backside alignment)</td>
</tr>
<tr>
<td>4.</td>
<td>Expose</td>
<td>DMO</td>
<td>Check dose (~350)</td>
<td>Straight walls. Put in glass dish in case of breakages. Measure in hole to confirm etch. Etch rate ~20nm/min. CF4 60sccm + Ar 4sccm, 60mT, 75W, DC bias voltage needs to be &gt;100V (else abort).</td>
</tr>
<tr>
<td>5.</td>
<td>PEB</td>
<td>Hotplate</td>
<td>115°C, 60 sec</td>
<td>DO NOT USE VACUUM</td>
</tr>
<tr>
<td>6.</td>
<td>Develop</td>
<td>Dish</td>
<td>AZ726, 2 min; rinse dH2O; dry N2</td>
<td>Track by eye</td>
</tr>
<tr>
<td>7.</td>
<td>Rinse and dry</td>
<td>Dish/N2 gun</td>
<td>Rinse gently in beaker of water, dry gently with N2 gun.</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check development</td>
<td>Do not continue unless developed right!</td>
</tr>
<tr>
<td>9.</td>
<td>Hard bake</td>
<td>Hotplate</td>
<td>130°C, 60 sec</td>
<td>DO NOT USE VACUUM</td>
</tr>
<tr>
<td>10.</td>
<td>Etch SiN</td>
<td>JLS</td>
<td>Prog 25 (Nitride), 8 min</td>
<td>Straight walls. Put in glass dish in case of breakages. Measure in hole to confirm etch. Etch rate ~2-8nm/min. Ar 25sccm, 30mT, 200W, DC bias voltage needs to be &gt;200V (else abort).</td>
</tr>
<tr>
<td>11.</td>
<td>Etch Pt</td>
<td>JLS</td>
<td>Prog 4 (Pt), 8 min</td>
<td>Put in glass dish in case of breakages. Measure in hole to confirm etch. Etch rate ~7</td>
</tr>
<tr>
<td>12.</td>
<td>Etch SiN</td>
<td>JLS</td>
<td>Prog 25 (Nitride), 10 min</td>
<td>Straight walls. Put in glass dish in case of breakages. Look in microscope/use light to see if holes are all the way down.</td>
</tr>
</tbody>
</table>
through. Etch rate ~20 nm/min. CF4 60 sccm + Ar 4 sccm, 60 mT, 75 W, DC bias voltage needs to be >100 V (else abort).

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>1</td>
<td>Wet strip resist</td>
<td>Solvent wet deck</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Careful, 1165 is nasty! If it doesn’t come off, move wafer to fresh 1165 after 15 mins and check again after extra 15 mins; IPA is to wash off ACT, DIW to wash off IPA</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>Rinse and dry</td>
<td>Dish/N2 gun</td>
</tr>
</tbody>
</table>
Runsheet for electrodes used for wafer-scale processing and testing, provided by Ilka.

**PROJECT DETAILS**

<table>
<thead>
<tr>
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<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Thermal oxidation</td>
<td>Furnace 1</td>
<td>WETOX14, 40 min (~0.5µm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Measure oxide thickness</td>
<td>Nanospec</td>
<td>Oxide on silicon</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Lower insulator**

<table>
<thead>
<tr>
<th>Step</th>
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<tbody>
<tr>
<td>1.</td>
<td>Thermal oxidation</td>
<td>Furnace 1</td>
<td>WETOX14, 40 min (~0.5µm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Measure oxide thickness</td>
<td>Nanospec</td>
<td>Oxide on silicon</td>
<td></td>
<td></td>
</tr>
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</table>

