Faculty of Science and Engineering  
Department of Chemistry

The Preparation and Electrochemical Characterization of Nanopore Array Membranes

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This thesis is presented for the Degree of  
Doctor of Philosophy  
of  
Curtin University

August 2014
Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: ...........................................
Date: .............................................
Contents Index

Declaration ...................................................................................................................... ii
Contents Index .............................................................................................................. iii
Acknowledgements ........................................................................................................ 2
Glossary of Major Symbols ............................................................................................. 4
Abstract ......................................................................................................................... 12
Chapter 1 ......................................................................................................................... 15
Introduction .................................................................................................................... 15
  1.1 Principles of electrochemistry ................................................................................ 15
  1.1.1 Background of electrochemistry ....................................................................... 15
  1.1.2 Solid-electrode electrochemistry ....................................................................... 15
  1.1.3 Electron transfer ................................................................................................. 16
  1.1.4 Faradaic and non-faradaic processes .................................................................. 17
  1.1.5 Polarisable and non-polarisable electrodes ......................................................... 18
  1.1.6 The electrical double layer ................................................................................. 19
  1.1.7 Mass transport ................................................................................................... 22
  1.2 Electrochemistry at the interface between two immiscible electrolyte solutions (ITIES) ......................................................................................................................... 24
  1.2.1 Background of liquid | liquid electrochemistry ................................................. 24
  1.2.2 Physical structure of the ITIES ......................................................................... 27
  1.2.3 Thermodynamics of the ITIES ......................................................................... 29
  1.2.4 Polarisable and non-polarisable ITIES ............................................................... 32
  1.2.5 Charge transfer processes at ITIES ................................................................. 35
  1.2.6 The voltammetric response of background electrolytes at the ITIES ............ 36
  1.2.7 The voltammetric response of analyte ion transfer at the ITIES ................. 39
  1.3 Miniaturisation of the ITIES: The development of micro- and nano-ITIES ................................................................................................................................. 41
  1.3.1 Theory of the development of miniaturised ITIES ........................................... 41
  1.3.2 History of the development of miniaturised ITIES ........................................... 42
  1.3.3 Methods of producing micro- and nano-ITIES ................................................. 45
  1.3.4 Gellification of the miniaturised ITIES ......................................................... 46
1.3.5 Diffusion regimes at singular micro- and nano-ITIES ........................... 47
1.3.6 Diffusion regimes at arrays of micro- and nano-ITIES .................. 52
1.3.7 Non-interacting diffusion zones .................................................. 54

1.4 Fabrication of solid-state nanopore membranes ......................... 56
1.4.1 Background of membrane technology ........................................ 56
1.4.2 Background of nanopore membrane .......................................... 56
1.4.3 Current methods of fabricating single and array solid-state nanopore membranes .............................................................. 58

1.5 Aim of the study ............................................................................. 61

Chapter 2 ............................................................................................. 63

Experimental methods and materials ............................................. 63

2.1 Micro- and nano-porous membrane experimental procedure .. 63
2.1.1 Micro- and nano-porous membranes ........................................... 63
2.1.2 Nanopore preparation via focused ion beam milling .................. 67
2.1.3 Nanopore preparation via transmission electron microscope instrument ............................................................... 69

2.2 The electrochemical cell ................................................................. 70
2.2.1 The four-electrode system at the ITIES ......................................... 70
2.2.2 The working electrode (WE) ........................................................ 71
2.2.3 The reference electrode (RE) ....................................................... 72
2.2.4 The counter electrode (CE) .......................................................... 73

2.3 Electrochemical techniques .............................................................. 74
2.3.1 Potentiodynamic ........................................................................ 74
2.3.1.1 Linear sweep voltammetry (LSV) ............................................... 74
2.3.1.2 Cyclic voltammetry (CV) ............................................................ 75
2.3.1.3 Stripping voltammetry (SV) ....................................................... 77
2.3.2 Potentiostatic ............................................................................ 79
2.3.2.1 Chronoamperometry (CA) ........................................................ 79

2.4 Experimental electrochemical procedures ..................................... 82
2.4.1 Reagents .................................................................................... 82
2.4.2 Preparation of micropore- and nanopore-supported ITIES ........ 83
2.4.3 The miniaturised ITIES experimental set-up: The four-electrode system ............................................................................. 85
2.4.4 The miniaturised ITIES experimental set-up: The two-electrode system ............................................................................. 86
2.4.5 Electrochemical procedure at the micro- and nano-ITIES arrays... 87
Chapter 3

Electrochemical Characterisation of Nanoscale-Liquid | Liquid Interfaces Confined within Silicon Nitride Membrane Prepared by Focused Ion Beam Milling

3.1 Introduction ........................................................................................................... 89
3.2 Experimental methods and materials ................................................................. 90
  3.2.1 Materials and reagents ...................................................................................... 90
  3.2.2 Nanopore preparation by FIB milling ............................................................... 90
  3.2.3 Contact angle study ......................................................................................... 91
  3.2.4 Preparation of nanopore-supported ITIES ...................................................... 91
  3.2.5 Electrochemical procedure ............................................................................... 91
3.3 Results and discussion .......................................................................................... 91
  3.3.1 Single and array nanopore preparation by FIB milling .................................... 91
  3.3.2 Electrochemical characterisation ...................................................................... 96
  3.3.2.1 TPrA$^+$ ion transfer across the nanopore membrane-modified ITIES .............. 96
  3.3.2.2 Influence of the concentration on the limiting current .................................. 101
  3.3.2.3 Influence of nanopore array geometry ......................................................... 103
  3.3.2.4 Influence of the TAA cation types on the transfer process ......................... 108
  3.3.2.5 Estimation of charging time ......................................................................... 110
3.4 Conclusion ............................................................................................................ 114

Chapter 4

Chronoamperometric Response at Nanoscale Liquid | Liquid Interface Arrays

4.1 Introduction .......................................................................................................... 115
4.2 Experimental methods and materials .................................................................. 119
  4.2.1 Reagents .......................................................................................................... 119
  4.2.2 Preparation of nano-interface arrays ............................................................... 119
  4.2.3 Experimental procedure .................................................................................. 119
4.3 Results and discussion .......................................................................................... 120
  4.3.1 Cyclic voltammetry of TPrA$^+$ transfer at the nano-ITIES array .................... 120
  4.3.2 Estimation of charging time .............................................................................. 123
  4.3.3 Chronoamperometry at the nanoITIES array ................................................ 128
4.4 Conclusions .......................................................................................................... 137
Chapter 5................................................................................................................. 139
Electrochemical Characterisation of Parylene C Porous Membrane Arrays...................... 139
5.1 Introduction........................................................................................................... 139
5.2 Experimental methods and materials ................................................................. 141
5.2.1 Reagents ........................................................................................................ 141
5.2.2 Preparation of ITIES ...................................................................................... 141
5.2.3 Experimental procedure .............................................................................. 142
5.2.4 Contact angle ............................................................................................... 143
5.2.5 Computational simulations .......................................................................... 143
5.3 Results and discussion ....................................................................................... 143
5.3.1 Cyclic voltammetry of TPrA\(^+\) transfer at parylene C porous membrane arrays ................................................................................................................. 143
5.3.2 CV of TPrA\(^+\) transfer at pure ITIES .......................................................... 151
5.3.3 Membrane porosity ...................................................................................... 159
5.3.4 Hydrophobicity and stability study ............................................................ 162
5.3.5 Simulations of membrane pore within an array ......................................... 163
5.4 Conclusion ........................................................................................................ 169
Chapter 6.................................................................................................................. 170
Electrochemical Detection of Ractopamine at Arrays of Micro-Liquid | Liquid Interfaces...................... 170
6.1 Introduction........................................................................................................... 170
6.2 Experimental methods and materials ................................................................. 173
6.2.1 Reagents ........................................................................................................ 173
6.2.2 Preparation of micro- and nano-interface arrays.......................................... 174
6.2.3 Experimental procedure at the micro-ITIES array ..................................... 175
6.2.4 Experimental procedure at the solid electrode ........................................... 176
6.3 Results and discussion ....................................................................................... 176
6.3.1 CV of RacH\(^+\) transfer at the micro-ITIES array ...................................... 176
6.3.2 Oxidation response of ractopamine ............................................................. 181
6.3.3 Thermodynamic parameters of RacH\(^+\) transfer at the water | DCH interface ................................................................................................................................. 185
6.3.4 Optimisation of the LSSV parameters for the detection of RacH\(^+\) .......... 188
6.3.5 LSSV analysis of RacH\(^+\) transfer at the micro-ITIES array ................. 192
6.3.6 Influence of the potentially interfering substances on RacH\(^+\) detection ................................................................................................................................. 193
6.3.7 Determination of RacH$^+$ in artificial serum ........................................ 196
6.3.8 CV of RacH$^+$ transfer at the nano-ITIES array ..................................... 200
6.3.9 CV of protonated salbutamol at the micro-ITIES array ..................... 203
6.4 Conclusion ................................................................................................. 205

Chapter 7 .............................................................................................................. 207

General Conclusions and Suggestions for Future Studies. 207
7.1 General conclusions ...................................................................................... 207
7.2 Suggestions for future studies ...................................................................... 210
References ........................................................................................................... 212
Appendix A ......................................................................................................... 228
Appendix B ......................................................................................................... 231
Appendix C ......................................................................................................... 233
Appendix D ......................................................................................................... 235
Appendix E ......................................................................................................... 245
Appendix F ......................................................................................................... 256
To my beloved family………
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# Glossary of Major Symbols

## Subscripts

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
<th>Symbol</th>
<th>Meaning</th>
</tr>
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<tbody>
<tr>
<td>$b$</td>
<td>bulk</td>
<td>$i$</td>
<td>ion, component</td>
</tr>
<tr>
<td>$c$</td>
<td>charging</td>
<td>$lit$</td>
<td>literature</td>
</tr>
<tr>
<td>$dl$</td>
<td>double layer</td>
<td>$p$</td>
<td>a) peak, b) pore</td>
</tr>
<tr>
<td>$eq$</td>
<td>equilibrium</td>
<td>$ss$</td>
<td>steady-state (or limiting)</td>
</tr>
<tr>
<td>$exp$</td>
<td>experimental</td>
<td>$u$</td>
<td>uncompensated</td>
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## Units

<table>
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<th>Symbol</th>
<th>Meaning</th>
</tr>
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<tr>
<td>$A$</td>
<td>Ampere</td>
<td>$min$</td>
<td>minute</td>
</tr>
<tr>
<td>Å</td>
<td>Angstrom</td>
<td>$mol$</td>
<td>mole</td>
</tr>
<tr>
<td>$C$</td>
<td>Celcius</td>
<td>$µL$</td>
<td>microlitre</td>
</tr>
<tr>
<td>$F$</td>
<td>Farad</td>
<td>$µM$</td>
<td>microMolar</td>
</tr>
<tr>
<td>$g$</td>
<td>gram</td>
<td>$µm$</td>
<td>micrometre</td>
</tr>
<tr>
<td>$J$</td>
<td>Joule</td>
<td>$µs$</td>
<td>microsecond</td>
</tr>
<tr>
<td>keV</td>
<td>kiloelectronVolt</td>
<td>nA</td>
<td>nanoAmpere</td>
</tr>
<tr>
<td>kV</td>
<td>kiloVolt</td>
<td>nF</td>
<td>nanoFarad</td>
</tr>
<tr>
<td>$M$</td>
<td>Molar</td>
<td>nm</td>
<td>nanometre</td>
</tr>
<tr>
<td>$MΩ$</td>
<td>megaOhm</td>
<td>pA</td>
<td>picoAmpere</td>
</tr>
<tr>
<td>$mL$</td>
<td>millilitre</td>
<td>pF</td>
<td>picoFarad</td>
</tr>
<tr>
<td>mM</td>
<td>milliMolar</td>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>mV</td>
<td>milliVolt</td>
<td>V</td>
<td>Volt</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
<td>% w/v</td>
<td>percentage weight per volume</td>
</tr>
<tr>
<td>$ms$</td>
<td>millisecond</td>
<td>$Ω$</td>
<td>Ohm</td>
</tr>
<tr>
<td>Symbol</td>
<td>Meaning</td>
<td>Usual unit</td>
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</tr>
<tr>
<td>--------</td>
<td>---------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>$A$</td>
<td>a) electrode area</td>
<td>a) cm², m²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) cross sectional area of pores</td>
<td>b) cm², m²</td>
<td></td>
</tr>
<tr>
<td>$a$</td>
<td>activity of the species</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$C$</td>
<td>a) capacitance</td>
<td>a) F</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) concentration</td>
<td>b) M, mol cm⁻³, mol m⁻³</td>
<td></td>
</tr>
<tr>
<td>$C_{dl}$</td>
<td>double layer capacitance</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>$C_{exp}$</td>
<td>experimental capacitance</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>$C_{exp}^0$</td>
<td>experimental specific capacitance, or experimental capacitance per unit area</td>
<td>F cm⁻²</td>
<td></td>
</tr>
<tr>
<td>$C_i$</td>
<td>bulk concentration of the transferring ions or electroactive species</td>
<td>M, mol cm⁻³, mol m⁻³</td>
<td></td>
</tr>
<tr>
<td>$C_{lit}$</td>
<td>capacitance from literature</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>$C_{lit}^0$</td>
<td>capacitance per unit area from literature</td>
<td>F cm⁻²</td>
<td></td>
</tr>
<tr>
<td>$C_O$</td>
<td>concentration of O</td>
<td>M, mol cm⁻³, mol m⁻³</td>
<td></td>
</tr>
<tr>
<td>$C_R$</td>
<td>concentration of R</td>
<td>M, mol cm⁻³, mol m⁻³</td>
<td></td>
</tr>
<tr>
<td>$\partial C(x, t)/\partial x$</td>
<td>concentration gradient at distance $x$ from the electrode surface and time $t$</td>
<td>M, mol cm⁻³, mol m⁻³</td>
<td></td>
</tr>
<tr>
<td>$\partial \phi(x)/\partial x$</td>
<td>potential gradient</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$C_b$</td>
<td>bulk concentration</td>
<td>M, mol cm⁻³, mol m⁻³</td>
<td></td>
</tr>
<tr>
<td>$C_s$</td>
<td>surface concentration</td>
<td>M, mol cm⁻³, mol m⁻³</td>
<td></td>
</tr>
<tr>
<td>$D$</td>
<td>diffusion coefficient</td>
<td>cm² s⁻¹, m² s⁻¹</td>
<td></td>
</tr>
<tr>
<td>$D_{aperture}$</td>
<td>aperture diameter</td>
<td>m</td>
<td></td>
</tr>
<tr>
<td>$D_i^a$</td>
<td>diffusion coefficients of the transferring ions in the aqueous phase</td>
<td>cm² s⁻¹, m² s⁻¹</td>
<td></td>
</tr>
<tr>
<td>$D_i^b$</td>
<td>diffusion coefficients of the transferring ions in the organic phase</td>
<td>cm² s⁻¹, m² s⁻¹</td>
<td></td>
</tr>
<tr>
<td>$d$</td>
<td>diameter</td>
<td>m, cm</td>
<td></td>
</tr>
<tr>
<td>$d_{inner}$</td>
<td>inner diameter</td>
<td>m, cm</td>
<td></td>
</tr>
<tr>
<td>$d_{outer}$</td>
<td>outer diameter</td>
<td>m, cm</td>
<td></td>
</tr>
<tr>
<td>$dE/dt$</td>
<td>potential variation with time</td>
<td>V s⁻¹</td>
<td></td>
</tr>
<tr>
<td>$E$</td>
<td>potential of an electrode</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$E^0$</td>
<td>standard ion transfer potential</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$E^{0'}$</td>
<td>formal potential of an electrode</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Unit</td>
<td></td>
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<td>--------</td>
<td>--------------------------------------------------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>$E_{eq}$</td>
<td>equilibrium potential</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$E_{rp}$</td>
<td>return peak potential</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$E_p$</td>
<td>peak potential</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$E_{p,W\rightarrow O}$</td>
<td>forward peak potential</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$E_{p,O\rightarrow W}$</td>
<td>reverse peak potential</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$E_1$</td>
<td>initial (or starting) potential</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$E_2$</td>
<td>final (or switching) potential</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$E_{1/2}$</td>
<td>half-wave potential in voltammetry</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$\Delta E$</td>
<td>a) potential interval</td>
<td>a) V</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) peak-to-peak separation</td>
<td>b) V</td>
<td></td>
</tr>
<tr>
<td>$e^-$</td>
<td>electron</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday constant</td>
<td>C mol$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\Delta G_{\text{transfer},i}^{0,\alpha \rightarrow \beta}$</td>
<td>standard Gibbs transfer energy of species $i$ from phase $\alpha$ into phase $\beta$</td>
<td>kJ mol$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\Delta G_{\text{transfer}}^{0',\text{w} \rightarrow \text{o}}$</td>
<td>formal Gibbs transfer energy of ion from aqueous to the organic phase</td>
<td>kJ mol$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$I$</td>
<td>diffusion-limited current</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>$I_c$</td>
<td>charging current</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>$I_{eos}$</td>
<td>current at the end of the forward scan</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>$I_p$</td>
<td>peak current</td>
<td>A</td>
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<td>$I_{p,W\rightarrow O}$</td>
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<td>$I_{p,O\rightarrow W}$</td>
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<td>A</td>
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</tr>
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<td>$I_{rp}$</td>
<td>return peak current</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>$I_{ss}$</td>
<td>steady-state (or limiting) current</td>
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</tr>
<tr>
<td>$I_{calc}$</td>
<td>calculated current</td>
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<tr>
<td>$I_{calc,total}$</td>
<td>calculated total current</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>$I_{exp}$</td>
<td>measured experimental current from cyclic voltammetry experiment</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>$I R$ or $I_{ss} R$</td>
<td>potential drop</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>$I^{z+}$</td>
<td>ion with charge $z^+$</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$I_{95%}$</td>
<td>current signal which is 95 % of the final signal value</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>$J$</td>
<td>flux</td>
<td>mol cm$^{-2}$ s$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$j$</td>
<td>current density</td>
<td>A m$^{-2}$, mA cm$^{-2}$</td>
<td></td>
</tr>
<tr>
<td>$J_{calc}$</td>
<td>calculated steady-state current density</td>
<td>A m$^{-2}$, mA cm$^{-2}$</td>
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</tr>
<tr>
<td>Symbol</td>
<td>Definition / Description</td>
<td>Unit</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>$j_{exp}$</td>
<td>experimental current density</td>
<td>$\text{A m}^{-2}, \text{mA cm}^{-2}$</td>
<td></td>
</tr>
<tr>
<td>$k^0$</td>
<td>standard heterogeneous rate constant</td>
<td>$\text{cm s}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$L$</td>
<td>working distance</td>
<td>m</td>
<td></td>
</tr>
<tr>
<td>$L^{z+}$</td>
<td>ligand with charge $z+$</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$l$</td>
<td>a) recess depth, or interface position within the pore channel</td>
<td>a) m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) membrane thickness</td>
<td>b) m</td>
<td></td>
</tr>
<tr>
<td>$N$</td>
<td>number of moles reacted in a system</td>
<td>mol</td>
<td></td>
</tr>
<tr>
<td>$N_p$</td>
<td>number of pores in the array</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>stoichiometric number of electrons involved in an electrode reaction</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$\eta$</td>
<td>viscosity</td>
<td>$\text{m}^2 \text{s}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>organic phase</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>oxidized form of the standard system, $0 + ne^- \rightleftharpoons R$</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$P_{DCH}^{0}$</td>
<td>partition coefficient of the ionised form in DCH</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$P_{n-oct}^{0}$</td>
<td>partition coefficient of the neutral form in $n$-octanol</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$Q$</td>
<td>charge</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>reduced form of the standard system, $0 + ne^- \rightleftharpoons R$</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>molar gas constant</td>
<td>$\text{J K}^{-1} \text{mol}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$R_{a,b}$</td>
<td>total bulk array resistance</td>
<td>$\Omega$</td>
<td></td>
</tr>
<tr>
<td>$R_b$</td>
<td>bulk solution resistance</td>
<td>$\Omega$</td>
<td></td>
</tr>
<tr>
<td>$R_{a,p}$</td>
<td>total pore array resistance</td>
<td>$\Omega$</td>
<td></td>
</tr>
<tr>
<td>$R_p$</td>
<td>pore resistance</td>
<td>$\Omega$</td>
<td></td>
</tr>
<tr>
<td>$R_u$</td>
<td>uncompensated resistance</td>
<td>$\Omega$</td>
<td></td>
</tr>
<tr>
<td>$R_u C_{exp}$</td>
<td>cell time constant</td>
<td>s</td>
<td></td>
</tr>
<tr>
<td>$R_u C_{dl}$</td>
<td>cell time constant</td>
<td>s</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>electrode radius</td>
<td>m, cm</td>
<td></td>
</tr>
<tr>
<td>$r_a$</td>
<td>pore radius</td>
<td>m, cm, nm</td>
<td></td>
</tr>
<tr>
<td>$r_c$</td>
<td>pore centre-to-centre separation</td>
<td>m, cm, nm</td>
<td></td>
</tr>
<tr>
<td>$r_c/r_a$</td>
<td>ratio of pore radius and pore-to-pore separation</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$r_d$</td>
<td>diffusion domain boundary/radius</td>
<td>m, cm</td>
<td></td>
</tr>
</tbody>
</table>
$S$  solubility
$T$  absolute temperature  K
$t$  time  s
$V(x,t)$  hydrodynamic velocity  cm s$^{-1}$
$v$  scan (or sweep) rate  V s$^{-1}$
$v^{1/2}$  square root of the scan rate  V$^{1/2}$ s$^{-1/2}$
$W$  aqueous phase  none
$x$  distance  m, cm
$x_1$  distance of the IHP from the electrode surface  m, cm
$x_2$  distance of the OHP from the electrode surface  m, cm
$z$  charge number of the transferring ion  none

**Greek symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
<th>Usual unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>a) denotes the aqueous phase of a liquid</td>
<td>a) none</td>
</tr>
<tr>
<td></td>
<td>liquid electrochemical setup</td>
<td>b) $^\circ$</td>
</tr>
<tr>
<td>$\beta$</td>
<td>denotes the organic phase of a liquid</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>liquid electrochemical setup</td>
<td></td>
</tr>
<tr>
<td>$\gamma$</td>
<td>a) activity coefficient</td>
<td>a) none</td>
</tr>
<tr>
<td></td>
<td>b) ratio of the diffusion coefficients of the transferring ions in the</td>
<td>b) none</td>
</tr>
<tr>
<td></td>
<td>aqueous and organic phases</td>
<td></td>
</tr>
<tr>
<td>$\delta$</td>
<td>diffusion zone extension</td>
<td>none</td>
</tr>
<tr>
<td>$\varepsilon_r$</td>
<td>dielectric constant</td>
<td>none</td>
</tr>
<tr>
<td>$\varepsilon_0$</td>
<td>vacuum permittivity</td>
<td>F m$^{-1}$ (or C$^{2}$ N$^{-1}$ m$^{-2}$)</td>
</tr>
<tr>
<td>$\eta$</td>
<td>overpotential, $E - E_q$</td>
<td>V</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>conductivity of a solution</td>
<td>S cm$^{-1}$ (or $\Omega^{-1}$ cm$^{-1}$)</td>
</tr>
<tr>
<td>$\mu$</td>
<td>chemical potential</td>
<td>kJ mol$^{-1}$</td>
</tr>
<tr>
<td>$\bar{\mu}$</td>
<td>electrochemical potential</td>
<td>kJ mol$^{-1}$</td>
</tr>
<tr>
<td>$\tau$</td>
<td>time variable</td>
<td>none</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Galvani (or inner) potential</td>
<td>V</td>
</tr>
<tr>
<td>$\Delta_{\phi}^{\alpha}$</td>
<td>Galvani interfacial potential difference at a liquid</td>
<td>V</td>
</tr>
<tr>
<td></td>
<td>liquid interface</td>
<td></td>
</tr>
</tbody>
</table>
\[ \Delta_{o}^{w} \theta^{0'} \] formal Galvani potential of ion transfer from aqueous to the organic phase

\[ \nabla^{2} \] Laplacian operator

\[ \theta \] membrane macroscopic coverage of pores or membrane porosity

### Standard abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>aq</td>
<td>aqueous</td>
</tr>
<tr>
<td>CA</td>
<td>chronoamperometry</td>
</tr>
<tr>
<td>C.A.</td>
<td>contact angle</td>
</tr>
<tr>
<td>ca.</td>
<td>circa</td>
</tr>
<tr>
<td>CBD</td>
<td>convergent-beam diffraction</td>
</tr>
<tr>
<td>CCP</td>
<td>cubic close-packed</td>
</tr>
<tr>
<td>CE</td>
<td>counter (or auxiliary) electrode</td>
</tr>
<tr>
<td>CT</td>
<td>charge transfer</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammetry</td>
</tr>
<tr>
<td>CVD</td>
<td>chemical vapour deposition</td>
</tr>
<tr>
<td>DPV</td>
<td>differential pulse voltammetry</td>
</tr>
<tr>
<td>DRIE</td>
<td>deep reactive ion etching</td>
</tr>
<tr>
<td>EBL</td>
<td>electron beam lithography</td>
</tr>
<tr>
<td>e-beam</td>
<td>electron beam</td>
</tr>
<tr>
<td>EIS</td>
<td>electrochemical impedance spectroscopy</td>
</tr>
<tr>
<td>ET</td>
<td>electron transfer</td>
</tr>
<tr>
<td>FEM</td>
<td>finite element method</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast Fourier Transform</td>
</tr>
<tr>
<td>FIB</td>
<td>focused ion beam</td>
</tr>
<tr>
<td>FIBSEM</td>
<td>focused ion beam scanning electron microscope/ microscopy</td>
</tr>
<tr>
<td>GCE</td>
<td>glassy carbon electrode</td>
</tr>
<tr>
<td>HCP</td>
<td>hexagonally close packed</td>
</tr>
<tr>
<td>IHP</td>
<td>inner Helmholtz plane</td>
</tr>
<tr>
<td>IPE</td>
<td>ideally polarised (or polarisable) electrode</td>
</tr>
<tr>
<td>IT</td>
<td>ion transfer</td>
</tr>
<tr>
<td>ITIES</td>
<td>interface between two immiscible electrolyte solutions</td>
</tr>
<tr>
<td>LOD</td>
<td>limit of detection</td>
</tr>
<tr>
<td>LSV</td>
<td>linear sweep voltammetry</td>
</tr>
</tbody>
</table>
LSSV | linear sweep stripping voltammetry
---|---
MD | molecular dynamics
MVN | modified Verwey-Niessen
OHP | outer Helmholtz plane
org | organic
pI | isoelectric point
pK_a | logarithmic of the acid dissociation constant
PMA | porous membrane arrays
PSCA | potential step chronoamperometry
R | linear correlation coefficient
RE | reference electrode
redox | reduction-oxidation
r.s.d. | relative standard deviation
SCE | saturated calomel electrode
SECM | scanning electrochemical microscopy
SEM | scanning electron microscope/microscopy
SWV | square wave voltammetry
SV | stripping voltammetry
TEM | transmission electron microscope/microscopy
WE | working electrode
µITIES | micro-interface between two immiscible electrolyte solutions

### Chemical abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>silver</td>
</tr>
<tr>
<td>Ag</td>
<td>AgCl</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>BTPPA⁺</td>
<td>bis(triphenylphosphoranylidene)ammonium cation</td>
</tr>
<tr>
<td>BTTPPACl</td>
<td>bis(triphenylphosphoranylidene)ammonium chloride</td>
</tr>
<tr>
<td>BTTPPATPBCl</td>
<td>bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>calcium chloride</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>chloride anion</td>
</tr>
<tr>
<td>1,2-DCE</td>
<td>1,2-dichloroethane</td>
</tr>
<tr>
<td>1,6-DCH</td>
<td>1,6-dichlorohexane</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
</tbody>
</table>
DNRH$^+$ daunorubicin cation
FeCl$_3$ ferric chloride
Hg | Hg$_2$Cl$_2$ | Cl$^-$ calomel reference electrode
KCl potassium chloride
KH$_2$PO$_4$ potassium phosphate monobasic
KTPBCl potassium tetrakis(4-chlorophenyl)borate
K$^+$ potassium cation
LiCl lithium chloride
Li$^+$ lithium cation
MeOH methanol
MgSO$_4$ magnesium sulfate
NaCl sodium chloride
Na$_2$SO$_4$ sodium sulfate
NaH$_2$PO$_4$ sodium phosphate monobasic
Na$^+$ sodium cation
NB nitrobenzene
NPOE 2-nitrophenyl octyl ether
parylene C poly(chloro-para-xylylene)
Pt platinum
PBS phosphate buffered saline
PET polyethylene terephthalate
PVC poly(vinylchloride)
Rac ractopamine
RacH$^+$ ractopamine cation
SalH$^+$ salbutamol cation
Si silicon
SiC silicon carbide
Si$_3$N$_4$ or SiN silicon nitride
SiO$_2$ or SiO silicon dioxide
TBA$^+$ tetrabutylammonium cation
TBABr tetrabutylammonium bromide
TEA$^+$ tetraethylammonium cation
TEACL tetraethylammonium chloride
TPBCl$^-$ tetrakis(4-chlorophenyl)borate anion
TPrA$^+$ tetrapropylammonium cation
TPrACl tetrapropylammonium chloride
Abstract

This thesis discusses the preparation, characterisation and application of ion transfer at the miniaturised interfaces between two immiscible electrolyte solutions (ITIES). Ion transfer electrochemistry of the model analyte, tetrapropylammonium cation (TPrA$^+$), and ionised drug, protonated ractopamine (RacH$^+$) across the water | 1,6-dichlorohexane (1,6-DCH) interface were studied by voltammetry and amperometry.

In Chapter 3, the electrochemical behaviour of single and arrayed nanoITIES formed at pores fabricated via focused ion beam (FIB)-milling of silicon nitride (SiN) membranes is presented. Nanopores in the range of ca. 30 to 80 nm in radii within a 500 µm x 500 µm SiN film were investigated towards the diffusion behaviour and the resulting current response. Nanopore arrays in cubic close-packed (CCP) arrangement were prepared with ratios of pore-to-pore separation to pore radius in the range of 16 to 32, at which the interfacial transfer of TPrA$^+$ across the single and array nanoITIES were characterised by cyclic voltammetry (CV). The limiting-current region of the CVs exhibited a steady increase of the current with applied potential up to the switching potential, referred to here as ‘sloping steady-state’ current behaviour. Overlapping diffusion profiles at adjacent nanoITIES resulted in lower experimental current. Despite this, the apparent steady-state behaviour may be explained with the radial diffusion dominance at the edge of the arrays, suppressing the linear diffusion within the arrays. In addition, analysis of the resistance and capacitance of the nanoITIES membrane system predicted that the resistance dominated the charging time measurement. FIB milling allows a maskless prototyping of nanopore arrays with different geometrical parameter and hence, is an attractive and simple method for the fabrication of membrane pores to support formation of nanoITIES.
In Chapter 4, potential step chronoamperometry (PSCA) was used to study the behaviour of arrays of nanoITIES formed at nanoporous SiN membranes prepared previously by electron beam lithography (EBL). The nanoITIES arrays contained 400 nanopores in a hexagonal close-packed (HCP) arrangement. Three membrane designs, with nanopore radii of 75, 50 and 17 nm, were studied by ion-transfer of TPrA\(^+\) across the nanopore array-supported water \(\mid\) 1,6-DCH interface. The cell time constants and charging time were determined prior to experimental PSCA. The three membrane designs studied exhibited charging times in the range of 0.08 s to 0.46 s, with the smallest pore configuration (17 nm radius) exhibiting the longest charging time. The experimental steady-state currents were 30 - 50 % lower than of the calculated inlaid disc current model, due to diffusion zone overlap at adjacent interfaces. The three nano-interface arrays studied also showed response times of 6 ± 1 s, being the time required to reach 95 % of the steady-state current.

Conformal vapour deposition of poly(chloro-p-xylylene), or parylene C, onto electron microscopy grids has been reported for the fabrication of highly ordered uniform porous membrane arrays (PMA). Chapter 5 presents the first results on parylene C PMA characterisation by electrochemistry at the ITIES. Parylene C PMA, with concave-shape pores (orifice and concave sizes of 8.3 \(\mu\)m and 0.7 \(\mu\)m, respectively) and interpore distance of 16.5 \(\mu\)m, was electrochemically characterised using CV of the transfer of TPrA\(^+\) across water \(\mid\) 1,6-DCH interfaces. Ion transfer from the aqueous to organic phase on the forward scan indicated a contribution of radial and linear diffusion control, while the strong peak-shaped voltammogram obtained on the reverse scan was attributed to linear diffusion control of the back-transfer of ion from organic to aqueous phase. Voltammetric response broadening suggested a contribution from residual uncompensated Ohmic drop. Computer simulation by finite element modelling demonstrated the possibility that the ITIES was located towards the organic phase side of the membrane. The current at the PMA-patterned interface was 48 % lower than that at a
pure ITIES. The results indicate the possibility to use organic membrane for the creation of miniaturised ITIES arrays for diverse application.

The final chapter of the thesis presented the behaviour of RacH⁺ at an array of µITIES, investigated via CV and linear sweep stripping voltammetry (LSSV). The µITIES array was formed at microporous silicon membranes, and containing 30 pores of radius 11.09 ± 0.12 µm, and pore centre-to-centre separation of 18.4 ± 2.1 times the pore radius. CV shows that RacH⁺ transferred across the water |1,6-DCH µITIES array at a very positive applied potential, close to the upper limit of the potential window. Nevertheless, CV was used in the estimation of some of the drug’s thermodynamic parameters, such as the formal transfer potential and the Gibbs transfer energy. Preliminary results on the oxidation response of ractopamine were also reported. LSSV was implemented by pre-concentration of the drug, into the organic phase, followed by voltammetric detection, based on the back-transfer of RacH⁺ from the organic to aqueous phase. Under optimised pre-concentration and detection conditions, a limit of detection of 0.1 µM was achieved. In addition, the impact of substances such as sugar, ascorbic acid, metal ions, amino acid, urea and uric acid on the available potential window was assessed. The detection of RacH⁺ in artificial serum indicated that the presence of serum protein interferes in the detection signal, so that sample deproteinisation is required for feasible bioanalytical applications. In this study, preliminary results on RacH⁺ and protonated salbutamol (SalH⁺) transfer at the nano- and micro-ITIES array, respectively, are briefly discussed. Due to the insufficient potential window for the ion transfer process, this study focussed on the drug detection at the micro-ITIES array to provide a basis for future studies at nano-ITIES arrays. Electrochemistry at the ITIES has proven a viable technique for drug detection.
Chapter 1

Introduction

1.1 Principles of electrochemistry

1.1.1 Background of electrochemistry
Electrochemistry is a subset of chemistry, which is concerned with the correlation between electrical activity and its chemical effects [1, 2]. The science of electrochemistry focuses on electron transfer process occurring at the electrolyte (an ionic conductor) | electrode (an electronic conductor) interface [1, 3, 4]. ‘Equilibrium electrochemistry’ and ‘dynamic electrochemistry’ are the two categories in this field. The first category occurs when an equilibrium potential difference is established between the electrode and electrolyte without the passage of a current, while the latter involves application of a potential difference to move the electrode away from its equilibrium value, which yields a current-potential (I versus E) curve or voltammogram [1, 4, 5].

1.1.2 Solid-electrode electrochemistry
Solid-electrode electrochemistry is defined as a heterogeneous chemical process of charge (electron) transfer between an electronic conductor or semiconductor (electrode) and an electro-active chemical species dissolved in an ionic conductor (electrolyte) [1, 2]. Examples of popular solid electrodes are carbon, platinum and gold [6], although a liquid conductor such as mercury can also be used.
1.1.3 Electron transfer

Electron transfer causes oxidation and reduction processes to occur at the electrode surface. The loss of electrons from the chemical species in the solution to the electrode is termed an oxidation or anodic process (Equation 1.1.1), while the gain of electrons from the electrode is termed a reduction or cathodic process (Equation 1.1.2) [1, 3, 5]:

\[ R \rightarrow O + ne^- \quad \text{(Eq. 1.1.1)} \]
\[ O + ne^- \rightarrow R \quad \text{(Eq. 1.1.2)} \]

where \( R \) and \( O \) represent the chemical species that undergo oxidation and reduction, respectively.

These oxidation/reduction (redox) reactions at the electrodes are driven by the application of a potential difference and are governed by Faraday’s law. The redox reactions will occur in a potential region where electron transfer is thermodynamically or kinetically favourable. In the case of thermodynamically controlled systems, the working electrode potential will reach a steady-state and equilibrium since no net current is permitted to flow through the cell [7]. The equilibrium potential of the working electrode, \( E_{eq} \), is given by the Nernst equation for the half reaction [1, 3]:

\[ E_{eq} = E^{0'} + \frac{RT}{nF} \ln \frac{C_O(0,t)}{C_R(0,t)} \quad \text{(Eq. 1.1.3)} \]

where \( E^{0'} \) is the formal potential of the redox couple (V), \( R \) is the molar gas constant (8.314 J K\(^{-1}\) mol\(^{-1}\)), \( T \) is the absolute temperature (K), \( n \) is the number of electrons transferred in the reaction, and \( F \) is the Faraday constant (96,485.4 C mol\(^{-1}\)). \( C_O \) and \( C_R \) represent the concentrations of \( O \) and \( R \), at the electrode surface, respectively. In the case where the potential of the working electrode is made more negative than the equilibrium potential, then \( C_O \) and \( C_R \) must take up new values (by reducing the ratio of \( C_O/C_R \)). This ratio change can only be made by the passage of a cathodic current [1, 3, 6].
Generally, electron transfer rate at the electrode surface is one key factor in determining the current or electrode reaction rate. The kinetics of electron transfer depends on the standard heterogeneous rate constant, \( k^0 \), in which a large \( k^0 \) value will achieve equilibrium faster; while, a small \( k^0 \) value will reach equilibrium slower [1].

### 1.1.4 Faradaic and non-faradaic processes

Two types of processes may occur in an electrochemical cell, faradaic and non-faradaic, and these processes contribute to the measured current [5]. The first term, ‘faradaic’, is the transfer of charge (e.g. electron) across the electrode | electrolyte interface which causes the oxidation or reduction process to occur, and is governed by Faraday’s law [1, 5, 6]. Faraday’s law correlates the amount of chemical reaction caused by the flow of current to the amount of electricity passed through a cell [1, 3]. The resulting current is termed as faradaic current, described by the following equation:

\[
\frac{dQ}{dt} = \frac{d}{dt}(nFN) = nF \frac{dN}{dt} \quad \text{(Eq. 1.1.4)}
\]

where \( Q \) (C) is the charge, \( t \) (s) is the time, \( n \) is the number of electrons transferred, \( F \) is Faraday’s constant, and \( N \) is the number of moles reacted [1, 3, 4]. The electrode where a faradaic process occurs is occasionally called a ‘charge transfer’ electrode [1].

The second term, ‘non-faradaic’, refers to the charging of the double layer (see Section 1.1.6) which requires a charging current (or background current) where no electron transfer process occurs at the electrode | electrolyte interface. It can be perturbed by adsorption and desorption processes, in which the structure of the electrode | electrolyte interface can vary with changes in potential or solution composition [1, 5, 6]. The resultant current is termed non-faradaic or charging current, \( I_c \), and is described by [5]:

\[
I_c = AC_{dl} \frac{dE}{dt} = AC_{dl} \nu \quad \text{(Eq. 1.1.5)}
\]
where $A$ is the electrode area ($m^2$), $C_{dl}$ is the double layer capacitance (F), $\frac{dE}{dt}$ is the potential variation with time, and $v$ is the scan rate ($V\ s^{-1}$). When a fast scan rate is applied, this non-faradaic charging current value can be undesirably high. Thus, small diameter electrodes, such as microelectrodes or nanoelectrodes, are essential to prevent the charging current effect from masking the faradaic current [4].

Even though the overall measured current is contributed by both faradaic and non-faradaic processes, the faradaic process is of main interest, while the non-faradaic contribution is minimised.

### 1.1.5 Polarisable and non-polarisable electrodes

Polarisation occurs when an electrode is able to deviate from its equilibrium potential value, $E_{eq}$, upon the passage of a faradaic current. The magnitude of polarisation is determined by the overpotential, $\eta$, given as:

$$\eta = E - E_{eq} \quad \text{(Eq. 1.1.6)}$$

An ideally polarised (or polarisable) electrode (IPE) is an electrode where no charge transfer can occur across the electrode $|$ solution interface, irrespective of the potential changes imposed by an external voltage. This category of electrode will show a very big change in potential upon application of an infinitely small current, depicted as horizontal region of the $I - E$ curve (Figure 1.1.1 (a)). Although impossible to behave as an IPE over the whole potential range, some electrode $|$ solution systems can demonstrate ideal polarisability over restricted potential ranges. As an example, mercury electrode $|$ deaerated potassium chloride solution system exhibited IPE behaviour over a 2 V potential range [1, 3].

On the other hand, an ideally non-polarisable electrode (or ideal depolarised electrode) is an electrode where the potential does not vary on passage of current. It is essentially an electrode of fixed potential. As illustrated in Figure 1.1.1 (b), the non-polarisability is characterised by a vertical region of the
$I - E$ curve. For example, saturated calomel electrode (SCE) built with a large-area mercury pool would approach ideal nonpolarisability at small currents [1].

![Figure 1.1.1: The current-potential curves for ideal (a) polarisable and (b) non-polarisable electrodes, with theory and practical plots are represented by solid and dashed lines, respectively](image)

### 1.1.6 The electrical double layer

The electrical double layer is composed of the electrical charge at the electrode surface and the charge of distributed ions in the solution in the immediate vicinity of the electrode, i.e. at the electrode | solution interface itself. This layer is created when a potential difference develops across the interface, causing non-faradaic/charging current to pass through the cell. The solution side of the double layer is assumed to be made up of several distinct layers, mainly inner and outer layers [1, 3].

Several models have been proposed in the literature to explain the electrical double layer structure. The first model was proposed by Helmholtz [8, 9], on charge separation at interfaces, where the counter-charge in solution resides at the electrode surface to maintain the electroneutrality. These two sheets of charge with opposite polarity were separated by a distance of molecular order, behaving as a capacitor (Figure 1.1.2). A capacitor is defined as an electrical circuit component made of a pair of metal electrodes, where charge will accumulate on its metal plates when a potential is applied, given by the following equation:
\[ \frac{Q}{E} = C \]  
(Eq. 1.1.7)

where \( Q \) represents the charge stored on the capacitor (coulombs, C), \( E \) represents the potential applied across the capacitor (volts, V) and \( C \) represents the capacitance (Farads, F) \([1]\). Charge will accumulate until \( Q \) in Equation 1.1.7 is satisfied. Concurrently, charging (or non-faradaic) current will flow. The double-layer capacitance, \( C_{dl} \), which is reliant on the applied potential, is a characteristic of the electrode | solution interface \([1]\).

\[ \text{Figure 1.1.2: The Helmholtz model of the electrical double layer. The electrode | electrolyte interface is equivalent to that of a capacitor} \]

Gouy \([10]\) and Chapman \([11]\) then proposed a more realistic model for the charge on the solution. In the case of low concentrations of electrolyte in which low density of charge carriers are present, a thick solution layer called the ‘diffuse layer’ would be necessary to counterbalance the charge of the electrode surface. The highest concentration of excess charge in solution would be next to the electrode surface, where the electrostatic forces are the strongest. As the distance from the electrode becomes greater, the concentration decreases due to weaker electrostatic forces. The charging of the electrode is related to the compactness of the diffuse layer. As the electrolyte concentration increases, a similar diffuse layer compression and a subsequent increment in capacitance is expected. This compact layer is responsible for the charging current. In the case of low solution
concentration, the charging current may impede the measurement of smaller faradaic current [1, 12].

Stern [13] later proposed a new model to overcome the shortcomings of the Helmholtz and Gouy and Chapman models. In the earlier models, the ions are considered as point charges that could approach the surface arbitrarily close. This understanding is not realistic since ions which have a finite size cannot approach the surface closer than the ionic radius. Stern introduced the concept of a ‘plane of closest approach’ for the centres of the ions at some distance, $x_2$. At high polarisation or high electrolyte concentration, the charge in solution would be more compactly compressed to the boundary at $x_2$, approaching the Helmholtz model. The plane $x_2$ is called the ‘outer Helmholtz plane’ (OHP). The outer layer, which consists of solvated ions, is totally bounded by solvent molecules (see Figure 1.1.3) [1, 3].