**Electrode metal 1: Ti/Pt deposition and patterning**

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<tr>
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<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Deposit TiPt</td>
<td>ANS</td>
<td>10nm Ti, 50nm Pt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Inspect</td>
<td>Nanospec</td>
<td>Measure reflectance</td>
<td>480nm line ~170% is good, &lt;160% is bad</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>IPA, DIW, N2 clean</td>
<td>Solvent wet deck</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Dry</td>
<td>Hotplate</td>
<td>90s, 110°C (parameters not critical)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>SPR220-3.0, 500RPM 5 sec, 2500RPM 60 sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 90 sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Expose</td>
<td>Karl Suss</td>
<td>Low vac / spacer, Exp 15 sec</td>
<td>Clean masks before and after</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Develop</td>
<td>Dish</td>
<td>MF26A, 60-90s; rinse dH2O; dry N2</td>
<td>Track by eye</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check development</td>
<td>Do not continue unless developed right!</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Measure oxide conductivity</td>
<td>Multimeter</td>
<td>Should be open circuit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Measure oxide thickness</td>
<td>Nanospec</td>
<td>Oxide on silicon</td>
<td>Should be = thickness of thermal oxide grown</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Wet strip resist</td>
<td>Solvent wet deck</td>
<td>ACT, 50°C, with sonication 2x15mins; 10min IPA then IPA wash, DIW wash, N2 dry</td>
<td>Careful, ACT is nasty! Should come off more or less right away when in ACT; if it doesn’t, move wafer to fresh ACT after 15min and check again after extra 15min; IPA is to wash off ACT, DIW to wash off IPA</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Inspect wafer</td>
<td>Microscope</td>
<td>Check resist fully removed</td>
<td></td>
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</tbody>
</table>

**Top insulator deposition and patterning**

<table>
<thead>
<tr>
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</tr>
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<tbody>
<tr>
<td>1.</td>
<td>O2 clean</td>
<td>Barrel asher</td>
<td>30 min</td>
<td></td>
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</tr>
<tr>
<td>2.</td>
<td>Deposit bi-layer</td>
<td>Furnace 3</td>
<td>300 nm si-rich nitride</td>
<td>Measure test wafer using Nanospec</td>
<td></td>
</tr>
<tr>
<td>Step</td>
<td>Description</td>
<td>Equipment</td>
<td>Process Parameters</td>
<td>Comments</td>
<td></td>
</tr>
<tr>
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<td>-----------</td>
<td>--------------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>IPA, DIW, N2 clean</td>
<td>Solvent wet deck</td>
<td>90s, 110°C (parameters not critical)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Dry</td>
<td>Hotplate</td>
<td>90s, 110°C (parameters not critical)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
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</tr>
<tr>
<td>6.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>SPR220-3.0, 500RPM 5 sec, 2500RPM 60 sec</td>
<td></td>
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<tr>
<td>7.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 90 sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Expose</td>
<td>Karl Suss</td>
<td>Low vac / spacer, Exp 15 sec</td>
<td></td>
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</tr>
<tr>
<td>9.</td>
<td>Develop</td>
<td>Dish</td>
<td>MF26A, 60-90s; rinse dH2O; dry N2</td>
<td>Track by eye</td>
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<td>10.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check development</td>
<td>Do not continue unless developed right!</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Etch SiN</td>
<td>JLS</td>
<td>Prog 25 (Nitride), 18 min</td>
<td>Straight walls, check time</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Measure oxide on silicon in dicing channels</td>
<td>Nanospec</td>
<td>Oxide on silicon</td>
<td>Expect just under thermal oxide thickness</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Wet strip resist</td>
<td>Solvent wet deck</td>
<td>ACT, 50°C, with sonication; then IPA wash, DIW wash, N2 dry</td>
<td>Careful, ACT is nasty! Should come off more or less right away when in ACT; if it doesn’t, move wafer to fresh ACT after 15min and check again after extra 15min; IPA is to wash off ACT, DIW to wash off IPA</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Inspect wafer</td>
<td>Microscope</td>
<td>Check resist removed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Electrode metal 2: Lift off patterning of Ag**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Equipment</th>
<th>Process Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>O2 clean</td>
<td>Barrel asher</td>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>nLOF AZ 2035 700RPM 10 sec, 3000RPM 45 sec</td>
<td>Approx. 3µm</td>
</tr>
<tr>
<td>4.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 1min</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Expose</td>
<td>Karl Suss</td>
<td>Low vac / spacer, 15s</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Post-exposure bake</td>
<td>Hotplate</td>
<td>110°C, 1 min</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Develop</td>
<td>Dish</td>
<td>AZ726, 2 min; rinse dH2O; dry N2</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check developed</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>O2 clean</td>
<td>Barrel asher</td>
<td>5 min</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Deposit Ag</td>
<td>ANS</td>
<td>250 nm silver</td>
<td>See Stewart Ramsey for ANS Try to leave in ANS and immediately strip resist to avoid exposure to air</td>
</tr>
<tr>
<td>11.</td>
<td>Resist strip</td>
<td>Dish</td>
<td>ACT 60°C until gone, short (~60s) bursts of US</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Rinse</td>
<td>Dish</td>
<td>Rinse IPA, rinse DIW, dry N2</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Inspect wafer</td>
<td>Microscope</td>
<td>Check resist fully removed</td>
<td>WAFFERS NEED TO BE KEPT FROM AIR NOW! The Ag/AgCl is photosensitive</td>
</tr>
<tr>
<td>Step</td>
<td>Description</td>
<td>Equipment</td>
<td>Process Parameters</td>
<td>Comments</td>
</tr>
<tr>
<td>------</td>
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<td>--------------------</td>
<td>----------</td>
</tr>
<tr>
<td>1.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>SPR220-3.0, 500RPM 5 sec, 2500RPM 60 sec</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>110°C, 90 sec</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Expose</td>
<td>Karl Suss</td>
<td>Low vac / spacer, 15s</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Develop</td>
<td>Dish</td>
<td>MF26A, 60-90s; rinse dH2O; dry N2</td>
<td>Track by eye</td>
</tr>
<tr>
<td>6.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check developed</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Dice wafer into chips</td>
<td>Disco</td>
<td>3.75 / 35</td>
<td>Talk to Ewan, Use protective resist</td>
</tr>
</tbody>
</table>
Runsheet for Nafion test structure fabrication provided by Jamie.