Further refinement to the Stern model was made by Grahame [14], even though the developed model is sufficient to describe the interfacial structure. Helmholtz previously has explained the non-specific adsorption of counter ions to the electrode surface by long range electrostatic effects, yet specific adsorption needs to be addressed too. Grahame reported that specific interaction is very short-range naturally, in which specifically adsorbed species and solvent molecules are compactly bound to the electrode surface. ‘Inner Helmholtz plane’ (IHP) defines the locus of the electrical centre of this layer and is located at distance $x_1$ from the electrode surface. The inner layer is also termed the compact, Helmholtz or Stern layer (see Figure 1.1.3) [1, 3].

The overall solution ionic concentration controls the diffuse layer thickness, with less than ~100 Angstrom for 10 mM concentration or higher [1].
1.1.3: A schematic diagram of the model of the electrical double layer under condition where anions are specifically adsorbed. The potential profile across the double layer region is illustrated in the absence of specific adsorption of ions. $\phi^m$, $\phi_2$, and $\phi^s$ represent the Galvani potentials of the metal surface, the solvated cation and the electrolyte solution, correspondingly.

1.1.7 Mass transport
Mass transport is defined as the movement of species in solution, from one location to another due to electrical and chemical potential differences. Three modes of mass transport important in electrochemistry are as follows [1, 5, 6]:

a) migration  
b) diffusion  
c) convection
The flux, $J$, in units of mol cm$^{-2}$ s$^{-1}$, measures the rate of mass transport at a fixed point and is described mathematically by the Nernst-Plank equation [1, 6], given here for one-dimensional mass transport to an electrode:

$$J(x, t) = -D \frac{\partial C(x, t)}{\partial x} - \frac{zF}{RT} DC \frac{\partial \phi(x, t)}{\partial x} + C(x, t) V(x, t) \quad \text{(Eq. 1.1.8)}$$

where $D$, $C$ and $z$ are the diffusion coefficient (cm$^2$ s$^{-1}$), concentration (mol cm$^{-3}$) and charge (dimensionless) of the electroactive species, respectively. $\frac{\partial C(x, t)}{\partial x}$ is the concentration gradient at distance $x$ from the electrode surface and time $t$, $\frac{\partial \phi(x)}{\partial x}$ is the potential gradient and $V(x, t)$ is the hydrodynamic velocity (cm s$^{-1}$) [1, 6]. $D$ in aqueous media is reported to range between $10^{-6}$ to $10^{-5}$ cm$^2$ s$^{-1}$ at 298 K [6, 15], and is reliant on factors such as temperature, solvent viscosity and diffusing species molecular size [4]. The generated current ($I$) is directly proportional to the flux [6]:

$$I = -nFAJ \quad \text{(Eq. 1.1.9)}$$

The right-hand side of Equation 1.1.8 denotes the diffusion, migration and convection components of a mass transport, respectively. The migration component is caused by the movement of charged particles under the influence of an electrical field. The convection component is the transport of species to the electrode by physical movement such as stirring, rotation or vibration. The diffusion component is the spontaneous movement under the influence of a concentration gradient [1, 6]. The migration and convection components can be suppressed by the addition of inert supporting electrolyte in an excess amount, and removing all stirring or hydrodynamic transport, so that the system is under stationary conditions, respectively. As a consequence, an electrochemical cell can be designed such that diffusion is the major contributor to mass transport [16, 17]. The reaction occurring at the electrode surface creates a concentration gradient, resulting in the generation of a diffusional flux [6].
This effect is described mathematically by Fick’s first law, in which diffusion rate or flux is directly proportional to the slope of the concentration gradient [5]:

\[ J(x, t) = \frac{-D}{x} \frac{\partial C(x,t)}{\partial x} \]  

(Eq. 1.1.10)

The flux of the species tends to transfer in the opposite direction to the concentration gradient, thus a negative sign arises in Equation 1.1.10. Combination of Equation 1.1.9 and 1.1.10 yields the current response, showing that the current (at any given time) is proportional to electroactive species’ concentration gradient:

\[ I = nFAD \frac{\partial C(x,t)}{\partial x} \]  

(Eq. 1.1.11)

The equation presented previously (Equation 1.1.10) indicates that the diffusional flux is time dependent, which may be described by Fick’s second law of diffusion in one dimension [5]:

\[ \frac{\partial C(x,t)}{\partial t} = D \frac{\partial^2 C(x,t)}{\partial x^2} \]  

(Eq. 1.1.12)

This equation can be written in general formulation for any geometry as [1]:

\[ \frac{\partial C(x,t)}{\partial t} = D \nabla^2 C(x,t) \]  

(Eq. 1.1.13)

where \( \nabla^2 \) is the Laplacian operator. The Laplacian operator is available in different forms depending on the electrode geometry.

1.2 Electrochemistry at the interface between two immiscible electrolyte solutions (ITIES)

1.2.1 Background of liquid | liquid electrochemistry
The interface between two immiscible electrolyte solutions or ITIES is formed when two liquid solvents of a low (or ideally zero) mutual miscibility, usually less than 1 % in weight, are brought into contact. For an aqueous | organic
interface system, the aqueous phase solvent contains a hydrophilic electrolyte salt (typically LiCl or Li$_2$SO$_4$), while the polar organic phase solvent contains a hydrophobic electrolyte salt (commonly bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate, (BTPPATPBCl). The organic solvent possess a moderate dielectric permittivity, such as nitrobenzene, 1,2-dichloroethane and 1,6-dichlorohexane, to permit at least a partial dissociation of dissolved electrolyte(s) into ions [18-21]. The application of ion transfer voltammetry at the ITIES overcomes the drawbacks of the solid | liquid (electrode | electrolyte) interface, where non-redox-active species may not be detected via conventional electroanalytical methods [18]. Additionally, in a conventional solid | liquid electrochemical system, the solid electrode is generally fixed, and only the liquid electrolyte is subject to variation. However, in a liquid | liquid interface system, both phases can be varied to suit the required functions, thus it is more flexible [22].

Electrochemistry at the ITIES is proving versatile in terms of its applications [21, 22], where it covers studies on ion pairing [23], charge transfer [24, 25], adsorption-desorption [24, 26], the voltammetric and amperometric detection of ions [27-29], separation and extractions [30, 31], phase-transfer catalysis [22, 32] and drug release and delivery in pharmacology [33-36]. Electrochemistry at the ITIES has moved from the transfer of model ions such as tetraalkylammonium ions to the detection of biologically important species such as proteins [26, 37], peptides [24, 38, 39], amino acids [40], ionised drugs [33-36, 41-43], neurotransmitters [44-46], food additives [47], carbohydrates [48] and deoxyribonucleic acid (DNA) [49]. In addition, electrochemical sensing based on ion transfer across the ITIES has examined the detection of a wide range of inorganic species such as alkali, alkaline earth, heavy metals and anions [50-53]. Thus it plays an important role in the fields of pharmaceutical chemistry, medicine, pharmacology and so on.
The earliest electrochemical investigation at the ITIES was carried out by Nernst and Riesenfeld in 1902 [54], who were interested in measuring the transport numbers of non-aqueous solvents. These authors observed the transfer of ions across the water | phenol | water interface system using coloured inorganic electrolytes [54, 55]. However, this field remained inactive until the 1960s, when several studies on ion transfer reactions at ITIES applying electrochemical methods were reported. Blank and Feig in 1963 [56] proposed that the water | oil structure of the ITIES could serve as a model for one-half of a biological membrane. A biological membrane by definition is a lipid bilayer composed of a double layer of phospholipids with the polar heads facing the aqueous intracellular and extracellular solutions. The lipophilic chains of the phospholipids create the oil-like inner layer of the membrane. Generally, biological membranes are 5 to 8 nm in thickness and permeable to nonpolar compounds [57].

Gavach and co-workers [58] demonstrated that the liquid | liquid interface can be polarised, and the Galvani potential difference between the two phases can be controlled for charge transfer reactions [59]. Later, Koryta and co-workers [60] also reported about the polarisability of the ITIES and postulated that the description of transport across ITIES was similar to the description of redox processes on solid-electrode surfaces immersed in a solution [55, 59, 60]. Samec et al. [61, 62] invented the concept of the 4-electrode potentiostat with ohmic drop compensation, to allow the use of voltammetry to study kinetics at the ITIES [59].

The conventional experimental studies with ITIES have limitations and a number of strategies have been developed to overcome the limitations and broaden its scope of application, as follows [20, 63]:

a) the issue of volatility of the organic phase is solved by replacing the conventionally used solvents (nitrobenzene and 1,2-dichloroethane) with 2-nitrophenyl octyl ether (NPOE), which encompasses excellent properties such as low vapour pressure, low mutual miscibility with water and medium permittivity [20]. Recently, ionic liquids are being used as the
organic phase solvents as they demonstrate low vapour pressure and high electrical conductivity [64]

b) the problem of mechanical instability of the liquid | liquid interface is solved by partial solidification of the organic phase (dissolving a polymer such as poly(vinyl chloride) (PVC) in it) [63, 65] and supporting the organic solution within micro- or nano-pore arrays [66, 67]

c) the issue of reduced width of the potential window is solved through proper selection of the electrolytes dissolved in both phases (for example highly hydrophobic organic ions and highly hydrophilic aqueous ions), and an organic phase constituted by mixed solvents (for example 1:1 mixture of 1,2-dichloroethane (1,2-DCE):cyclohexane), which can lead to a wider potential window of up to 1.3 V [68]

Numerous literatures in this field have been published, namely review articles [18, 21, 22, 26, 69], books/book chapters [59, 70] and a technical report [19].

1.2.2 Physical structure of the ITIES

The electrical double layer at the interface between two immiscible electrolyte solutions was first described by Verwey and Niessen [71] as two back-to-back diffuse layers, which is based on the Gouy and Chapman theory [59, 69, 71]. Gavach and co-workers [72] pioneered the electrochemical investigation of the structure of the ITIES by modifying the Verwey and Niessen model to incorporate a ‘compact layer’ of orientated dipole molecules to separate the two back-to-back diffuse layers. The developed model is known now as the modified Verwey-Niessen (MVN) model [59, 72].

Girault and Schiffrin [73] who investigated the surface excess of water at the interface between pure aqueous electrolytes and organic solvents, observed that the water surface excess was less than the equivalent of one monolayer and proposed that ions penetrate the interfacial region. These authors concluded that the interfacial layer should be considered as a mixed solvent layer [59, 69, 73]. Samec et al. [74] also reported on the mixed solvent layer separating the two diffuse layers and suggested that ions can penetrate into the inner layer over a distance [59]. The extent of the penetration of ions into
the layer was observed by Schiffrin et al. as a function of the ionic radii [59, 69]. A schematic representation of the mixed solvent layer model at the ITIES is illustrated in Figure 1.2.1.

\[ \text{Mixed solvent layer} \]

- Organic diffuse layer
- Aqueous diffuse layer
- Organic bulk
- Aqueous bulk
- Mixed solvent layer
- organic solvent molecule
- aqueous solvent molecule
- electrolyte cation
- electrolyte anion

**Figure 1.2.1: Schematic diagram of the mixed solvent layer model**

In recent years, new experimental approaches have been reported to probe the liquid | liquid interfacial region. Synchrotron x-ray reflectivity was employed by Schlossman and co-workers [75, 76] to study the interfacial width of water | alkane interfaces, based on the exponential dependence of the reflectivity on the interfacial electron density profile. The measured interfacial widths from this study were in the range between 3.5 to 6.0 Å. Further studies by this same group employed molecular dynamics (MD) simulations, which include the molecular-scale structure in the liquid solution, to estimate the ion distributions, which agree with their x-ray reflectivity measurements [77]. These ion distributions were predicted using potential of mean force of a single ion in a generalised Poisson-Boltzmann equation. Recently, this group reported the influence of the ion-ion coupling strength (from 0.8 to 3.7) on ion distributions at the nanoscopic scales, employing x-ray reflectivity measurements [78]. This work provided confirmation of many ion correlation models with a sharply defined electrical double layer for strong
coupling strength, in contrary to the diffuse distributions predicted by mean field theory. On the other hand, Strutwolf et. al [79] employed neutron reflection and scanning electrochemical microscopy (SECM) to probe the liquid | liquid interface. The reflectivity profile of the 1,2-dichloroethane | aqueous potassium hydroxide interface suggested a smooth interface with a root mean square roughness less than 10 Å, in agreement with molecular dynamics simulations and capillary wave theory.

1.2.3 Thermodynamics of the ITIES

When two electrically conducting liquid phases are brought into contact, an interface termed the ITIES is formed. The charge carriers of the conducting liquids, partition between the two adjacent phases. This partition occurs due to the Galvani interfacial potential difference, \( \Delta \beta \phi \), across the interface:

\[
\Delta \beta \phi = \phi^\alpha - \phi^\beta
\]  

(Eq. 1.2.1)

where \( \phi \) is the Galvani (or inner) potential of the two electrically conducting phases, \( \alpha \) (water phase) and \( \beta \) (organic phase) [19, 20].

The work required to transfer ion \( i \) from a vacuum phase to a liquid phase, \( \alpha \), is termed the electrochemical potential, \( \bar{\mu}_i^\alpha \):

\[
\bar{\mu}_i^\alpha = \mu_i^\alpha + z_i F \phi^\alpha
\]  

(Eq. 1.2.2)

where \( \mu_i^\alpha \) is the chemical potential of ion \( i \) in phase \( \alpha \) and \( z_i \) is the charge of ion \( i \). The \( z_i F \phi^\alpha \) term is the electrical work required to transport the charge that the ion has into the phase \( \alpha \). In solution, the chemical potential, \( \mu_i^\alpha \) is represented by:

\[
\mu_i^\alpha = \mu_i^{\alpha,0} + RT \ln a_i^\alpha
\]  

(Eq. 1.2.3)

where \( \mu_i^{\alpha,0} \) is the standard chemical potential and \( a_i^\alpha \) is the activity of the ion \( i \). The activity is a measure of the effective concentration of component \( i \) in solution which depends on factors such as temperature, pressure and solution composition. The activity can be written in terms of concentration with \( \gamma_i^\alpha \) as the activity coefficient of ion \( i \) in phase \( \alpha \):
\[ a_i^\alpha = \gamma_i^\alpha c_i^\alpha \]  
(Eq. 1.2.4)

Equation 1.2.2 now can be re-written as follows:

\[ \mu_i^{\alpha,0} = \mu_i^{\alpha,0} + RT \ln a_i^{\alpha} + z_i F \phi^{\alpha} \]  
(Eq. 1.2.5)

At a constant temperature and pressure, the condition of thermodynamic equilibrium of the system is achieved when the component \( i \) is equal in the phases \( \alpha \) and \( \beta \), thus the electrochemical potential in both phases must be equal [3]:

\[ \bar{\mu}_i^{\alpha} = \bar{\mu}_i^{\beta} \]  
(Eq. 1.2.6)

Equation 1.2.5 and 1.2.6 can be combined to produce [3]:

\[ \mu_i^{\alpha,0} + RT \ln a_i^{\alpha} + z_i F \phi^{\alpha} = \mu_i^{\beta,0} + RT \ln a_i^{\beta} + z_i F \phi^{\beta} \]  
(Eq. 1.2.7)

Re-arranging Equation 1.2.7 leads to the Galvani potential difference equation, established at the interface between the two phases, \( \Delta_i^\beta \phi \) [3]:

\[ \Delta_i^\beta \phi = \phi^{\alpha} - \phi^{\beta} = \frac{\mu_i^{\beta,0} - \mu_i^{\alpha,0}}{z_i F} + \frac{RT}{z_i F} \ln \left( \frac{a_i^{\beta}}{a_i^{\alpha}} \right) \]  
(Eq. 1.2.8)

The difference in the standard chemical potentials is also known as the standard Gibbs energy of ion transfer from phase \( \alpha \) to phase \( \beta \), \( \Delta G_{\text{transfer},i}^{0,\alpha \to \beta} \). This provides information on the difference in ion solvation and hydration in the two phases. Ions with a great positive or negative \( \Delta G_{\text{transfer},i}^{0,\alpha \to \beta} \) value are signified as hydrophilic or hydrophobic ions, correspondingly.

The standard Gibbs energy of ion transfer between the two phases, \( \Delta G_{\text{transfer},i}^{0,\alpha \to \beta} \), can be defined in terms of a standard ion transfer potential, \( \Delta_i^\beta \phi_i^{0} \) (or the standard Galvani potential difference of ion transfer of species \( i \) from phase \( \alpha \) to phase \( \beta \)) [1]:

\[ \Delta_i^\beta \phi_i^{0} = \frac{\Delta G_{\text{transfer},i}^{0,\alpha \to \beta}}{z_i F} = \frac{\mu_i^{\beta,0} - \mu_i^{\alpha,0}}{z_i F} \]  
(Eq. 1.2.9)
By combining Equation 1.2.8 and 1.2.9, the Nernst-type equation is reached [1, 18-21]:

\[ \Delta_{\beta}^{\alpha} \Phi = \Phi^{\alpha} - \Phi^{\beta} = \Delta_{\beta}^{\alpha} \Phi_{i}^{0} + \frac{RT}{z_{i}F} \ln \left( \frac{a_{i}^{\beta}}{a_{i}^{\alpha}} \right) \]  

(Eq. 1.2.10)

Equation 1.2.10 is the equivalent of the classical Nernst equation for electron transfer reaction at the solid electrode | electrolyte solution interface. Since this equation does not involve any redox reactions, it is recognized as the Nernst equation for the ion transfer at the ITIES. As the \( \Delta_{\beta}^{\alpha} \Phi_{i}^{0} \) term remains constant when the interfacial potential at the ITIES is altered, the ratio \( a_{i}^{\beta} / a_{i}^{\alpha} \) has to change. As a result, a portion of equilibrated ions will transfer into the other phase, thus stimulating an electrical current to move across the interface. Voltammograms are attained by plotting current as a function of the applied potential difference, similar to the solid electrode | electrolyte solution set-up. On the other hand, by changing the relative activities of a common ion on either side of the interface, the interfacial potential difference at the ITIES can be manipulated [18].

By combining Equation 1.2.10 and 1.2.4, the Nernst equation can be expressed in terms of concentration and activity coefficients of component \( i \):

\[ \Delta_{\beta}^{\alpha} \Phi = \Delta_{\beta}^{\alpha} \Phi_{i}^{0} + \frac{RT}{z_{i}F} \ln \left( \frac{\gamma_{i}^{\beta} C_{i}^{\beta}}{\gamma_{i}^{\alpha} C_{i}^{\alpha}} \right) \]  

(Eq. 1.2.11)

Finally, Equation 1.2.11 can be expressed individually in terms of concentration of component \( i \) in either phase by replacing the standard Galvani ion transfer potential, \( \Delta_{\beta}^{\alpha} \Phi_{i}^{0} \), and activity coefficients with the formal Galvani ion transfer potential, \( \Delta_{\beta}^{\alpha} \Phi_{i}^{0'} \) [59].

\[ \Delta_{\beta}^{\alpha} \Phi = \Delta_{\beta}^{\alpha} \Phi_{i}^{0'} + \frac{RT}{z_{i}F} \ln \left( \frac{C_{i}^{\beta}}{C_{i}^{\alpha}} \right) \]  

(Eq. 1.2.12)

with:
\[ \Delta_{\beta}^a \phi_{i}^{0'} = \Delta_{\beta}^a \phi_{i}^{0} + \frac{RT}{z_i F} \ln \left( \frac{y_i^\beta}{y_i^{\alpha}} \right) \]  
(Eq. 1.2.13)

### 1.2.4 Polarisable and non-polarisable ITIES

The polarisable and non-polarisable ITIES can be distinguished, similar to the solid (electrode) | liquid (electrolyte) interface. The polarisation of the liquid | liquid interface is an ionic process, where one phase demonstrates an excess of positive charge, while the other an excess of negative charge. The interface acts as a working electrode, where transfer of importance is occurring [16, 70].

A polarisable ITIES occurs when the constituent electrolyte ions in both the aqueous and organic phases have infinite Gibbs energy of transfer, resulting in no current flow irrespective of the applied polarisation voltage, which is controlled by an externally supplied charge (potentiostat). Nevertheless, in the real world, no such system occurs since real ions have a restricted solubility in any solvent. Generally, a polarisable interface forms between a very hydrophilic salt in the aqueous phase (e.g. LiCl, HCl, MgCl₂, MgSO₄) with a very hydrophobic (lipophilic) salt in the organic phase (e.g. tetrabutylammonium, tetraphenylarsonium, or bis(triphenyl phosphoranylidene)ammonium cations with tetrphenylborate, tetrakis(4-chlorophenyl)borate, or tetrakis[3,5-bistrifluoromethyl]phenyl] borate anions) [70, 80]. \( A^+B^- \) and \( C^+D^- \) in Figure 1.2.2 represent the highly hydrophilic and hydrophobic ions, respectively, in order to achieve a polarisable interface [60].

![Figure 1.2.2: Constituent ionic electrolytes of a polarisable ITIES](image-url)
\( \Delta^\alpha_\beta \emptyset^0 \) of every electrolyte ion present is maximised. Under these conditions, the ITIES is polarised within a certain potential window, defined by the formal transfer potentials of the electrolyte ions.

Non-polarisable ITIES may occur in two conditions. In the first type, \( A^+B^- \) ions are present in both the aqueous and organic phases [60], as depicted in Figure 1.2.3.

\[
\begin{array}{c|c}
A^+B^- & A^+B^- \\
\text{Aqueous phase (}\alpha\text{)} & \text{Organic phase (}\beta\text{)}
\end{array}
\]

*Figure 1.2.3: Constituent ionic electrolytes of a non-polarisable ITIES (type 1)*

The Nernst expression introduced previously (see Equation 1.2.10) can be written for the cation \( A^+ \) (charge, \( z_{A^+} = +1 \)) and the anion \( B^- \) (charge, \( z_{B^-} = -1 \)) as follows:

\[
\Delta^\alpha_\beta \emptyset = \Delta^\alpha_\beta \emptyset^0_{A^+} + \frac{RT}{F} \ln \left( \frac{\alpha^\beta_{A^+}}{\alpha^\alpha_{A^+}} \right) \quad \text{(Eq. 1.2.14)}
\]

\[
\Delta^\alpha_\beta \emptyset = \Delta^\alpha_\beta \emptyset^0_{B^-} + \frac{RT}{F} \ln \left( \frac{\alpha^\beta_{B^-}}{\alpha^\alpha_{B^-}} \right) \quad \text{(Eq. 1.2.15)}
\]

The following expressions are true with regard to solubility of the cation and anion in each phase:

\[
S_{A^+}^\alpha \neq S_{B^-}^\alpha, \quad S_{A^+}^\beta \neq S_{B^-}^\beta, \quad S_{A^+}^\alpha \neq S_{B^-}^\beta, \quad S_{A^+}^\beta \neq S_{B^-}^\beta,
\]

where \( S_{A^+}^\alpha \) and \( S_{B^-}^\alpha \) are the solubility of the cation and anion in the aqueous phase, respectively. \( S_{A^+}^\beta \) and \( S_{B^-}^\beta \) represent the solubility of the cation and anion in the organic phase, respectively.
The cation $A^+$ has different solubilities in the aqueous and organic phases, leading to the establishment of distribution potential across the ITIES. However, the distribution potential is independent of concentration. When an ITIES is established, an equilibration will be achieved based on $A^+$’s solubility in either phase, irrespective of the initial $A^+$ concentration in the aqueous and organic phases.

By combining Equation 1.2.14 and 1.2.15, the interfacial potential difference can be re-written in terms of the activity coefficients of the ions (i.e. independent of concentration), where $\Delta^{a}_{\beta} \phi$ is controlled by the ion distribution across the ITIES. This is often termed as a ‘distribution potential’.

$$
\Delta^{a}_{\beta} \phi = \frac{\Delta^{a}_{\beta} \phi_A^{0'} + \Delta^{a}_{\beta} \phi_B^{0'}}{2} + \frac{RT}{2F} \ln \left( \frac{y_A^B y_B^{-}}{y_A^A y_C^-} \right) 
$$
(Eq. 1.2.16)

In the second type, one common ion, $A^+$, is present in both the aqueous and organic phases (Figure 1.2.4). The anions $B^-$ and $C^-$ must be sufficiently hydrophilic and hydrophobic to remain in the aqueous and organic phases, respectively.

In this case, $\Delta^{a}_{\beta} \phi$ is controlled by the distribution of the cations $A^+$ in the two phases, and is, in principle, a basic version of the processes taking place in Figure 1.2.3. A distribution potential will be established between the two phases. However, this case it is only dependent on the activity of the cations $A^+$ in either phase:

$$
\Delta^{a}_{\beta} \phi = \Delta^{a}_{\beta} \phi_{A^+}^{0} + \frac{RT}{F} \ln \left( \frac{a_{A^+}^B}{a_{A^+}^A} \right) 
$$
(Eq. 1.2.17)
1.2.5 Charge transfer processes at ITIES

In contrast to solid | liquid electrochemistry which focus on electron transfer (ET), charge transfer (CT) reactions at the liquid | liquid system involve both ET and ion transfer (IT). All CT processes occurring at macroscopic ITIES can also be observed at a micro- and nano-ITIES [81]. Ion transfers are composed of either simple IT or facilitated (or assisted) IT. A simple IT is a one-step process, where an ion, \( I^{z+} \) is transferred directly from one phase to another (i.e. from water to organic phase) [81]:

\[
I^{z+}(W) \rightleftharpoons I^{z+}(O) \quad (\text{Eq. 1.2.18})
\]

Besides simple IT, this process can also involve ion pairs [82] or ion clusters [83].

On the other hand, the facilitated IT process requires the addition of a reagent (ligand, \( L \)) in the second phase (i.e. 1,6-dichlorohexane (1,6-DCH)) which can react with the ion \( I^{z+} \) to form a complex, resulting in ion transfer [81]:

\[
I^{z+}(W) + nL(O) \rightleftharpoons I_n^{z+}(O) \quad (\text{Eq. 1.2.19})
\]

The reagent lowers the transfer energy required for that ion and takes it to within the accessible potential window [18, 81].

ET occurs across the phase boundary via redox reaction of a species in one phase with a species in the other phase. The ET process between redox molecules confined to ITIES is given as [81]:

\[
O_1(W) + R_2(O) \rightleftharpoons R_1(W) + O_2(O) \quad (\text{Eq. 1.2.20})
\]

Difficulty in the identification of redox couples has been the primary problem in the investigation of ET at ITIES. The products of the ET process should not transfer across the liquid | liquid interface, otherwise ionic transfer currents are generated that mask the measurements [59].
1.2.6 The voltammetric response of background electrolytes at the ITIES

Figure 1.2.5 represents a typical experimental cyclic voltammogram (CV) for a liquid | liquid system that contains only the background electrolyte ions. In the aqueous and organic phases, the supporting electrolytes are LiCl dissolved in high-purity water, and bis(triphenylphosphoranylidene) ammonium tetrakis(4-chlorophenyl)borate (BTPPATPBCl), dissolved in 1,6-dichlorohexane (1,6-DCH), respectively. The transfer of the background electrolyte ions, from one phase to the other at the extremes of the potential window, determines the available window. Thus electrolytes selection is crucial as their transfer at the extremes of potential define the available potential window. Highly hydrophobic organic electrolyte ions for the organic phase and highly hydrophilic inorganic electrolyte ions for the aqueous phase are more likely to provide wide potential window. This study employed (BTPPATPBCl), a hydrophobic cation and anion pair, both of which are difficult to transfer into the aqueous phase [18].

Two conventions need to be considered when looking at the CV in Figure 1.2.5, as follows:

a) the current convention, and

b) the potential convention

a) When a positively charged ion (cation) transfers from the aqueous phase, W, to the organic phase, O, a positive current is generated, and vice-versa when a cation transfers from O to W, a negative current is produced. Transfer of a negatively charged ion (anion) from W to O, generates a negative current, and the reverse, where the transfer of anion from O to W takes place, produces a positive current.

b) Travelling from the left-hand side to the right-hand side of the voltammogram, the aqueous phase becomes more positively charged relative to the organic phase

The CV in Figure 1.2.5 has been divided into three distinct regions - A, B and C. Regions A and C represent the negative and positive potential regions,
respectively, while region B is the intermediate (polarised) region. In this figure, the starting potential is set at the lowest potential in region A, whereas the switching potential is fixed at the highest (most positive) potential in region C. The polarisation potential is scanned in the positive direction, from the left-hand side to the right-hand side, and then reversed. Realistically, this CV could start in the middle potential, scanned to one extreme potential, and then reversed to the other extreme. Later, it is scanned back to the middle potential.

On starting a CV, a lower positive starting potential (0.05 V) is applied (region A). The positively charged organic cation (BTPPA\(^+\)) is transferred from O to the more negative polarised W phase, while the negatively charged aqueous anion (Cl\(^-\)) is transferred from W to O phase instantaneously. As the scanning potential becomes more positive (from 0.05 V to approximately 0.20 V), BTPPA\(^+\) and Cl\(^-\) ions transfer back to the organic and aqueous phases, respectively. Scanning in the region between 0.20 V to approximately 0.85 V (region B), no transfer of background electrolyte ions (or faradaic process) takes place and this is termed the 'polarisation region'. In this region, the current measured is solely contributed by current charging at the ITIES (due to non-faradaic process). On increasing the potential to highly positive value in the forward scan, from 0.85 V to approximately 1.00 V (region C), the aqueous cation (Li\(^+\)) will transfer from W to O phase and the organic anion (TPBCl\(^-\)) will transfer from O to W phase. As a consequence, a positive increase in the current is observed.

The CV is switched at the highest potential achieved, 1.00 V. Then it is subsequently reversed. In the potential region of 1.00 V to 0.85 V, Li\(^+\) and TPBCl\(^-\) ions are back transferred from the organic and aqueous phases, respectively. Scanning through the 'polarisation region' again, from 0.85 V to 0.20 V, the current recorded is due to non-faradaic processes occurring at the ITIES. At the lowest positive applied potentials, 0.20 V to 0.05 V, BTPPA\(^+\) transfers from O to W phase, while Cl\(^-\) transfers from W to O phase.
Simultaneous transfer reactions of hydrophilic cation (in this case, Li\(^+\)) and hydrophobic anion (in this case, TPBCl\(^-\)) are occurring at the positive potential and generally limit the positive end of the potential window. On the other hand, simultaneous transfer reactions of hydrophilic anion (in this case, Cl\(^-\)) and hydrophobic cation (in this case, BTPPA\(^+\)) are occurring at the negative potential and generally limit the negative end of the potential window.

The ‘polarisation range’ is the region most suited to ion detection at the ITIES. Thus, the transfer potential of the detected ions must fall within the available ‘polarisation range’ window. Generally, a wide operating potential window is desirable and could simply be achieved by proper selection of the electrolytes dissolved in both the aqueous and organic phases [12, 20].

![Diagram](image)

**Figure 1.2.5:** Characteristic cyclic voltammetry (CV) response for a ‘blank’ system containing 10 mM LiCl and 10 mM BTPPATPBCl dissolved in water and 1,6-DCH, respectively. The interface is formed within an array of micropores in a silicon membrane fabricated by lithographic patterning and wet and dry silicon etching methods. The micropores are 11.09 ± 0.12 µm in radius, \(r_a\), 30 pores in a hexagonal close-packed arrangement, and with pore
centre-to-centre separation, \( r_c \), of 18.4 ± 2.1 times the pore radius, \( r_a \) (i.e. \( r_c = 18.4r_a \)). The reference electrodes used in the aqueous and organic phase were Ag/AgCl wires. The arrows indicate the direction of the potential scan (positive direction for the forward sweep and negative direction for the reverse sweep). The potential is reported with respect to the experimentally-used reference electrodes.

1.2.7 The voltammetric response of analyte ion transfer at the ITIES

Figure 1.2.6 represents a typical experimental cyclic voltammogram for a liquid | liquid system that contains the analyte ions of interest (100 \( \mu \)M tetrapropylammonium cations, TPrA\(^+\)) to explain ion transfer (IT) signal generation at the ITIES, in this case the water | 1,6-DCH interface. The interface is formed within an array of micropores and is assumed inlaid, in which the pore is filled with the organic phase. This geometry enables the spherical diffusion of transferring species from the aqueous phase to the organic phase, resulting in a steady-state voltammetric response (Figure 1.2.6). On the other hand, linear diffusion occurs when the transferring species transfers from the organic phase to the aqueous phase, producing a peak-shaped voltammetric response (Figure 1.2.6). This will be discussed in detail later in Section 1.3.5.

In order for ion transfer to occur across the ITIES, the formal Galvani applied potential must exceed the Gibbs energy of ion transfer on a voltage scale. To obtain a clear IT signal, the Gibbs energy of ion transfer must fall within the ‘polarisation range’ where insignificant supporting electrolyte transfer takes place [12].

Theoretically, ion transfer process consists of three situations [12, 16]:

a) mass transport of the ion in one of the phases (aqueous or organic) to the interface
b) the ion transfer reaction, and
c) mass transport of the transferred ion away from the interface in the other phase
As discussed in Section 1.1.7, mass transport, or formally defined as the flux, $J$, of an ionic species to the interface is established by a combination of diffusion, migration and also convection. Mass transport to the interface is primarily diffusion controlled with minimisation of migration and convection effects. The employment of excess supporting electrolyte in both the aqueous and organic phases lessens migration effects. On the other hand, convection is minimised by avoiding stirring, rotation and vibration to the cell, while conducting the experiment on a short time scale helps to minimise the natural convection influences [1, 6].

Ion transfer processes of the background electrolytes in region A and C of Figure 1.2.6 occur as explained in the previous section. When a potential is applied in the forward (positive) direction and achieves a potential positive of the Gibbs energy of ion transfer on a voltage scale of TPrA$^+$ (approximately 0.45 V), TPrA$^+$ concentrations on either side of the ITIES rearranged to maintain electroneutrality at the interface. The TPrA$^+$ ions start to transfer across the interface, resulting in an increase in the current observed (region B). The steady-state current response observed indicates the establishment of radial diffusion field of ion transfer [17]. On the reverse (negative) sweep, when the applied potential reaches a value negative of the Gibbs energy of ion transfer of TPrA$^+$, the back extraction of TPrA$^+$ occurs from O to W phase, producing a negative peak current. This peak-shaped response signifies the linear diffusion-controlled process of ion transfer [17].

The shape and size of the voltammogram observed are dependent on the diffusion regime shape, the position of the liquid | liquid (l | l) interface within the pore channel and the magnitude of the diffusion zone extension ($\delta$) [84-86].
Figure 1.2.6: CV demonstrating a system with addition of transferring analyte. The positively charged ion (100 µM tetrapropylammonium, TPrA⁺) transfers across the liquid/liquid interface containing 10 mM LiCl dissolved in purified water in the aqueous phase and 10 mM BTPPATPBCI dissolved in 1,6-DCH in the organic phase. The interface was assumed inlaid where each pore is filled with the organic electrolyte solution, such that the interface is positioned at the aqueous side of the pore mouth. The reference electrodes used in both phases were Ag/AgCl wires. The arrows represent the forward and reverse potential scans. The potential is reported with respect to the experimentally-used reference electrodes.

1.3 Miniaturisation of the ITIES: The development of micro- and nano-ITIES

1.3.1 Theory of the development of miniaturised ITIES

Miniaturisation of the interfaces between two immiscible electrolyte solutions from a macro-scale ITIES to a micro- and nano-scale ITIES offers many advantages, analogous to the solid | liquid interface electrochemistry on
introduction of ultramicroelectrodes (UMEs) (micro- and nano-electrodes) [85, 87, 88].

The small interfacial electroactive area of the micro- and nano-ITIES results in very small currents generated at the ITIES, in turn minimising the double layer charging current and $IR$ or Ohmic drop effect, which is very crucial in the measurement of kinetic parameters [22, 80, 89]. Furthermore, reducing the size of the ITIES enhances the rate of diffusional mass transport, providing greater current densities and the prospect of enhanced sensitivity of the analytical response [17, 67, 87, 89]. Employing a single ITIES at the micro- and nano-scale has significantly enabled voltammetric measurements in low polarity media or media without supporting electrolytes [90-92].

The miniaturisation of the ITIES also offers prospects for measurements in microenvironments (e.g. the study of living cells) and as a probe for scanning electrochemical microscopy (SECM) [93, 94]. In addition, it assists to simplify the electrochemical measurement instrumentation on introduction of a two-electrode potentiostat setup to replace the conventional four-electrode setup [22]. Preparing several miniaturised ITIES in parallel as arrays creates micro- or nano-ITIES arrays, which are beneficial to amplify the electroanalytical current signal [80, 87]. Furthermore, supporting the micro- or nano-ITIES arrays within solid-state membranes allows improved control over pore size and the geometry of the array, together with higher mechanical stability at the interface [67, 80, 87].

1.3.2 History of the development of miniaturised ITIES
The concept of miniaturisation of the ITIES was first introduced by Taylor and Girault in 1986 [95], where pulled glass micropipettes were applied to support the micro-scale liquid | liquid interface. The transfer of tetraethylammonium cation (TEA$^+$) from the organic phase to the aqueous phase via 25 µm internal diameter micropipette was reported [95]. This study displayed the advantages analogous to the solid microelectrodes, where formation of spherical mass transfer pattern, leading to a high steady-state mass transport, and a low $IR$ drop were observed [22, 66, 95]. Following this
invention, several research groups have also investigated the use of micropipettes at the ITIES [96-98].

In 1991, Shao and co-workers [99] described the observation of asymmetric diffusion regimes when studying the micropipette-based ITIES. ‘Spherical diffusion’ is observed due to the transfer of transferring species into the micropipette, while ‘linear diffusion’ is observed due to the transfer of transferring species out of the micropipette. ‘Linear diffusion’ occurred when the micropipette wall limited the diffusion boundaries [80, 99].

The major disadvantage of using micropipettes during that period was the poor reproducibility of the tip geometry. This problem prompted Beattie and co-workers to develop a more advanced pipette puller and thinner glass in 1995 [96]. The borosilicate glass or quartz materials employed in the micropipette fabrication process possess hydrophilic properties, thus only the aqueous phase could be inserted in the micropipette. However, Shao and Mirkin in 1998 revealed that this hydrophilic nature could be transformed to hydrophobic via silanization [98]. Silanizing the inner or outer wall of the micropipette produced hydrophobic conditions, respectively. The silanized inner wall allows the organic phase to be inside the micropipette, while the silanized outer wall aids to prevent the formation of a thin film of water, and therefore, kept the interface at the mouth of the micropipette [98].

Single (and dual) microITIES established by glass micropipettes have proven successful to overcome the Ohmic drop problem, yet the low current measured (basically in the nanoamperes region) can create problems for some applications. Hence, an array of microITIES was introduced with the development of membranes perforated by an array of microholes (or micropores) [66, 100-102].

In 2007, Arrigan and co-workers established the fabrication of microporous silicon membranes from silicon wafers [66]. The fabrication process involved photolithographic patterning in conjunction with potassium hydroxide wet etching and deep reactive ion etching (DRIE). The micropores prepared by
this process are observed to be hydrophobic due to the fluorocarbon-coated pore walls. This technique has successfully produced a regularly aligned and reproducible geometry of micropore arrays [66, 80].

Another approach in the development of miniaturised ITIES was developed by Cunnane and co-workers in 1995 [103]. This approach was based on the establishment of a micro-cavity by the chemical dissolution of a glass-encapsulated micro-silver wire to support a microITIES [22, 103].

In the subsequent years, many groups focused on the improvement and extension of the applications of microITIES with the development of nanoITIES. The pioneers in the development of nanopipettes to support a liquid | liquid interface are Shao and Mirkin [89] in 1997. In their study, a standard laser-heated pipette pulling technique (P-2000 laser puller from Sutter Instruments Co., USA) with optimised pulling parameters was employed in the fabrication of the nanopipettes from borosilicate or quartz capillaries. Currently, several studies have reported nanopipette-based ITIES with a diameter of less than a few nanometres [89, 104].

Dryfe and co-workers introduced track etched membranes, followed by chemical etching, to fabricate arrays of nanoITIES [101, 105]. The track etching procedures drove nuclear particles through a membrane, and this track was subsequently etched using chemical etching procedures. This opens up pores in the membranes. Besides, \( \gamma \)-alumina membranes which are commercially available for nanofiltration systems, have also been applied to create an array of nanoITIES [106]. However, the lack of control over pore geometry and spacing between neighbouring pores resulted in complications during data interpretation. Thus, this type of membrane is difficult to be applied in the electrochemistry at ITIES.

In the latest development, fabrication of nano-dimension pores are conducted employing methods normally used in the semiconductor fabrication facilities. Several techniques, including focused ion beam (FIB) machining and electron beam milling (using transmission electron microscopy) have been
applied in the fabrication of solid-state nanopore membranes [107]. A study conducted by Kasianowicz and co-workers in 1996, on the biological pore α-haemolysin, has triggered interest in the application of the fabricated solid-state membranes for use in DNA sequencing, where electrophoretic translocation of DNA is utilised [108]. From a genomics point of interest, if DNA transfer through a nanopore could be conducted in a linear mode, this might serve as a device to read the DNA sequence in an ultrarapid approach [107]. Discussion of this rapidly expanding solid-state membrane fabrication field employing ion and electron beams, with emphasis on engineered nanopores in silicon-based materials, is given in Section 1.4.3.

Arrigan and co-workers employed the fabrication of nanopore arrays in silicon nitride (Si₃N₄) membranes [67, 80, 87, 109]. In this study, combinations of photo- and electron-beam lithography, etching and deposition procedures were employed to fabricate arrays of nanopores with diameter between 30 and 500 nm. The final Si₃N₄ membrane chip had a 500 µm × 500 µm area and was 100 nm in thickness, supported on a silicon wafer.

1.3.3 Methods of producing micro- and nano-ITIES
To date, two approaches for establishing microITIES have been reported [66, 80, 84]. The first is based on the use of micropipettes in which the liquid | liquid interface is created at the tip of a pulled glass pipette [97, 98, 110]. The second is based on placement of the aqueous and organic phases on either sides of membranes containing arrays of micron-sized pores or holes [66]. For the first time, microhole was developed by Girault’s group on an inert polyester thin-film substrate, Melynex, employing UV laser photoablation technology [111]. Numerous studies have reported the advantages of using microITIES arrays formed on an inert membrane. Various membrane fabrication materials have been investigated, such as polymers (polyimide, polyester, polyethylene terephthalate (PET) and cellulose) [100-102, 112-116] and silicon [38, 66, 117]. Cellulose was observed to be unsuitable as it became swollen when in contact with the aqueous phase, while polyester, which is chemically inert in the aqueous and organic phases, is suitable.
As for microITIES, nanoscale ITIES and their arrays can also be formed by two approaches, some of which are produced using advance nanofabrication techniques and instrumentation [67, 80]. One is those supported at the tip of single [89, 90, 104, 118, 119] or dual [90, 120, 121] nanopipettes, thus producing single- or double nanoITIES. Another one is produced by placing nanoporous materials containing geometrically regular or irregular pore arrays at the ITIES. Silicon nitride membranes [67, 87, 109], prepared by electron beam lithography and chemical etching methods, are used to create regular nanoITIES arrays. On the other hand, track-etched polyester [101, 105] and γ-alumina ultrafiltration [106] membranes, which contain high pore densities (ca. up to $10^9$ pores per cm$^2$), are used to form the irregular nanoITIES arrays. Even though solid-state nanopore membranes from a variety of fabrication materials have been established in the past decade, those membranes have not been utilised for nanostructuring of the ITIES [67] until recently. These membranes are primarily used as sensors for DNA and other biological molecules such as peptides and proteins [38, 107].

1.3.4 Gellification of the miniaturised ITIES
Senda and co-workers [122, 123] introduced the gellification of either phase used to form the ITIES. This approach, which incorporates gellification of one of the phases (normally the organic phase) was developed to overcome the problem of mechanical instability at the liquid | liquid interface. However, gellification of the organic phase also results in an increase of the system resistance. Miniaturisation of the ITIES has been observed to minimise the high interfacial resistance problem. In 1985, Senda’s group introduced the organic phase gellified with PVC-nitrobenzene (PVC-NB). Since then, various papers have been reported on gellified-ITIES, notably, PVC-nitrophenyl octyl ether (PVC-NPOE) [50, 100, 124, 125], PVC-1,2-DCE [30, 31] and PVC-1,6-dichlorohexane (PVC-1,6-DCH) [38, 65, 87]. Besides, the aqueous phase can be gellified too, using agarose [126, 127] or even in frozen condition [128].
### 1.3.5 Diffusion regimes at singular micro- and nano-ITIES

Several parameters are found important in describing the shape and magnitude of the voltammogram observed at the micro- and nano-ITIES: the diffusion regime shape, the magnitude of the diffusion zone extension (δ), the recess depth or interface position within the pore channel, ℓ, and the ratio of the diffusion coefficients of the transferring species in the organic and aqueous phases [80, 84-86]. All the effects will be discussed.