### PROJECT DETAILS

<table>
<thead>
<tr>
<th>Step</th>
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<th>Process Parameters</th>
<th>Comments</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>O₂ clean</td>
<td>Barrel asher</td>
<td>30 mins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Deposit substrate material</td>
<td>PECVD</td>
<td>HFSIO, 10 mins</td>
<td>Target 500 nm</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Deposit substrate material</td>
<td>PECVD</td>
<td>HFSIN, 50 mins</td>
<td>Target 500 nm</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Deposit substrate material</td>
<td>PECVD</td>
<td>LFSIO, 10 mins</td>
<td>Target 500 nm</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Deposit substrate material</td>
<td>PECVD</td>
<td>LFSIN, 10 mins</td>
<td>Target 500 nm</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Deposit substrate material</td>
<td>ANS</td>
<td>10 nm Ti and 50 nm Pt on oxidised wafer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>O₂ clean</td>
<td>Barrel asher</td>
<td>30 mins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Prime</td>
<td>Dish</td>
<td>Silane A174 1:50 in ethanol, 5 min</td>
<td>Withdraw Silane A174 from bottle under N₂. Dab excess liquid from wafer edge after treating</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Dry</td>
<td>Oven</td>
<td>60°C, 30 min</td>
<td>Place wafer on glass box for support</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Coat Nafion</td>
<td>Polos</td>
<td>Nafion 5%, 150RPM 10s, 500RPM 30s; dry at RT</td>
<td>Use liner</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Anneal</td>
<td>Hotplate</td>
<td>120°C, 5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Prime</td>
<td>Box</td>
<td>HMDS, 10 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Coat resist</td>
<td>Polos</td>
<td>SPR350, 700 RPM 10 s, 4000 RPM 60 s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Soft bake</td>
<td>Hotplate</td>
<td>90°C, 90s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Expose</td>
<td>Karl Suss</td>
<td>Spacer / Prox (5µm), texp 10 s</td>
<td>Clean mask between wafers</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Develop</td>
<td>Dish</td>
<td>MF26A 2:1 water, 1.5min, rinse DIW, N₂ dry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Inspect resist</td>
<td>Microscope</td>
<td>Check development and alignment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Etch Nafion</td>
<td>Plasmalab</td>
<td>Prog #1 (O₂ 50sccm, RF 100W) 5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Inspect wafer</td>
<td>Microscope</td>
<td>Check etch complete</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>Washing resist off</td>
<td>Solvent wet deck</td>
<td>30s in acetone bath, move to another acetone bath for 30s without drying in between, move to big water dish for 5min, gently dry with N₂</td>
<td>Be very gently with them, e.g. no pointing squeezy bottles or N₂ guns at them, or the Nafion will fall off</td>
<td></td>
</tr>
</tbody>
</table>