In this thesis, two types of arrays are employed, namely the micro- and nano-ITIES arrays. The diffusion regimes that may occur at these types of interface are presented. Girault and co-workers have reported that the theory established for solid microelectrode arrays could also be applied to microITIES arrays [115, 129]. Results from the Girault and Campbell study have shown that the microITIES diffusion regime at membrane-based pore was similar to those experienced at micropipettes, where spherical (or radial) and linear diffusion occur [111, 130].

An inlaid micro- or nano-ITIES geometry, as depicted in Figure 1.3.1, is observed when the pore is fully (100%) filled with the organic phase, such that the interface is positioned at the end of the pore channel, on the aqueous phase side. In addition, the interface formed is planar (i.e. flat). This geometry enables the spherical diffusion of transferring species to the interface. Enhanced mass transport due to dominance of spherical diffusion is one of the key advantages of employing microITIES in electrochemical studies, and this is expected to be achieved to a greater extent when employing nanoITIES [85]. The resulting steady-state current at an inlaid electrode (in this case equivalent to an inlaid ITIES) is defined by the Saito equation [131]:

\[
I_{ss} = 4|z_i|F D_i C_i r_a
\]  
(Eq. 1.3.1)

where \(I_{ss}\) is the steady-state (or limiting) current (A), \(F\) is the Faraday constant, \(r_a\) is the pore radius (m), and \(z_i, D_i\) and \(C_i\) are the charge, the diffusion coefficient (cm² s⁻¹) and the bulk concentration (mol m⁻³) of the transferring ions, \(i\), respectively. To obtain the total current for the array, the
current calculated for one pore must be multiplied by the number of pores, $N_p$.

![Schematic diagram of the geometry and diffusion regime of inlaid miniaturised ITIES with spherical diffusion process for ions transferring from the aqueous phase to the organic phase](image)

*Figure 1.3.1: Schematic diagram of the geometry and diffusion regime of inlaid miniaturised ITIES with spherical diffusion process for ions transferring from the aqueous phase to the organic phase*

Diffusion to a single micro- or nano-pore occurs in two-stages at sufficiently long times. The first diffusion is normal to the plane of the interface (linear diffusion), and the second diffusion is radial with respect to the axis of symmetry (spherical diffusion) [1]. As a result, current density is non-uniform across the interface. The diffusion and current density is largest at the interface edge, which is due to the enhanced mass transport rate of the transferring species, resulting from spherical diffusion. This phenomenon is termed the ‘edge effect’ [88] and is the major contributor to the total diffusion current. As compared to a recessed interface which exhibits linear diffusion control with no ‘edge effect’, currents produced by an inlaid interface are several times higher.

Linear diffusion occurs when the transferring species transfers from the organic phase to the aqueous phase (Figure 1.3.2), due to the boundary set by the pore wall, and this results in a peak-shaped voltammetric response. This linear diffusion control is analogous to a larger ITIES in which the contribution from spherical diffusion is masked by the linear diffusion.
A recessed micro- or nano-ITIES geometry is observed when the interface level is lower than the pore mouth. The pore is partially filled with the aqueous and organic phases, such that the interface is positioned somewhere along the pore channel. When the interface level is positioned at the bottom of a pore channel, as illustrated in Figure 1.3.3, the pore is fully filled with the aqueous phase. This geometry produces a more complex diffusion regime. Spherical diffusion is observed at the pore opening, while linear diffusion dominates within the confines of the channel. Compared to the inlaid interface, recessed interface demonstrates lower steady-state current ($I_{ss}$), by a factor equal to $(4l + \pi r_a) + 1$, as reported by Bond and co-workers [132]. This is due to shielding effect of the surrounding pore wall. The steady-state current ($I_{ss}$) at a recessed interface is given by [132]:

$$I_{ss} = \frac{4\pi |z_i|FD_iC_i r_a^2}{(4l + \pi r)}$$  \hspace{1cm} (Eq. 1.3.2)

where $l$ is the recess depth (m). The total current for the array is obtained by multiplying the current calculated for one pore by the number of pores, $N_p$. When $l$ is zero, Equation 1.3.2 will be simplified to Equation 1.3.1.
Figure 1.3.3: Schematic diagram of the geometry and diffusion regime of recessed miniaturised ITIES with spherical and linear diffusion process for ions transferring from the aqueous phase to the organic phase

The position of the liquid | liquid interface determines whether the resultant voltammogram is symmetric or asymmetric. The hydrophobicity and wetting properties of the membrane are two primary factors that influence this position [84]. The interface can be either inlaid at the aqueous side of the pore channel, inlaid at the organic side of the pore channel or located at the centre of the pore channel, as depicted in Figure 1.3.4 (A), (B) and (C), respectively. In the case of identical diffusion coefficients, $D$, of the transferring species in both the aqueous and organic phases, symmetrical CVs are only possible if the ITIES position is in the centre of the pore channel (Figure 1.3.4 (C)). In this condition, diffusion patterns (and also the shielding effect of the surrounding pore wall) produced from the forward and reverse sweeps are equal [16]. The transfer processes from both W to O, and O to W may be treated similar to the recessed ITIES condition, where W and O represent aqueous and organic phases, respectively.

When the interface is inlaid at the aqueous side of the pore channel (Figure 1.3.4 (A)), the transfer process from W to O is treated similarly to the inlaid ITIES condition, and the transfer process from O to W is treated similar to the recessed ITIES condition, as described previously. On the contrary, when the interface is inlaid at the organic side of the pore channel (Figure 1.3.4 (B)), the transfer process from W to O is now treated similar to the recessed ITIES
condition, and the transfer process from O to W is treated similar to the inlaid ITIES condition.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Ion transfer direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>The organic phase fills the micro- or nanopore</td>
</tr>
<tr>
<td>B</td>
<td>The aqueous phase fills the micro- or nanopore</td>
</tr>
<tr>
<td>C</td>
<td>The micro- or nano-pore is equally filled with aqueous and organic phases</td>
</tr>
</tbody>
</table>

**Figure 1.3.4:** Schematic diagrams of the ion transfer diffusion regimes from W to O and O to W at a singular solid-state miniaturised ITIES. A represents an inlaid interface at the aqueous side of the pore channel, B is an inlaid interface at the organic side of the pore channel and in C, the interface is located at the centre of the pore channel. The diffusion coefficients of the transferring species are identical in both phases.

Another parameter that influences the symmetric/asymmetric shape of the voltammograms for ion transfer across a singular miniaturised ITIES is the ratio of the diffusion coefficients of the transferring ions in the aqueous ($D_i^a$) and organic ($D_i^b$) phases [80, 84]:

\[ \gamma = \frac{D^\beta_i}{D^\alpha_i} \]  \hspace{1cm} (Eq. 1.3.3)

Since the organic phase in the microITIES experimental setup is regularly partially solidified or gellified, so as to increase the mechanical stability of the interface [20], this results in the reduction of \(D^\beta_i\). Previously, when the ITIES position is in the centre of the pore channel and \(D^\alpha_i = D^\beta_i\), symmetrical ion transfer may be observed. However, in the case of \(D^\alpha_i \gg D^\beta_i\), the value of \(\gamma\) is substantially lower than 1. For ion transfer from W to O phase, the diffusion field can extend out of the pore, resulting in spherical diffusion dominance and an enhanced steady-state current. In contrast, the reduction of \(D^\beta_i\) value causes the ions transferred initially from W to O phase to not diffuse out of the pore on the timescale of the voltammetric experiment. Thus, the reverse sweep current is dominated by linear diffusion only and causing an enhanced peak current [16]. Further decrease in \(\gamma\) value leads to an increase of the peak current during the reverse sweep, for the back-transfer of transferring ions from the organic to aqueous phase [80, 84].

1.3.6 Diffusion regimes at arrays of micro- and nano-ITIES

A diffusion zone is created around each individual ITIES in the array when there is a change in concentration of the transferring species at the liquid | liquid interface during a voltammetric experiment [84]. Several factors have been identified to influence the magnitude of the diffusion zone extension, \(\delta\), and the possibility for individual diffusion zone overlapping, as follows:

a) the geometric parameters of the micro- and nano-ITIES arrays: pore radii, \(r_\alpha\), and pore centre-to-centre separation, \(r_c\);

b) the voltammetric timescale of the experiment

c) the diffusion coefficients of the transferring ions in the aqueous and organic phases

The emphasis will be given to the effect of interface centre-to-centre separation.

The separation between adjacent interfaces in micro- or nano-ITIES arrays should be sufficient so as to ensure the individual diffusion zones remain
independent of each other, and yield a maximum current signal. In the case where the separation between these interfaces becomes smaller, the individual diffusion zones will gradually overlap and results in the individual interfaces depleting the same region of the solution (termed an exclusion zone) (Figure 1.3.5). Consequently, the spherical diffusion contribution will decline, while the linear diffusion contribution will increase, resulting in the appearance of a peak-shaped voltammogram. If the diffusion zones are heavily overlapped (Figure 1.3.6), a purely linear diffusion will be observed, and the resulting peak current is defined by the Randles-Sevcik expression [1, 106]:

\[
I_p = 0.4463 z_i F A C_i \sqrt{\frac{z_i F v D_i}{RT}}
\]  
(Eq. 1.3.4)

where \(I_p\) is the peak current (A), \(A\) is the total membrane area (m²) and \(v\) is the sweep rate (V s\(^{-1}\)).

Figure 1.3.5: Schematic diagram of the overlap of spherical diffusion zones with exclusion zone highlighted in red. The interface is inlaid at the aqueous side of the pore channel with diffusion occurs from the aqueous phase to the organic phase. The dotted lines present diffusion zone.
Figure 1.3.6: Extensively overlapping diffusion zones resulting in linear diffusion from the aqueous phase to the organic phase. The interface is inlaid at the aqueous side of the pore channel. The dotted lines present diffusion zones

The diffusion zones occurring along the boundaries of the array will be dissimilar from the zones inside the array. Increasing the number of interfaces in the array, however, will reduce the effect of the different diffusion zones along the boundaries [80, 133, 134].

In the special case of an array of nanoelectrodes occupying a few µm², Godino and co-workers [135] have demonstrated via experimental and simulation studies that substantial diffusion zone overlapping at neighbouring nanoelectrodes occurs in the timescale of a potential sweep experiment, for electrode centre-to-centre separation of 60 times the radius. The measured voltammograms show sigmoidal steady-state characteristics. The underlying principle is that, at a small size array, an array of nano-electrodes behave as if the entire array were a single microelectrode of the same interfacial surface area with equivalent properties, and a spherical diffusion zone is associated with that microelectrode [135].

1.3.7 Non-interacting diffusion zones

The size of the individual diffusion zone (δ) is not solely influenced by the pore radius (rₐ). Other factors, such as the diffusion coefficient (D) and the
duration of the experiment ($t$), also have an influence. The following expression describes this relationship [1]:

$$\delta \propto \sqrt{Dt} \quad \text{(Eq. 1.3.5)}$$

Using species with a small diffusion coefficient and fast experimental times (i.e. scan rate), the diffusion zone will be small in size, hence eliminate diffusion zone overlap. Therefore, varying the scan rate can shift from overlapping diffusion zones to non-interacting diffusion zones [12].

Many research investigations conducted previously estimate the conditions for the size of the diffusion zone ($\delta$) based on linear relationship between $r_a$ and $r_c$. An example is the work by Saito et al., based on the assumption of purely steady-state voltammetry at microelectrode (equivalent to microinterface) arrays, therefore independent of the scan rate [84, 86, 131]:

$$r_c > 12r_a \quad \text{(Eq. 1.3.6)}$$

Based on investigation conducted by Alfred and Oldham [136], Fletcher and Horne formulated the following approximation [86, 137]:

$$r_c > 20r_a \quad \text{(Eq. 1.3.7)}$$

Recently, Davies and Compton have formulated an expression to estimate the diffusion zone extension ($\delta$) under voltammetric conditions at microdisk electrode (equivalent to microinterface) arrays [84, 86]:

$$\delta \approx \sqrt{2Di \frac{\Delta E}{v}} \quad \text{(Eq. 1.3.8)}$$

where $\Delta E$ is the potential interval from the onset of (Faradaic) current to the attainment of the steady-state current. This equation included the effect of time (via the potential scan rate) on the diffusion layer thickness. However, the effect of the interface (electrode) radius is excluded since this equation was derived with the assumption of one-dimensional diffusion.
On the matter of the timescale of the experiment, the microinterface exhibits non-linear diffusion when the diffusion zone does not exceed half of the interface centre-to-centre separation ($\delta < 0.5r_c$). The system voltammetric response can be calculated by multiplying the response of a single interface by the number of interfaces in the array. In contrast, when $\delta > 0.5r_c$, overlapping diffusion zones occurred and the microinterface array exhibited linear diffusion. The adjacent pores deplete the same region in the solution, resulting in a lower flux to an individual interface compared to an isolated interface [80, 84, 86]. All the described assumptions, proved to be suitable for $r_c$ or $\delta$ calculations when $r_a > 1 \mu m$ and can be readily applied to microITIES experiments described in this thesis. However, these equations are found to be unsuitable for the discussion of the overlapping diffusion zones created at nanoITIES when $r_a < 1 \mu m$.

1.4 Fabrication of solid-state nanopore membranes

1.4.1 Background of membrane technology
Membrane science and technology has evolved from basic applications in the laboratory to high impact industrial utilisation. Membrane processes offer a diverse range of applications in modern society, including in the areas of chemical sensor, biosensor, food and pharmaceutical industries processing, desalination, gas separation and so on [138].

1.4.2 Background of nanopore membrane
Approximately one century ago, the development of fluid-separation (or liquid filtration) membranes began [139]. The fabricated membranes, initially, were relatively thick (several micrometres) and consisted of non-uniform and random pore diameters of porous organic or inorganic materials. At this stage, the membranes fabricated had limited selectivity and strength due to the fairly broad pore diameter range, despite the success in liquid filtration and gas separation applications. Thus, the development of uniformly perforated nano-scale membranes, which exhibit adequate mechanical strength with respect to transmembrane pressure, possess low flow.
resistance to facilitate high flux, and have sufficient thermal and chemical stability towards severe conditions, is required as it offers prospects for improvements in diverse areas of technology [139]. Furthermore, from a fundamental science point of view, the construction of nanoscale pores in inert membrane materials would allow for the emulation of the capability of protein pores to transfer materials in and out of cells as observed in living organisms [107, 108].

In nanoscience and nanotechnology, ‘nano’ is defined as the length scale in the range of 1 to 100 nm. In electrochemical studies, nanoelectrode (in this case equivalent to nanopore or nanohole or nanochannel or nanointerface) may be termed as an electrode (or pore) with a critical dimension in the nanometre range too, where the critical dimension means the dimension that controls the electrochemical response. For a disc electrode or a pore, the radius is the critical dimension which controls the signal generated [85, 140].

In recent years, nanopores have gained great interest for applications in biosensing, molecular separation, molecular electronics, optical devices, nanofluidics and others [140-145]. The recent class of nanosensors, developed from single nanometre-sized pore embedded in an insulating membrane, is used for fast electrical detection and characterization of biomolecules [141]. In biology, the transfer of individual molecules across naturally occurring nanopores is nothing new. However, only recently researchers have been successfully transferring single DNA molecules across artificial solid-state nanopores fabricated in insulating membrane [107].

Generally, nanopores fall into two distinct categories [107, 140, 141]:

a) biological nanopores [108, 143] (for example, $\alpha$-hemolysin protein nanopores in lipid membranes), and

b) solid-state nanopores [67, 146] (for example, silicon nitride and silicon oxide membranes)
Solid-state materials offer more advantages in fabrication of nanopores compared to biological materials. The solid-state nanopores exhibit high stability (mechanical, chemical and electrical) and rigidity, have adjustable surface properties, capable of control of geometry with nanometre precision (diameter and channel length) and have the potential for integration into devices and arrays [107, 140, 147-149]. On the other hand, biological nanopores do exhibit several drawbacks, such as a fixed size and offering limited stability, even though they has been employed widely in translocation experiments. Any variations in external parameters, for example, pH, salt concentration, temperature and mechanical stress, can affect the pores and the embedding lipid bilayer [107, 140, 150].

1.4.3 Current methods of fabricating single and array solid-state nanopore membranes

The fabrication materials generally used in the microelectronics industry are employed in the creation of the solid-state nanopore membranes, since those materials have been well characterised. Silicon (Si) [151], silicon nitride (Si₃N₄) [139, 146-148, 150, 152-154], silicon dioxide (SiO₂) [141, 149, 152] and silicon carbide (SiC) [155] are among the widely used membrane materials, with ultra-thin films of Si₃N₄ and SiO₂ being the most common materials used in the fabrication of solid-state nanopores and nanopore devices by FIB [140]. Several remarkable exclusions exist, namely nanopores in track-etched polymers [156-158] and thermoplastics [159, 160].

Currently, various approaches have been investigated in the challenge to fabricate solid-state nanopores with real nanometre dimensions, with emphasis on the fabrication of single nanopore membranes. These approaches can be classified into several categories [140, 148, 152]:

a) ‘ion beam sculpting’ by FIB milling [139, 161]

b) direct drilling by the high-energy focused electron beams of transmission electron microscopes (TEM) [147, 153, 154, 162]

c) electron beam lithography and chemical etching [67, 87, 150], followed by controlled shrinkage of pore by chemical transition or field migration [107, 141, 149]
d) reactive gas- or water vapour-assisted charge-beam etching [148, 152]
e) track etching by high-energy heavy metal ion and chemical etching

The following part discusses in detail selected solid-state nanopore membrane fabrication methods via focused ion beam, electron beam of TEM and electron beam lithography. Although several studies have reported the possibility of using solid-state nanopore membranes prepared by FIB and TEM for rapid and sensitive DNA sequencing, these engineered nanopore membranes have not been examined as the basis for nano-ITIES formation and nanoelectrochemical sensing, which provide the platform for this study.

Li and co-workers [146] at Harvard University employed low energy ion beams with feedback-controlled sputtering to fabricate a molecular-scale hole (or nanopore) in a thin insulating silicon nitride membrane supported on a silicon frame. This approach used 3 keV argon (Ar+) beam and was termed ‘ion beam sculpting’. Sputtering or atomic-scale erosion process occurred when enormous ions with several thousand electron-volts energy impinged on a surface (in this case Si₃N₄ flat surface) [147]. Every incident ion, then removes approximately one atom from the surface, later resulting in pore formation [141, 154, 163]. In this study, Li’s group implemented a feedback-controlled ion sputtering system to determine the stop time of the erosion process, thus a molecular-scale pore is created on the 500 nm thickness Si₃N₄ surface. The parameters crucial for the ‘ion beam sculpting’ process were controlled, such as sample temperature, ion beam duty cycle and instantaneous ion beam flux. This research proved that ‘ion beam sculpting’ is able to create a single nanopore in Si₃N₄ membrane for use as a single-molecule electronic detector of DNA [146].

Tong and co-workers [139] employed FIB etching to fabricate an array of identical geometry cylindrical nanopores in an ultrathin micromachined silicon nitride membrane. In this study, a dual FIB system with 30 kV gallium ion beam was used. A coating process was conducted prior to FIB drilling, using 2 nm of chromium (Cr) to prevent charging during the exposure to electron and ion beams, which can be removed using a Cr etching solution [139]. The
The smallest possible FIB current of 1 pA is employed to obtain a small beam diameter (10 nm, corresponding to a current density of 1.2 A/cm²) and a narrow beam-diameter distribution. The application of this small ion beam current was successful to produce highly uniform small pores of 25 nm in diameter, which were then further reduced to below 10 nm by coating with another silicon nitride layer. In addition, the time taken for the milling process was still short because an ultrathin layer (10 nm) is used, despite the smallest FIB current. In this research, a Matlab program was applied to control the FIB milling process, so that an array of pores with dwell time of 10 µs was made. High-resolution scanning electron microscopy (HRSEM) imaging of the top and bottom pores showed that the geometries are almost identical therefore proving the pore wall slope angle was nearly 90°. The fabricated membranes showed satisfactory mechanical strength (up to several bars of transmembrane pressure), high temperature tolerance (up to 900 °C) and stability towards aggressive chemicals, for example nitric acid and piranha solution (sulfuric acid (H₂SO₄) + hydrogen peroxide (H₂O₂)).

Ho and co-workers [162] employed a transmission electron microscope that produces a tightly focused electron beam as small as 0.5 nm in diameter (measured in Gaussian width) and of high-energy (200 keV (1 eV = 1.602 x 10⁻¹⁹ J)) to fabricate single nanometer-diameter pores. The TEM used was able to sputter atoms in 10 nm thickness silicon nitride membranes. Unfortunately, it produced uneven pore diameters throughout the membrane thickness, where the pore resembles two intersecting cones with a 10° cone angle. The pore exhibits wider diameter at the top and bottom openings compared to the centre.

Dekker and co-workers [107, 141] at Delft used electron-beam lithography and subsequent anisotropic etching to pattern pores in free-standing Si, Si₃N₄ or SiO₂ membranes. The group discovered that high-intensity wide-field illumination with electrons during imaging using TEM slowly modified the nanopore size, in which large pores (pore diameter greater than thickness) grew in size, while small pores shrunk. A commercial TEM operated at high
accelerating voltage of 300 keV, was used to damage or deform the specimen. They managed to fabricate pores of less than 20 nm diameter.

1.5 Aim of the study

The aim of the study presented in this thesis is to explore the electrochemical performances of micro- and nano-porous membranes located at liquid | liquid interface (with emphasis on the nanopore array membrane) as a basis for new sensor technologies. In particular, this thesis covers:

a) the preparation of nanopores by focused ion beam milling
b) the formation of nanoITIES arrays and their characterisation by various electrochemical methods, such as cyclic voltammetry, stripping voltammetry and chronoamperometry
c) the use of electrochemistry at the ITIES for the detection of drug molecule (ractopamine)

In Chapter 3, single and array pore membranes will be described based on solid state membranes, specifically silicon nitride membranes, using FIB milling. The FIB instruments employed were available at the John de Laeter Centre, Curtin University and the FIB Centre, University of Ulm, Germany. The prepared pore membranes will be characterised physically and electrochemically. In the electrochemical characterisation study, a simple voltammetric ion transfer of model analyte tetrapropylammonium cation (TPrA+) will be implemented and the effects of pore size (pore radius), pore centre-to-centre separation, and total number of pores in the array, will be investigated. This is the first reported electrochemical characterisation at the liquid | liquid interface of solid state nanopore array membranes prepared by FIB.

In Chapter 4, nano-ITIES confined within solid state silicon nitride nanopore arrays, fabricated at the Tyndall National Institute [67], will be electrochemically characterised by the amperometric transfer of TPrA+. Important information that will be generated from this study are charging
time, capacitance, resistance and response time, which will be beneficial in the design of chemical and biochemical sensors.

The use of electrochemical techniques for the characterisation of a micro-/submicro-pore organic membrane will be discussed in Chapter 5. Parylene C membrane, which was fabricated by conformal vapour deposition of parylene C onto electron microscopy grids, will be characterised by voltammetric transfer of TPrA\(^+\) across the interface held within the pores of the organic membrane. The effects of diffusion regime shape, magnitude of the diffusion zone extension (\(\delta\)) and position of the liquid\(\mid\)liquid interface within the pore channel towards the shape of the voltammetric responses and the size of the steady-state currents will be determined. These will be the first results on parylene C organic membrane characterisation by electrochemistry at ITIES which offers in situ characterisation of membrane properties as well as the possibility for detection of biologically-important species.

Chapter 6 will focus on the possibility of employing micro-ITIES arrays for the detection of cationic \(\beta\)-agonist drug, protonated ractopamine (RacH\(^+\)), using cyclic and linear sweep stripping voltammetries techniques. The solid-state silicon micropore array membrane used to form the micro-ITIES was developed at Tyndall National Institute [66]. The effects of optimising linear sweep stripping voltammetry operating parameters such as sweep rate, pre-concentration potentials and pre-concentration times towards lowering the drug limit of detection will be investigated. This chapter will also present how electrochemical investigations can be employed to deduce a drug’s fundamental thermodynamic parameters. Finally, the influence of the potentially interfering substances (such as sugar, ascorbic acid, metals, amino acid, urea and uric acid), and serum protein towards the detection of RacH\(^+\) will be investigated too. In this chapter, only preliminary results on protonated ractopamine transfer detection at the nano-ITIES array will be addressed. Due to the insufficient potential window range for the ion transfer process, this study will focus on the drug detection at the micro-ITIES array which will provide a basis for future studies at nano-ITIES array.
Chapter 2

Experimental methods and materials

2.1 Micro- and nano-porous membrane experimental procedure

2.1.1 Micro- and nano-porous membranes
Two types of nano-porous membranes, both from silicon nitride (Si₃N₄) material, were employed in this thesis. The first type of Si₃N₄ membrane was DuraSiN™ film, purchased from Electron Microscopy Sciences, Pennsylvania, USA, this was used for pore preparation. The rigid silicon frame (2.65 mm × 2.65 mm × 300 µm) is used to support the Si₃N₄ film area (500 µm × 500 µm) in which single and array pores were prepared using focused ion beam (FIB) milling and transmission electron microscopy (TEM) drilling. The schematic diagram with membrane specifications is given in Figure 2.1.1. The membrane thickness was one of 50, 100 or 200 nm.
Figure 2.1.1: The DuraSiN™ silicon nitride membranes ((a) 50, (b) 100 and (c) 200 nm in thickness) are supported by 300 µm thick silicon wafer with 500 µm × 500 µm window for nanopore preparation study (top). Schematic diagram (not to scale) of the DuraSiN™ film (DTF-05523) (bottom)

The second type of Si₃N₄ membrane was fabricated at Tyndall National Institute, University College Cork, Cork, Ireland (shown in Figure 2.1.2 and 2.1.3). A combination of photo- and electron-beam lithography, etching and deposition procedures was employed to fabricate the arrays of nanopores [67]. The 100 nm thickness Si₃N₄ nanopore array membrane was supported on a square silicon chip support (5 mm × 5 mm). The pores were one of three different radii ($r_a = 75, 50$ and 17 nm). Each membrane contains 400 pores in a hexagonal block packing arrangement with pore centre-to-centre separation, $r_c = 20r_a$. 
Figure 2.1.2: Tyndall nanoporous membranes. The left and right hand sides in the image represent the top (in contact with aqueous phase) and bottom (in contact with organic phase) of the membranes, respectively.

Figure 2.1.3: SEM images of part of Si$_3$N$_4$ nanopore arrays with 75 nm (left) and 17 nm (right) pore radii.

The microporous silicon membrane used in the application study in Chapter 6 was fabricated from silicon wafers at Tyndall National Institute, University College Cork, Cork, Ireland, similar to the nanoporous membrane (shown in Figure 2.1.4 and 2.1.5). The procedure involves standard photolithographic patterning, in conjunction with potassium hydroxide wet etching and deep reactive ion etching (DRIE) [66]. The membrane thickness was 100 µm with pores of $11.09 \pm 0.12$ µm in radius, $r_a$. The membrane contains 30 pores in a hexagonal close-packed (HCP) arrangement with $r_c$ of approximately $18.4 \pm 2.1$ times the pore radius, $r_a$ ($r_c = 18.4r_a$) [66].
Figure 2.1.4: Tyndall microporous membranes. The left and right hand sides in the image represent the top (in contact with aqueous phase) and bottom (in contact with organic phase) of the membranes, respectively. The bottom of the membrane will be sealed onto the lower orifice of a borosilicate glass tube using silicone rubber sealant, later on.

Figure 2.1.5: Scanning electron micrographs of a silicon membrane with 30 pores of ca. 22.18 µm in diameter, \(d\). The left image represents a complete array, and the right image represents a close-up of individual pore.

In addition, an organic micro-/submicro-pore parylene C membrane supplied by Indiana University, USA was used in the electrochemical characterisation study (Figure 2.1.6 and 2.1.7). ‘Submicro’ is defined as the length scale in the range of less than a micrometer but more than 100 nm. This membrane, which was developed by conformal vapour deposition of parylene C onto electron microscopy grids, producing highly ordered PMA with concave-shape pores in a cubic close-packed (CCP) arrangement. The pores have wide openings at the top and bottom, and a narrow channel in the middle. Each individual pore measured 8.3 µm at the top and bottom openings, 0.7
µm in the central constriction diameter and 11.0 µm in thickness. The pore centre-to-centre distance measured 16.5 µm [164]. An electron micrograph of a silica structure that replicates the membrane pore geometry and size has been reported [164].

![Image of Parylene C porous membrane arrays (PMAs) positioned within slits between PDMS elastomer](image1)

Figure 2.1.6: Parylene C porous membrane arrays (PMAs) positioned within slits between PDMS elastomer

![Image of SEM micrograph of a parylene C porous membrane arrays with cubic close-packed arrangement](image2)

Figure 2.1.7: SEM micrograph of a parylene C porous membrane arrays with cubic close-packed arrangement

2.1.2 Nanopore preparation via focused ion beam milling

The nanopore preparation work in this study utilised a FIB. This beam was employed to physically drill holes in silicon nitride membranes to form the nanopore membranes that are the subject of investigation.

The focused ion beam scanning electron microscope (FIBSEM) instruments, Zeiss Neon 40EsB (Carl Zeiss Nano Technology Systems, Oberkochen, Germany) at John de Laeter Centre, Curtin University or DualBeam Helios Nanolab 600 FIB/SEM facilities (FEI Company, Eindhoven, NL) at FIB
Centre, Institute of Analytical and Bioanalytical Chemistry, University of Ulm, Germany were employed for this study. These instruments serve a dual function: FIB for milling process and SEM for imaging. The instruments are equipped with gallium ion beam for the pore milling process. The instruments have a beam acceleration voltage (the speed or energy of electrons) in the range of 0.2 to 30 kV in which the highest acceleration voltage is employed in this study (30 kV). The ‘feature milling’ mode was applied in the pore formation process with 50 pA imaging and milling current which corresponds to an ion beam probe size of 25.0 nm, utilising the Zeiss Neon 40EsB instrument, while the DualBeam Helios Nanolab 600 FIB instrument utilised 10 pA milling current resulting in an ion beam probe size of 12.8 nm. Single and array nanopores were milled in the 500 µm × 500 µm Si₃N₄ film window. The sputtering of the Si and N atoms due to impingement of the high intensity ion beam on the solid state silicon nitride membrane resulted in pore formation [141, 147, 154].

The silicon nitride membranes for the nanopore preparation work, DuraSiN™ membranes, were placed on aluminium SEM stubs' using copper tape or silver paint to facilitate easy removal. As they will be used for electrochemical characterisation study, later on, the membranes were not coated with a conductive layer, a typical of SEM samples preparation. The membranes for FIB milling process are 50 nm in thickness, and are supported by a 300 µm thick silicon wafer. The physical characteristics of the prepared nanopore membranes were obtained from SEM imaging. The milled pores were imaged and characterised by SEM with a beam of 5 kV or 3 kV, utilising the Zeiss Neon 40EsB or DualBeam Helios Nanolab 600 instruments, respectively. Nanopore geometry measurement from the SEM images was conducted utilising ImageJ software (National Institutes of Health, Maryland, USA).

SEM, which is a tool for observing the surface of micro- and nano-sized specimens by using a focused beam of electrons, operates under vacuum. The specimen is irradiated with an electron probe to emit secondary electrons from its surface that produce an image when observed by two-
dimensional scanning of the probe over the surface. The specimen in SEM can be magnified from 10 to 300,000 times, as shown with a scale bar. Acceleration voltage, beam current, working distance and objective aperture are among the variables that need to be considered when conducting SEM imaging.

2.1.3 Nanopore preparation via transmission electron microscope instrument

The transmission electron microscope (TEM) instrument is widely used for imaging, with limited literature reported on its ability for pore formation [147, 153, 154]. This instrument utilises a high energy electron beam to irradiate and pass through a sufficiently thin sample, typically 100 nm in thickness.

In this study, a JEOL 3000F field emission gun transmission electron microscope (FEGTEM) (JEOL Ltd., Tokyo, Japan) is employed to prepare single or low-number nanopore arrays in the Si₃N₄ membranes (500 µm × 500 µm film window), using direct electron beam writing with the beam of TEM. This instrument is available at Centre for Microscopy Characterisation and Analysis (CMCA), The University of Western Australia (UWA), Australia. The field emission gun provides the main source of electrons and, operates at an acceleration voltage of 300 kV. The intense electron beam (e-beam) is used to directly fabricate nanopores with pore diameters less than 30 nm. The convergent-beam diffraction (CBD) mode (2.4 nm - 9) was implemented in the drilling process, where 2.4 nm represents the spot/probe size and 9 represents the beam divergence angle, α. In CBD mode, α (as depicted in Figure 2.1.8) ranges from 1 (smallest, ion beam is focused) to 9 (biggest, ion beam is spread). The sputtering of the Si and N atoms on both membranes sides, due to the impinging high intensity electron beam on the solid state silicon nitride membrane resulted in the pore formation [141, 147, 154].

Prior to the drilling process, the membrane is allowed to stabilize for approximately 5 minutes to reduce the effect of drifting that could be checked using Fast Fourier Transform (FFT) analysis. From the manufacturer’s data, the probe drift is recorded to be 0.9 nm/min [165]. Since the pore drilling
process via TEM has been reported to be lengthy (frequently requires 400 to 600 s for individual pore drilling [152]), as compared to FIB machining (0.1 to 4.0 s [166]), the drifting effect should be as minimum as possible. The physical characteristics of the prepared porous membranes were obtained from TEM imaging. The membranes for TEM-drilled process are 50, 100 and 200 nm in thickness, and supported by a rigid silicon wafer, 300 µm in thickness.

![Beam divergence angle (α)](figure.png)

**Figure 2.1.8: Beam divergence angle (α)**

## 2.2 The electrochemical cell

### 2.2.1 The four-electrode system at the ITIES

The four-electrode potentiostat system at the ITIES consists of two counter electrodes (CE) and two reference electrodes (RE), with one of each electrode in each phase, respectively. It was first introduced by Samec and co-workers in late 1970s [61, 62]. This system allows for the polarisation of the interface, therefore the Galvani interfacial potential difference can be monitored and controlled while recording the current response. In a conventional experimental set-up, the denser phase which is the organic phase, is positioned at the bottom of the electrochemical cell, while the lighter phase (the aqueous phase) is positioned at the top. For electrochemistry at the ITIES, the interface itself is considered the working electrode since the observed current is due to charge transfer across the interface.
2.2.2 The working electrode (WE)

In a conventional solid | liquid electrochemical system, the working electrode (WE) is the electrode where the redox reaction of importance occurs. This type of electrode is fabricated from electrically conducting materials, for example carbon, platinum and gold. However, a liquid conductor such as mercury can also be used, even though the use is decreasing due to environmental concerns [5, 6]. The working electrode material has a great impact on the performances of the voltammetric techniques, in which high signal-to-noise ratio and repeatability of response are expected. Various factors such as the redox behaviour of the target analyte, potential window, the reproducibility of the surface, mechanical properties, cost and toxicity need to be considered for its selection [6]. On the other hand, the quality of the voltammogram obtained depends on several factors such as surface cleanliness, employment of convection-free environment and ability to minimise problems associated with resistance. Since the Ohmic drop (or \( IR \) drop) term is associated with the uncompensated resistance arising from the solution between the WE surface and the RE, small working to reference electrode separations and also sufficiently conducting solutions are generally employed to minimise this undesirable drop [5].

In the case of the four-electrode ITIES set-up, the working ‘electrode’ is the interface itself. Similarly, in the miniaturised two-electrode ITIES set-up where the liquid | liquid interface is modified by immobilizing porous solid-state inert membrane at the ITIES, the working ‘electrode’ is defined by the interface, as by definition, this is where the charge (ion and electron) transfer of importance occurs. As compared to a solid electrode, the ITIES offers the advantage of reproducibility of the interface ‘surface’ where no mechanical or electrical cleaning is required. The geometry/physical properties of the working ‘electrode’ has a big effect on the electrochemical response, and may be modified using micro- and nano-pore membranes, or by using micro- and nano-pipettes [16].
2.2.3 The reference electrode (RE)

In a conventional solid | liquid electrochemical system, the reference electrode (RE) provides a stable and reproducible reference potential, against which the potential of the WE is measured [3, 5, 6]. The most common examples of the RE in voltammetric studies are silver | silver chloride | chloride electrode (Ag | AgCl | Cl') and calomel reference electrode (Hg | Hg₂Cl₂ | Cl'). Respectively, the half-cell reactions utilized in these electrodes are expressed as follows [1, 5]:

\[
\text{AgCl}(\text{solid}) + e^- \rightleftharpoons \text{Ag}(\text{solid}) + \text{Cl}^- (\text{solution}) \quad (\text{Eq. 2.2.1})
\]

\[
\frac{1}{2}\text{Hg}_2\text{Cl}_2(\text{solid}) + e^- \rightleftharpoons \text{Hg}(\text{liquid}) + \text{Cl}^- \quad (\text{Eq. 2.2.2})
\]

Typically, NaCl or KCl provides the source of aqueous solution-phase chloride ions for both the Ag | AgCl | Cl' and calomel electrodes, with concentration ranging from 1.0 mM to saturated. The constant makeup of the RE guarantees it operates at a fixed potential [1].

In the case of liquid | liquid electrochemical system, the reference electrodes are positioned in two Luggin capillaries, located close to the interface to minimise the IR drop effects [19]. These electrodes monitor the potential of the whole electrochemical cell, inclusive of the liquid | liquid interface. Normally, Ag | AgCl | Cl' is used as the aqueous phase reference electrode, and this phase contains a constant chloride composition to maintain a constant electrode potential [16, 167]. On the other hand, the organic phase reference electrode is more complicated since it consists of two interfaces. Again Ag | AgCl | Cl' electrode is used, and in this case, aqueous chloride solution contains a common cation with the organic phase electrolyte salt [21]. This latter solution is termed the organic phase reference solution. In this study, the organic electrolyte salt is bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate (BTPPATPBCl), thus the cation in the reference solution is BTPPA⁺. The interface where the organic phase reference solution and organic phase are in contact is termed the reference interface [21]. The common cation
(BTPPA\(^{+}\)) equilibrates between the two solutions and creates an interfacial potential expressed in Equation 1.2.12. The potential applied by the potentiostat and observed on a cyclic voltammogram normally differs from the Galvani standard ion transfer potential, \(\Delta_\beta^0 \phi_i^0\). However, it is the sum of the interfacial potential, the potential of the two reference electrodes and the potential of the reference interface. For that reason, on completion of an experiment, a suitable reference ion such as tetrapropylammonium chloride (TPrACl) may be added to the aqueous phase to reference the half-wave potentials \(E_{1/2}^{TPrA+}\) and hence to the Galvani potential scale.

2.2.4 The counter electrode (CE)

The third electrode normally present in a voltammetric experiment is termed the counter (or auxiliary) electrode (CE) [1, 5]. It is typically a good electronic conductor (e.g. metal), which performs as electron source or sink in the electrochemical circuit. The presence of this electrode in the electrochemical cell permits the potentiostat to pass current through the electrolyte solution without the need to pass current into or out of the reference electrode. This avoids any interferences to the reference electrode potential, either by electrode polarisation or \(IR\) drop [1, 6]. The counter electrode is usually made from an inert conductive material, such as platinum (wire or gauze) or carbon (disc or rod), placed directly in the test solution [5]. The design should incorporate a sufficiently large surface area, greater than the WE surface area to ensure current flowing in the total circuit is not limited, since current measured in a voltammetric experiment flows between the WE and CE. In addition, a small surface area would introduce extra resistance to the system [1, 5]. These CEs can be achieved by spot-welding Pt-mesh to a Pt wire [4, 16]. The counter electrode platinum wire in the organic phase is enclosed by materials such as glass or Teflon to avoid any contact with the aqueous phase and the interface [21].
2.3 Electrochemical techniques

2.3.1 Potentiodynamic

2.3.1.1 Linear sweep voltammetry (LSV)

Linear sweep voltammetry is a technique where the potential of the working electrode is swept linearly from an initial potential, \( E_1 \), where no current flows, to a final potential, \( E_2 \), where the charge transfer process occurs (illustrated in Figure 2.3.1). The applied potential, \( E \), is a function of the speed at which the potential is swept (\( v \)) and the sweep time (\( t \)). During the linear sweep experiment, the current is measured continuously (illustrated in Figure 2.3.2). In this case, the geometry of the interface is of macro-scale ITIES where linear diffusion occurs, resulting in a peak-shaped voltammetric response. The maximum current or also known as the peak current, \( I_p \) is directly proportional to the concentration of the transferring species and increases with voltage sweep rate [7]. Several publications have reported the application of LSV technique for charge transfer study at the miniaturised-ITIES [110] and electrodes [86, 168].

![Figure 2.3.1: Linear sweep voltammetry potential-time excitation signal](image-url)
2.3.1.2 Cyclic voltammetry (CV)

Cyclic voltammetry is the most widely employed voltammetric technique for gaining qualitative information about electrochemical processes [15, 20]. CV can provide information on reversibility, diffusion, adsorption, lipophilicity, kinetics and the number of electrons involved in the respective processes [1, 7, 12].

This technique is basically the extension of linear sweep voltammetry technique (discussed in the previous section). CV measures the current passing through a working electrode as the applied potential is varied from an initial (starting) potential, $E_1$, to a final (switching) potential, $E_2$, then reversed to $E_1$, at a fixed scan rate, $v$. This produces a triangular potential waveform, as illustrated in Figure 2.3.3 [7]. The sweep can be terminated after the first cycle, or it can be repeated for many cycles. $E_1$ is typically chosen at a potential where no electrochemical reaction occurs, while $E_2$ is normally selected so that the potential interval ($E_2 - E_1$) contains the electrochemical process of interest. The construction of a plot of current versus potential yields a ‘voltammogram’ (as shown in Figure 2.3.4) [15]. The interface geometry is assumed to be a macro-scale ITIES where linear diffusion occurs, resulting in a peak-shaped voltammogram. The shape of the voltammogram on the forward sweep is analogous to the case of linear sweep voltammetry.

Figure 2.3.2: Linear sweep voltammogram for the transfer of a cation from aqueous (W) to organic (O) phase
Voltammetric data analysis is not limited to peak current, peak-to-peak potential separation and limiting current measurement only. However, additional information, for instance rate constants, diffusion coefficients and transfer coefficients, can be extracted via numerical simulation [15, 17].

Information about the species being investigated can be generated from parameters measured from a cyclic voltammetry experiment, namely the peak current, \( I_p \) (W \( \rightarrow \) O or O \( \rightarrow \) W) and the peak potential, \( E_p \) (W \( \rightarrow \) O or O \( \rightarrow \) W) (Figure 2.3.4), in the case the diffusion is linear. The peaks on both forward and reverse sweeps are equally important. W is the aqueous phase, and O is the organic phase.
aqueous phase on the reverse sweep. $I_{p, W \rightarrow 0}$ and $I_{p, 0 \rightarrow W}$ represent the peak currents, while $E_{p, W \rightarrow 0}$ and $E_{p, 0 \rightarrow W}$ represent the peak potentials for the forward and reverse peaks, respectively.

The peak current is expressed by the Randles-Sevcik equation, as discussed earlier in Section 1.3.6. Assumptions that the diffusion is linear, has large excess of supporting electrolyte and that the interface is flat, are included in this equation [12]. Regardless of voltammogram reversibility (reversible, irreversible or quasi-reversible), the absolute magnitudes of the peak currents, $I_p$ in the forward and reverse sweeps depend on the voltage scan rate [7]. To verify system reversibility, a graph of $I_p$ measured versus the square root of the scan rate, $v^{1/2}$ can be plotted. For a reversible system, the ratio of $I_{p, W \rightarrow 0} / I_{p, 0 \rightarrow W}$ must be $\sim 1$ and $I_p$ for both peaks are proportional to $v^{1/2}$. In addition, the peak-to-peak separation, $\Delta E_p (E_{p, W \rightarrow 0} - E_{p, 0 \rightarrow W})$ is approximately $(59/z)$ mV (at 25 ºC). For an irreversible system, no reverse peak is observed. For a quasi-reversible system, the peak-to-peak separation is greater than $(59/z)$ mV and depends on the scan rate. Also, $I_p$ is not proportional to $v^{1/2}$ [7]. On the other hand, the slope of $I_p$ versus $v^{1/2}$ can give information on the diffusion coefficients of the transferring ions in the aqueous phase, $D^a$ [12].

Earlier in Section 1.2.6 and 1.2.7, the generation of voltammetric response of the background electrolyte and analyte at the micro-ITIES array, respectively, have been discussed. In addition, the two types of arrays employed in this thesis, namely the micro- and nano-ITIES arrays, the possible diffusion regimes and the resulting current equation, have been presented in Section 1.3.5 and 1.3.6.

2.3.1.3 Stripping voltammetry (SV)
Stripping voltammetry is a two-step electrochemical technique which has proven to be valuable for improving the detection capabilities [6, 26, 169]. SV involves a potential-controlled pre-concentration step, followed by a stripping (or detection) step, as depicted in Figure 2.3.5. In the case of stripping
voltammetry at the ITIES, in the pre-concentration step, the target species is selectively extracted from the aqueous phase then accumulated into the organic phase during the pre-concentration time. In the second step, the accumulated species is back-transferred to the aqueous phase by application of a suitable voltammetric method. The scanning of the potential can either be in the positive or negative direction. The stripping (back-extraction) process results in peak current or charge which is the analytical response, and is proportional to the analyte concentration [12, 16, 80]. The stripping voltammetric experiments at the ITIES can detect not only cationic or anionic species, even neutral molecules whose transfer process is assisted by ions [80]. Besides, redox activity is not compulsory provided that the Gibbs energy of transfer is within the available potential window for the transfer of background electrolyte ions [80].

Linear sweep, staircase, square-wave or differential pulse voltammetry are among the most frequently used detection techniques for the stripping step. In order to reduce the limit of detection (LOD) achieved, the pre-concentration time can be manipulated. In addition, background subtraction step may further improve the sensitivity of this technique [12, 16, 80]. Stripping voltammetry at miniaturised-ITIES arrays is suitable for the detection of analytes in real samples, such as water and biological fluids (blood and saliva), since it offers high specificity, sensitivity (detection limits as low as nM or ppb level) and reliability [169].
2.3.2 Potentiostatic

2.3.2.1 Chronoamperometry (CA)
Chronoamperometry (CA) is an electrochemical technique that studies current variation with time under potentiostatic control. In CA, the potential of the working electrode is stepped from an initial potential (where no current flows), $E_1$, to a final potential (where the charge transfer process occurs), $E_2$ (as shown in Figure 2.3.6). Initially, a large current is generated, and as charge transfer process occurs, the transferring species depletes, and the current decays exponentially with time, ultimately to zero \cite{7}. The plot of current versus time from a chronoamperometric experiment produces a ‘chronoamperogram’ (as shown in Figure 2.3.7) \cite{1, 4, 7}.

Figure 2.3.6: A typical waveform of potential step chronoamperometry
Several analytical expressions have been developed to describe the change in electrical current response with respect to time in a controlled potential experiment. The Cottrell equation serves as the basic expression to describe the current-time transient for linear diffusion control process which is the situation occurring at a macroelectrode [1, 7, 170]:

$$|I| = \frac{nFAD^{1/2}C}{\pi^{1/2}t^{1/2}}$$  \hspace{1cm} (Eq. 2.3.1)

where $|I|$ is the diffusion-limited current (A), $n$ is the stoichiometric number of electrons involved in an electrode reaction, $F$ is the Faraday constant, $A$ is the electrode surface area (m$^2$), $t$ is the time (s), and $D$ and $C$ are the diffusion coefficient (m$^2$ s$^{-1}$) and the bulk concentration (mol m$^{-3}$), respectively.

When spherical diffusion dominates, such as at a microelectrode, the experimental transient decays to a limiting current, as opposed to zero for Cottrellian characteristic. The current-time expressions are given by Aoki and Osteryoung [171], Shoup and Szabo [172], and Mahon and Oldham [173, 174]. The first expression by Aoki and Osteryoung describes current as a function of a dimensionless time parameter, $\tau$:

$$I = 4nFrtDcf(\tau)$$  \hspace{1cm} (Eq. 2.3.2)

where:
\[ \tau = \frac{4Dt}{r^2} \]  
(Eq. 2.3.3)

\( r \) in the expression is the electrode radius (m).

Two different expressions for \( f(\tau) \) has been developed. At short times when \( \tau \) is less than 1.44:

\[ f(\tau) = \sqrt{\frac{\pi}{4\tau}} + \frac{\pi}{4} + 0.094\sqrt{\tau} \]  
(Eq. 2.3.4)

while at longer times when \( \tau \) is more than 0.82:

\[ f(\tau) = 1 + 0.71835\tau^{-1/2} + 0.05626\tau^{-3/2} - 0.00646\tau^{-5/2} \]  
(Eq. 2.3.5)

Shoup and Szabo [172] later derived a single expression to describe current over the full range, with a maximum error of less than 0.6 %, since the developed expressions were not sufficient to describe the variation of \( I \) over the entire range of \( \tau \):

\[ f(\tau) = 0.7854 + 0.8862\tau^{-1/2} + 0.2146\exp(-0.7823\tau^{-1/2}) \]  
(Eq. 2.3.6)

The expressions developed by Mahon and Oldham [173, 174] are the most exact closed-form expressions to describe the current-time transient, with a maximum error of 0.02 %. For times less than 1.281 \( r^2 / D \), the expression is:

\[ \frac{I(t)}{n\pi FrDC} = \frac{r}{\sqrt{\pi Dt}} + 1 + \frac{1}{2r} \sqrt{\frac{Dt}{\pi}} - \frac{0.12003Dt}{r^2} + \frac{0.013273(Dt)^{3/2}}{r^3} \]  
(Eq. 2.3.7)

and for times greater than 1.281 \( r^2 / D \), the expression is:

\[ \frac{I(t)}{n\pi FrDC} = \frac{4}{\pi} + \frac{8r}{\sqrt{\pi^5Dt}} + \frac{0.0089542r^3}{(Dt)^{3/2}} - \frac{0.00025664r^5}{(Dt)^{5/2}} - \frac{0.00022312r^7}{(Dt)^{7/2}} + \frac{0.000027628r^9}{(Dt)^{9/2}} \]  
(Eq. 2.3.8)

These equations can be readily applied to the ITIES, with \( n \) replaced by the charge number of the transferring ion, \( z \). This potential step experiment can
be employed to calculate the diffusion coefficient of the transferring species [7].

2.4 Experimental electrochemical procedures

2.4.1 Reagents
A complete list of all the chemicals used for the duration of this study is given in Appendix A. All reagents were obtained from Sigma Aldrich, Australia, and were used as received, unless stated otherwise.

10 mM lithium chloride (LiCl) which was prepared in ultrapure water (resistivity of 18 MΩ cm) from a Milli-Q water purification system (Millipore Pty Ltd, North Ryde, NSW, Australia) was employed as the aqueous phase solution during the course of the studies outlined in this thesis. The organic phase used was 10 mM BTPPATPBCl dissolved in 1,6-dichlorohexane (DCH) in the nano-ITIES study, while in the micro- or submicro-ITIES study, the organic phase was present as an organogel with incorporation of low molecular weight PVC, to increase the mechanical stability of the interface [20, 38, 63, 65], generally at (10 % w/v), unless stated otherwise. The organic electrolyte salt was prepared by metathesis reaction of bis(triphenylphosphoranylidene)ammonium chloride (BTPPACl) and potassium tetrakis(4-chlorophenyl)borate (KTPBCl), as outlined in Appendix B. Prior to the experiments, both the aqueous and organic phase solvents were mutually pre-saturated. This step was conducted to prevent instability associated with mixing when contacting the ultrapure water and 1,6-DCH solutions. The organic reference solution consisted of 10 mM BTPPACl dissolved in 10 mM LiCl (aqueous). The investigated analytes were prepared in 10 mM LiCl, unless stated otherwise.

As mentioned, the organic phase in the micro- or submicro-ITIES experiments (the cationic β-agonist drug detection studies, as well as the characterisation of an organic membrane) was present as an organogel. The organogel composed of an organic solvent (1,6-dichlorohexane (1,6-DCH)),
an organic electrolyte (BTPPATPBCl), and the low molecular weight poly(vinylchloride) (PVC). The procedure to prepare an organogel is detailed as follows:

a) 2 mL of 1,6-DCH solution was added to a pre-weighed amount of organic electrolyte salt in a 25 mL beaker, producing a 10 mM concentration of BTPPATPBCl solution

b) The electrolyte solution was warmed on a hot plate to approximately 60 °C and stirred continuously using a magnetic stirrer, until the solution went clear

c) 0.2 g of PVC was added in stages to produce a 10 % w/v solution. In the case of parylene C organic membrane characterisation study, only 1 % w/v solution is used. Initially, a cloudy solution was observed, which was then allowed to become clear over a period of 20 minutes, with the assistance of stirring. The temperature was increased regularly to a maximum of approximately 105 °C

d) The organogel was transferred into the cylindrical borosilicate glass tube with micro-/submicro-porous membrane attached to the bottom, using a pasteur pipette. The prepared organogel was allowed to cure at room temperature for 1 hour prior to use

e) This procedure allowed the organogel to fill the hydrophobic pores, resulting an inlaid interface at the ITIES

2.4.2 Preparation of micropore- and nanopore-supported ITIES

The micro- and nano-porous membranes were sealed onto the lower orifice of a cylindrical borosilicate glass tube (as depicted in Figure 2.4.1) using silicone rubber sealant (Selleys, Australia and New Zealand) and allowed to cure for 72 hours prior to the first usage.

In the case of the Tyndall microporous and nanoporous membranes, the glass tube inner and outer diameters were 2.5 mm and 4.0 mm, respectively. On the other hand, the glass tube inner and outer diameters for DuraSiN™ nanoporous membrane and parylene C micro-/submicro-porous membrane were 1.4 mm and 3.0 mm, respectively.
Figure 2.4.1: DuraSiN™ (left) and Tyndall (right) nanopore membranes attached to a glass tube photographed using Canon EOS 600D digital SLR (Canon Inc., Tokyo, Japan)

In the experiment with nanoITIES, the glass tube contained approximately 10 to 50 µL of the organic phase solution with 200 µL of the organic reference solutions placed on top of it. In the experiment with micro- or submicro-ITIES, approximately 100 µL of the organogel was used. This glass tube was then immersed in the 6 mL of the aqueous phase solution contained in a 10 mL glass beaker. Figure 2.4.2 illustrates the organic phase-filled nanopore arrays in contact with the aqueous phase.

Figure 2.4.2: Schematic diagram (not to scale) showing the cross sectional of the nanopore array membrane filled with the organic phase and in contact
with the aqueous phase. Arrows represent diffusion of TPrA$^+$ ions across the nanoITIES. The electrochemical cell employed a two-electrode system, and the details of the system will be discussed in Section 2.4.4.

2.4.3 The miniaturised ITIES experimental set-up: The four-electrode system

The miniaturised ITIES employing a four-electrode system is shown in Figure 2.4.3. This set-up is used in the characterisation of a micro-/submicro-pore parylene C organic membrane to compensate the resistance associated with the organic phase and the membrane [105]. This system comprises of two counter electrodes and two reference electrodes. The interfacial potential difference was employed between the two Ag | AgCl reference electrodes (each in the aqueous and organic phases). The resulting current was measured by two Pt mesh counter electrodes (one each in the aqueous and organic phases). The interface itself acts as the working electrode since the current measured is due to charge transfer across the interface.

The Ag | AgCl reference electrodes were prepared by oxidising Ag wires in ferric chloride (FeCl$_3$) solution (concentrated). Upon contact with the solution, a visible redox reaction occurred immediately. This resulted in the formation of a silver chloride layer. The redox reaction occurring is given by the following equation:

$$Ag(s) + FeCl_3(aq) \rightarrow AgCl(s) + FeCl_2(aq)$$  \hspace{1cm} (Eq. 2.4.1)

The counter electrodes were prepared by spot-welding a mesh of platinum to one end of a platinum wire. The platinum counter electrode in the organic phase is covered by glass or Teflon-coating to avoid any contact with the organic reference solution and the interface. The electrochemical cell was clean in a suitable solvent, typically acetone and dried using an air gun to evaporate off any remaining solvent before and after each electrochemical experiment.
Figure 2.4.3: The four-electrode system used for electrochemistry at the miniaturised ITIES

2.4.4 The miniaturised ITIES experimental set-up: The two-electrode system

The electrochemical cell used to support the two-electrode set-up is illustrated in Figure 2.4.4. In contrast to macroscopic ITIES, the interfacial CT current is small, therefore it is possible to perform the potentiostatic experiments at micro- and nano-ITIES by applying voltage between the two reference electrodes, thus a four-electrode set-up is not necessary [138].

In this two-electrode electrochemical cell, two Ag | AgCl electrodes were employed and functioned as reference and counter electrodes in their respective phases. The applied potential, \( E \), is defined as the potential difference between the two electrodes, with positive current due to the transfer of cations from the aqueous to the organic phase [33]. The Ag | AgCl electrodes were prepared by oxidising silver wire in concentrated ferric chloride (FeCl\(_3\)) solution.
2.4.5 Electrochemical procedure at the micro- and nano-ITIES arrays

Voltammetry and amperometry experiments at micropore- and nanopore-supported liquid-liquid interface arrays were performed using an Autolab PGSTAT 302N (Metrohm Autolab B. V., Utrecht, The Netherlands) interfaced to a personal computer. Nova 1.7 or Nova 1.8 software package supplied with the instrument was used for data processing. A potentiostat by definition is an instrument that has voltage control over the WE-CE pair, and adjusts the voltage to maintain the potential difference between the WE-RE pair [1]. The methodology applied when preparing the set-up for microITIES was similar to nanoITIES with the major difference being the membrane used to support the miniaturised ITIES. The electrochemical cell set up was placed in a Faraday cage for the duration of the experiments to minimise electrical noise.

In the voltammetric studies, a sequence of background voltammograms (i.e. no analyte present in the aqueous phase) was run over a wide potential range to establish the limits of the available potential window before injection.
of the required amount of analyte concentrated solution into the aqueous phase with a micropipette to achieve the required analyte concentration. On injection of each analyte aliquot, a sequence of three CVs was obtained. A 5 s quiet time was employed at the initial applied potential prior to each scan to stabilize and minimize the background charging current. The voltammetric sweep rate implemented throughout the duration of this study was 5 mV s\(^{-1}\), unless stated otherwise. The selection of a proper scanning rate in voltammetry is vital to obtain a very minimum charging current and a steady-state [138]. The membrane used in this study was generally cleaned with acetone and dried with air before and after each electrochemical experiment. All the experiments were conducted at room temperature (20 °C).

2.4.6 Contact angle study

Contact angle study was conducted to investigate membrane hydrophobicity. The measurements employed a Contact Angle Meter, CAM 101 (KSV Instrument Ltd., Helsinki, Finland) and the software supplied with the instrument (KSV Contact Angle Measurement System). The contact angle (water/air/membrane) between the dispensed drop (approximately 1 µL) and the substrate surface was measured directly after the contact was created.
Chapter 3

Electrochemical Characterisation of Nanoscale-Liquid | Liquid Interfaces Confined within Silicon Nitride Membrane Prepared by Focused Ion Beam Milling

3.1 Introduction

In this chapter, the preparation of single and array nanopore membranes via FIB milling and characterisation of ITIES localised within the FIB-generated nanopores is presented. The behaviour of the nanoITIES was characterised by cyclic voltammetry (CV) with the transfer of tetrapropylammonium cation (TPrA⁺) across a water | 1,6-dichlorohexane (DCH) interface chosen as a model system. The voltammetric responses in terms of the number of pores in the array, and the ratio between the pore-to-pore separation and the pore radius were investigated, besides the estimation of the charging time. FIB milling serves as a platform for simple and fast prototyping of nanopore membrane which are potentially useful in chemical and biochemical sensing applications, as well as fundamental electrochemical studies.

The fabrication of solid-state nano-dimension pores with true nanometre dimension is challenging and several approaches have been tested such as, ‘ion beam sculpting’ by focused ion beam (FIB) milling [139, 161], direct drilling using transmission electron microscopy (TEM) with high-energy focused electron beams [147, 153, 154, 162], electron beam lithography
(EBL) and chemical etching [67, 150], reactive gas- or water vapour-assisted charge beam etching [148, 152], and track etching by high-energy heavy metal ion and chemical etching approaches [101, 105], as discussed earlier in Section 1.4.3. FIB milling provides an alternative to EBL since it is a direct-write method, and thus facilitates the fast prototyping of nanopore membranes [175]. EBL, on the other hand, involves more complex and lengthy procedures, which prove to be time-consuming [175]. SiN and silicon oxide (SiO) are the most widely used materials of nanopore and nanopore structures generated by FIB [140]. To the best of our knowledge, little information is available on these engineered nanopore membranes as platforms for nano-interface between two immiscible electrolyte solutions (nanoITIES) and nanoelectrochemical sensing, which is the focus of this study.

3.2 Experimental methods and materials

3.2.1 Materials and reagents
The silicon nitride membranes (DuraSiN™ film) used for nanopore preparation via FIB milling had a film thickness of 50 nm. The membrane specifications are as outlined in Section 2.1.1 (Chapter 2).

The chemical reagents used in this chapter are as explained in Section 2.4.1 of Chapter 2. The primary tetraalkylammonium salt used as model analyte in this study was the chloride salt of TPrA⁺ in 0.01 M LiCl in 1,6-dichlorohexane (DCH)-saturated water. In addition, tetraethylammonium (TEA⁺) and tetrabutylammonium (TBA⁺) used as analytes were in their chloride and bromide forms, respectively.

3.2.2 Nanopore preparation by FIB milling
Single and array nanopores were milled in the 500 µm × 500 µm SiN film window of the DuraSiN™ membranes using the FIBSEM instruments detailed in Section 2.1.2 of Chapter 2. Nanopore preparation by FIB milling
presented in this chapter was assisted by Dr. Nigel C. Tan (Curtin University, Australia) and Gregor Neusser (University of Ulm, Germany).

3.2.3 Contact angle study
A contact angle study was conducted to investigate DuraSiN™ membrane hydrophobicity, and the procedures have been described in Section 2.4.6 of Chapter 2.

3.2.4 Preparation of nanopore-supported ITIES
The single and array nanoITIES were formed at a water | DCH interface. The procedures to attach nanopore membrane onto borosilicate glass tube, and the nanopore-supported ITIES assembly have been detailed in Section 2.4.2 (Chapter 2).

3.2.5 Electrochemical procedure
Following assembly of the electrochemical cell, voltammetric studies were conducted employing procedures outlined in Section 2.4.5 of Chapter 2. A two-electrode electrochemical cell with two Ag | AgCl electrodes was employed in this work. The scheme of the electrochemical cell set-up is as depicted in Figure 2.4.4 of Chapter 2.

The electrochemical cell can be summarized as follows:

\[
\text{Ag} \mid \text{AgCl} \mid x \text{ mM TAA}^+ + 0.01 \text{ M LiCl}_w \mid 0.01 \text{ M BTPPATPBCI}_{\text{DCH}} \mid 0.01 \text{ M BTPPACI in } 0.01 \text{ M LiCl}_w \mid \text{AgCl} \mid \text{Ag}
\]

where \(x\) is the concentration of tetraalkylammonium salt (TAA) solution in the aqueous phase (TEACl, TPrACl and TBABr).

3.3 Results and discussion

3.3.1 Single and array nanopore preparation by FIB milling
Figure 3.3.1 (a) to (h) represents SEM images of the FIB-milled nanopore arrays, which are in cubic close-packed (CCP) arrangement. The geometric characteristics of the nanopore arrays are listed in Table 3.3.1. Nanopores
with radii in the range of approximately 30 to 80 nm were successfully milled into the SiN membrane. Arrays of 9, 16, 25, 100 and 400 pores, featuring the ratio between the pore-to-pore separation, $r_c$, and the pore radius, $r_a$, in the range of 16 to 32, were prepared. A large ratio $r_c/r_a$ has been previously reported minimising the overlap of diffusion zones formed at adjacent interfaces in electrochemical experiments [67, 109], as will be discussed in Section 3.3.2.
Figure 3.3.1: SEM images of the FIB-milled single and array nanopore membranes with 50 nm film thickness. (a) $1 \times 1$, $r_a = 62 \pm 3$ nm (b) $3 \times 3$, $r_a = 80 \pm 15$ nm (c) $4 \times 4$, $r_a = 66 \pm 9$ nm (d) $5 \times 5$, $r_a = 66 \pm 8$ nm (e) $10 \times 10$, $r_a = 47 \pm 6$ nm and (f) $20 \times 20$, $r_a = 39 \pm 6$ nm nanopore arrays prepared via Zeiss Neon 40 EsB. (g) $r_a$ of $31 \pm 3$ nm, and (h) $r_a$ of $30 \pm 1$ nm, represent $10 \times 10$ nanopore arrays prepared via DualBeam Helios Nanolab 600 with pore centre-to-centre separation, $r_c$ ca. 1000 nm and 500 nm, respectively. All the nanopore membranes featured a cubic close-packed (CCP) arrangement.
### Table 3.3.1: Characteristics of the eight different nanopore array membranes prepared by FIB milling

<table>
<thead>
<tr>
<th>Design</th>
<th>Arrays</th>
<th>Number of pores in the array, $N_p$</th>
<th>Pore radius, $r_a$ (nm) $^a$ (± x)</th>
<th>Pore centre-to-centre separation, $r_c$ (nm) $^b$ (± x)</th>
<th>$r_c/r_a$ $^c$ (± x)</th>
<th>Porosity, % $^c$ (± x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>3 x 3</td>
<td>9</td>
<td>80 ± 15</td>
<td>1312 ± 27</td>
<td>16 ± 3</td>
<td>7.24 ± 2.71 x 10^5</td>
</tr>
<tr>
<td>b</td>
<td>4 x 4</td>
<td>16</td>
<td>66 ± 9</td>
<td>1197 ± 41</td>
<td>18 ± 3</td>
<td>8.76 ± 2.39 x 10^5</td>
</tr>
<tr>
<td>c</td>
<td>5 x 5</td>
<td>25</td>
<td>66 ± 8</td>
<td>1162 ± 26</td>
<td>18 ± 2</td>
<td>1.37 ± 0.33 x 10^4</td>
</tr>
<tr>
<td>d</td>
<td>10 x 10</td>
<td>100</td>
<td>47 ± 6</td>
<td>1019 ± 65</td>
<td>22 ± 3</td>
<td>2.78 ± 0.71 x 10^4</td>
</tr>
<tr>
<td>e</td>
<td>20 x 20</td>
<td>400</td>
<td>39 ± 6</td>
<td>778 ± 20</td>
<td>20 ± 3</td>
<td>7.65 ± 2.35 x 10^4</td>
</tr>
<tr>
<td>f</td>
<td>10 x 10</td>
<td>100</td>
<td>31 ± 3</td>
<td>996 ± 4</td>
<td>32 ± 3</td>
<td>1.21 ± 0.23 x 10^4</td>
</tr>
<tr>
<td>g</td>
<td>10 x 10</td>
<td>100</td>
<td>30 ± 1</td>
<td>497 ± 2</td>
<td>17 ± 1</td>
<td>1.13 ± 0.08 x 10^4</td>
</tr>
</tbody>
</table>

x: Standard deviation

$^a$ Pore radius is average values over 5 pores with 3 measurements in each pore, with the exception of design (a) where 3 measurements were taken of the pore.

$^b$ Pore-to-pore separation is average values over 5 measurements.

$^c$ The standard deviation of $r_c/r_a$ ratio and porosity were calculated using the method of propagation of random errors [176].

The time for pore formation is dependent on the milling rate, which is greatly influenced by the ion beam parameters such as beam current and dwell time [154, 175]. The sputter rate mainly depends on ion flux to the sample, the incidence angle of the ion beam, and the probability that atoms are ejected from the sample [141, 147, 154]. In this study, the nanopores of design (a) to (f) (Figure 3.3.1; Table 3.3.1) which employed similar milling parameters, required approximately 0.5 s for individual pore milling, as measured from a plot of total milling time versus the number of pores (Figure 3.3.2). The sheer magnitude of the increment in total milling time with the 20 x 20 pore arrays necessitated the use of log scales to compare all the pore arrays data directly. The FIB milling applied here is a serial process in which each pore in an array is being prepared individually [175], resulting in a dependence of the
overall milling time on the numbers of pores in the targeted array size, unlike EBL, enabling the formation of pores in a parallel way [67]. Single and array nanopore electrodes were previously fabricated via FIB milling through a SiN layer over a buried platinum electrode. The time to mill through the 500 nm SiN layer with pore radii in the range of ca. 75 to 200 nm was typically 40 s per pore [175]. Patterson et al. [166] reported the time to mill through 200 nm of SiN was typically within the range 0.1 to 4.0 s, depending on the beam current.

Figure 3.3.2: Plot of the dependence of total milling time versus number of pores milled in 50 nm thick SiN film with 30 kV acceleration voltage and 50 pA milling current. Both axes are in log scales

The hydrophobicity of the SiN membrane surface was investigated via water contact angle measurement. The contact angle (C.A.) by definition is a quantitative measure of the wetting of a solid (in this case the SiN membrane) by a liquid (in this case deionised water). The measured value was 93.3 ± 0.5 ° confirming a hydrophobic SiN membrane surface [67]. An assumption was made that the nanopore walls were analogously hydrophobic, since it was not possible to determine the contact angle inside the nanopores. In addition, due to the high surface-to-volume ratio within the nanopores, it may be difficult to permeate with water, and consequently they behave more hydrophobically in practice compared to the membrane surface.
Consequently, the organic phase is assumed to fill the pores in the electrochemical studies presented here.

Membrane porosity, \( \Theta \), is one of the parameters that influence the voltammetric response to ion transfer across the ITIES. It is defined by the cross-sectional area of the pores relative to the whole membrane area. The porosity of the SiN membrane was calculated from the total area of the membrane surface (in this case 0.25 mm\(^2\)) and the total cross-sectional area of the pores, as described by [106]:

\[
\Theta = \frac{\pi r_a^2 N_p}{A_{total}} \times 100 \% \quad \text{(Eq. 3.3.1)}
\]

where \( r_a \) is pore radius (nm) and \( N_p \) is the number of pores of the membrane. The porosity of each membrane studied is shown in Table 3.3.1. The values ranged between 0.00048 % and 0.077 %, demonstrating the minute fraction of the membrane surface occupied by nanopores. In comparison, a \( \gamma \)-alumina ultrafiltration membrane previously used for voltammetric experiments exhibited porosity in the range of 13 % to 30 % [106].

### 3.3.2 Electrochemical characterisation

#### 3.3.2.1 TPRA\(^+\) ion transfer across the nanopore membrane-modified ITIES

The silicon nitride-based single and array nanopore membranes were electrochemically characterised using ion-transfer CV with TPRA\(^+\) ion as the model analyte. CV of the background electrolyte solution was recorded prior to the addition of the analyte ions. Typically, the potential window ranged from 0 to 1 V. In this study, analyte concentrations were varied between 0.1 and 2.0 mM in a background solution of 0.01 M LiCl. CVs of five different concentrations of TPRACl were recorded to obtain a calibration curve correlating currents with ion concentrations in the aqueous phase. Background-subtracted voltammograms were attained by subtracting the background CV from the CV obtained in the presence of TPRA\(^+\).
The shape of the voltammetric responses and the magnitude of the current observed were dependent on several important parameters, which include: (i) the number of nanopores (equivalent to the number of nanointerfaces), (ii) the pore-to-pore separation, \( r_c \), (iii) the pore radius, (iv) the recess depth of the liquid | liquid interface position within the pore channel, \( l \), which primarily depends on the hydrophobicity or wetting properties of the membrane material, (v) the diffusion field, whether spherical or linear diffusion occurs at the nanoITIES, and (vi) the magnitude of the diffusion zone extension, \( \delta \) [67, 85, 86, 135].

Figure 3.3.3 (a) and (b) represent the voltammograms corresponding to the transfer of TPrA\(^+\) ion from aqueous to organic phase, then back to aqueous phase. Recordings were obtained with 2.0 mM and 0.1 mM TPrACl in the aqueous phase, for Figure 3.3.3 (a) and (b), respectively. Figure 3.3.3 (c) and (d) represent the forward scan background-subtracted curves for all concentrations studied. Figure 3.3.3 (a) and (c) represent single nanoITIES with a radius of ca. 62 nm, while Figure 3.3.3 (b) and (d) represent an array nanoITIES comprised of 10 × 10 interfaces of ca. 47 nm radius for the individual pores.
Figure 3.3.3: CVs of (a) 2.0 mM, and (b) 0.1 mM tetrapropylammonium cation (TPrA\(^+\)) transfer across water/1,6-dichlorohexane (DCH) interface formed within a nanoporous silicon nitride membrane, at 5 mV s\(^{-1}\) sweep rate. The dotted and solid lines represent blank and analyte voltammograms, respectively. (c), (d) Background-subtracted voltammograms (forward scan only) of 0.1, 0.5, 1.0, 1.5 and 2.0 mM TPrA\(^+\) transfer across the nanolITIES with arrows indicate increasing analyte concentrations. Figure (a) and (c) represent single nanopore membrane, \(r_a\) of 62 ± 3 nm (design (a) in Table 3.3.1), while figure (b) and (d) represent array (10 × 10) nanopore membrane, \(r_a\) of 47 ± 6 nm (design (e) in Table 3.3.1). The potentials are reported with respect to the experimentally-used reference electrodes.

Voltammograms of the nanopore membrane-modified ITIES in the forward scan (TPrA\(^+\) ion transfer from the aqueous to the organic phase) showed that the current rose steadily with applied potential up to the switching potential. This result is in agreement with previous reports [109], where no steady-state
(or limiting) current plateau was reached in the diffusion-limited region. The nanoITIES based on design (a) (Figure 3.3.3 (a) and (c)) clearly shows this behaviour. The nanoITIES based on design (e) (Figure 3.3.3 (b) and (d)) demonstrated diffusion-limited current initially, which then increased gradually with applied potential up to the switching potential. This phenomenon might be explained by a combination of the influence of background electrolyte ion transfer at higher potential [109] and reversible expansion of the interface during the ion transfer process [178]. Dale and Unwin [178] demonstrated that the polarised liquid | liquid micro-interface was neither flat nor static during voltammetric experiments, as imaged by confocal laser scanning microscopy. The interface underwent significant potential-induced movement. The increase in interface area may contribute to the observed current response not achieving a limiting plateau, but instead continuing to increase in magnitude. Additionally, the lack of a true limiting current plateau may be due to artefacts introduced by the background subtraction procedure employed. It is important to note that the sloping current in the diffusion-limited region did not occur throughout the voltammogram; instead, it is only observable when the ions start to transfer, as reported previously [109]. In addition, the nanoITIES based on design (a) (as in Figure 3.3.3 (a) and (c)) which is a single nanoITIES, exhibited an analyte ion transfer wave that was indistinguishable from the rising current response, in particular, at lower analyte concentration.

As no true limiting current plateau is achieved in the diffusion-limited region, the experimental current was determined at a potential ca. 200 mV positive of the foot of ion transfer wave [109]. The foot of the ion transfer wave was at ca. 0.50 V and 0.45 V. It was observed that the ion transfer wave foot progressively shifted to a more negative applied potential with increasing analyte concentration.

In the case of the nanoITIES array (Figure 3.3.3 (b) and (d)), the observed forward scan behaviour suggests that there is no diffusion zone overlap as this would introduce linear diffusion to the interfaces, and result in peak-shaped voltammograms. Since the voltammetric behaviour is neither steady-
state nor peak, it is best approximated as ‘sloping steady-state’ current behaviour, with establishment of radial (or spherical) diffusion to the interfaces. A recent simulation study demonstrated when radial diffusion to electrodes at the edges of the array is dominant, steady-state characteristics are achieved at nanoelectrode (in this study equivalent to the nanointerface) arrays, despite the presence of overlapping diffusion zones at nanoelectrodes within the array [135]. The simulation results were supported with experimental data, and together are in good agreement with the results presented in this study, as well as other studies [135, 175]. The underlying principle of the observed behaviour at nanoelectrode arrays of a few micrometers in size, may be explained that such an array of nano-electrodes can be treated as a single microelectrode of the same interfacial surface area, with the equivalent properties, and associated spherical diffusion zone [67, 109, 135].

Voltammograms of the nanopore membrane-modified ITIES in the reverse scan (TPrA$^+$ ion transfer from the organic to the aqueous phase) showed the absence of peak behaviour. This observation is related to the aspect ratio of the pores, and the ratio of the diffusion coefficients of the transferring ion in the organic and aqueous phases. The pore aspect ratio is defined as the ratio of the pore radius to the pore depth $= r_a/l$, with pore depth controlled by membrane thickness [179]. In the case of the nanoITIES array employed in this study, the pore aspect ratio was approximately 1 (if $r_a$ is taken as 50 nm, and $l$ is always 50 nm). In the case of a microITIES array, employing the geometric properties of micropore array detailed in Section 2.1.1 of Chapter 2 ($r_a = 11.09 \mu m$, $l = 100 \mu m$), the pore aspect ratio was approximately 0.11. The reverse peak current is enhanced by the use of relatively deep pores (i.e. a low pore aspect ratio), as in the case of the microITIES array (as will be apparent in the discussion of Section 6.3.1 of Chapter 6), as compared to the nanoITIES array (i.e. a high pore aspect ratio) employed here. In addition, the organic phase at the nanoITIES array is a liquid in which close to identical diffusion coefficients of a target analyte in both the aqueous and organic phases can be assumed. Hence, in this study, no reverse peak-shaped behaviour at the nanoITIES was observed, due to easier escape of
the ions from the interface region (aspect ratio impact) and more rapid
diffusion, relative to a microITIES array (noting that the latter use a viscous
organic solution) [67, 179].

3.3.2.2 Influence of the concentration on the limiting current

The calibration curves correlating the measured current with the
concentration of aqueous phase TPrA\(^+\) are plotted in Figure 3.3.4 (a) and (b),
formed by single and array \((10 \times 10)\) nanoITIES, respectively. Theoretically,
for the transfer of ions from the aqueous phase to the organic phase, the
liquid|liquid interface can be considered as inlaid or a recessed disk
electrode. This geometry depends on whether the nanopores are filled with
the organic or aqueous phase. An inlaid nanoITIES is observed when the
pore is fully (100 %) filled with the organic phase, such that the interface is
positioned at the pore orifice and the interface formed is planar. A recessed
nanoITIES is observed when the interface level is lower than the pore orifice.

The current at a single interface can be calculated according to one of the
following equations; the inlaid disc current model (given by the Saito
expression, Equation 3.3.2) or the recessed disc current model (Equation
3.3.3) [131, 132]:

\[
I = 4|z|FDCr_a \quad \text{(Eq. 3.3.2)}
\]

\[
I = 4\pi|z|FDCr_a/(4l + \pi r_a) \quad \text{(Eq. 3.3.3)}
\]

where \(I\) is the current, \(F\) is the Faraday constant, and \(z\), \(D\) and \(C\) are the
charge, the diffusion coefficient and the bulk concentration of transferring
ions, respectively. \(r_a\) and \(l\) have their usual meaning. To obtain the total
current for the array, the current calculated for one pore must be multiplied by
the number of pores, \(N_p\). Equation 3.3.2 varies from Equation 3.3.3 by the
factor of \((4l/\pi r_a) + 1\). When \(l\) is zero, Equation 3.3.3 becomes analogous to
Equation 3.3.2 [109].

In the present study, the interface formed was assumed to be inlaid (organic
phase solution filled 100 % of the pores) [109], thus the theoretical current
was estimated employing Equation 3.3.2. As expected, a linear relationship exists between the experimental current and TPrA\(^+\) concentration for both sets of data in Figure 3.3.4. The single nanoITIES (Figure 3.3.4 (a)) showed a good agreement of the experimental current and the calculated current based on the inlaid disc model. This observation supports the hypothesis that the single liquid | liquid interface created was co-planar with the aqueous side of the silicon nitride membrane and consistent with organic phase filling the pores as SiN membrane surface and pore walls are hydrophobic. This is an important result as it shows that an inlaid interface is formed and rules out the possibility that lower current for the arrays may be due to recessed interfaces.

The 10 × 10 nanoITIES array (Figure 3.3.4 (b)) resulted in measured currents that were approximately 50 % of the theoretical currents calculated using the inlaid disc model modified to include the number of nanointerfaces. This is again in agreement with previous studies \[109, 135\]. Those studies demonstrated that the overlap of diffusion zones at adjacent interfaces within the array was attributed to non-independent diffusion to each individual interface. The extent of overlap between neighbouring diffusion zones at the nanopores decreased as the pores became more separated (large ratio of \(r_c/r_a\)), hence yielding a higher current due to the dominance of radial diffusion over linear diffusion \[67, 109\].

This diffusion zone overlap results in the discrepancy between experimental and calculated currents (Figure 3.3.4 (b)). Even though the reduced current values reported here could also be associated with recessed rather than inlaid interfaces, previous studies have demonstrated that the interfaces are indeed inlaid and thus implicating diffusion zone overlap as the primary reason for the lower currents \[67, 109\]. The fact that a single nanoITIES produces a current in agreement with inlaid theory also support the assumption that the arrayed interfaces suffer from diffusion zone overlap.
Figure 3.3.4: The calibration curve plots of the experimental currents (forward scans) against TPrA$^+$ concentrations, represented by squares. The dashed lines represent the theoretical inlaid disc current model based on Equation 3.3.2 while the solid lines are the best linear fit to the experimental data. (a) and (b) represent single ($r_a$ of 62 ± 3 nm, design (a)) and array (10 × 10) ($r_a$ of 47 ± 6 nm, design (e)) nanopore membranes, respectively. Correlation coefficient refer to the linear fit of the experimental data.

3.3.2.3 Influence of nanopore array geometry

The influence of nanopore array geometries on the electrochemical behaviour of the single and array nanoFTIES was investigated in terms of the number of pores, the average pore-to-pore separation and the average pore radius. The study on the effect of increasing pore numbers was conducted employing $N_p$ of 1, 100 and 400 pores (designs (a), (e) and (f) in Table 3.3.1). However, in this case, the pore radius and the $r_c/r_a$ ratio were not
constant as a result of the milling process. The pore radii varied in the range of 39 to 62 nm, with a slight variation in the ratio of $r_c/r_a$ from 20 to 22. The CV responses (background-subtracted forward scan only) for the three nanopore array designs presenting the transfer of 0.1 mM TPrA$^+$ across the nanoITIES array are presented in Figure 3.3.5. For all array designs investigated, the shape of the voltammograms is as discussed in the preceding section. As expected, the current increased as $N_p$ increased, although not in a linear fashion [67]. As the number of pores in the array increased, the average current per nanopore decreased [67]. This is a characteristic of overlapped diffusion zones in an array. Also, it was seen that when $N_p$ increased, $E_{1/2}$ value shifted to lower potentials, due to the lower resistance of the array.

**Figure 3.3.5:** Background-subtracted CV (forward sweep only) of 100 µM TPrA$^+$ ion transfer at single and array nanoITIES created using nanopore design (a: $r_a$ of 62 ± 3 nm), (e: $r_a$ of 47 ± 6 nm) and (f: $r_a$ of 39 ± 6 nm) with increasing number of pores in the array, $N_p$ of 1, 100 and 400 pores, accordingly. Arrow indicates an increase in $N_p$. Scan rate used was 5 mV s$^{-1}$. The potential is reported with respect to the experimentally-used reference electrodes.

Miniaturisation of the liquid | liquid interfaces from a millimetre-scale ITIES to a micro-, then nano-scale ITIES is associated with significant reduction in the interfacial area, which in turn results in an overall reduced current, $I$ [22, 67,
Therefore, arrays of interfaces were introduced, to increase current signal. However, reducing the size of the ITIES enhances the diffusional flux, providing a greater current density, $j$.

Figure 3.3.6 summarises the effect of varying the $r_c/r_a$ ratio on the electrochemical ion transfer signal (here reported as current densities, $j_{exp}$), measured at $10 \times 10$ nanoITIES arrays with radii of ca. 30 nm. In this study, design (g) and design (h) were applied, with $r_c/r_a$ ratios of 32 and 17, respectively. The background-subtracted CV responses (not shown) for both designs were employed. The current density is the ratio of the current, $I$ (A) and the total geometric interfacial area, $A$ ($m^2$), which can be derived from Equation 3.3.2, in the case of radial diffusion field:

$$j = 4|z|FDC/\pi r_a$$

(Eq. 3.3.4)

The total current density for the array is obtained by multiplying the current calculated for one interface by $N_p$.

The nanoITIES membrane with the higher ratio of $r_c/r_a$ of 32 (design (g) Table 3.3.1) exhibited higher current densities compared to design (h) with $r_c/r_a$ of 17 (Figure 3.3.6). It can be seen that the array with the greater ratio $r_c/r_a$ produces a larger current density, which is consistent with the individual nanointerfaces exhibiting less diffusion zone overlap as they are further displaced from their neighbours. The experimental current density was approximately 5 % and 30 % lower than the calculated current densities for nanoITIES arrays based on design (g) and (h), respectively. The 5 % difference is well within experimental error. Thus, when designing nanoITIES arrays, it is crucial to ensure that the patterned pores are sufficiently separated so that the individual diffusion zones remain independent of each other, and yield a maximal current. In the case where the separation between these pores becomes smaller, as in the case of design (h), the individual diffusion zones will overlap and result in the individual pores depleting the same region of the solution (termed as exclusion zone). Consequently, the radial diffusion contribution will decline, while the linear diffusion contribution
will increase, resulting in measured current density that was approximately 30 % of the theoretical current density. If the diffusion zones are heavily overlapped, a purely linear diffusion will be observed, resulting in the appearance of a peak-shaped voltammogram [81, 106]. Godino and co-workers [135] have demonstrated, via simulations and experiments, that substantial diffusion zone overlap at neighbouring nanoelectrodes occurs, on the timescale of a potential sweep experiment, even for the ratio $r_c/r_a$ of 60, which is larger than the commonly used approximation ($r_c/r_a$ of 20) formulated by Fletcher and Horne [86, 137] for microelectrode arrays. This latter value is also the approximate ratio applied in the present investigation (designs (b), (c), (d), (e), (f) and (h) in Table 3.3.1).

Figure 3.3.6: Comparison of the current densities, $j_{\text{exp}} \, (\text{mA cm}^{-2})$ featuring the ratio of $r_c/r_a$ of 32 (design (g), solid line) or 17 (design (h), dashed line). TPrA$^+$ concentration range = 0.1 to 2.0 mM; 10 × 10 nanolTIES arrays of $r_a$ = 30 nm; scan rate = 5 mV s$^{-1}$. The error bars were standard deviations obtained from two current measurements. In this figure, the propagation of random errors due to the standard deviations of pore radii was not taken into account in the calculation of the standard deviations of the current density.

Table 3.3.2 presents the summary of the current, $I$ and current density, $j$ obtained with each of the six nanolTIES arrays studied (designs (a), (b), (e), (f), (g) and (h) in Table 3.3.1) for transfer of 100 µM TPrA$^+$. $I_{\text{calc}}$ and $I_{\text{calc, total}}$
are obtained using Equation 3.3.2, while $I_{exp}$ is experimentally retrieved from CV. $j_{calc}$ and $j_{exp}$ are obtained employing Equation 3.3.4. All the experimental data presented (with the exception of design (g)) show an agreement with calculated data employing the inlaid disc model (Equation 3.3.2). The individual experimental current and current densities were in the range of 23 to 61 % lower than the calculated values, which is supportive of the possibility of diffusion zone overlap. In the case of design (g), the experimental current was within 60 % of the calculated current (Equation 3.3.2), suggesting the possibility that the interfaces formed at the nanopore orifices were not flat. This in turn enhanced the radial diffusion to the interfaces, resulted in current increment for that particular concentration. However, the experimental data for the full range concentrations agreed with the theoretical inlaid disc current model with minimal diffusion zone overlapping, as discussed previously in the effect of varying the $r_c/r_a$ ratio. In general, it can be seen that as pore size changes, so do the current and the current density, in line with expectations.
Table 3.3.2: Summary of the current, $I$ and current density, $j$ obtained by CV of 100 µM TPrA$^+$ of nanoITIES formed at nanoporous membrane

<table>
<thead>
<tr>
<th>Design</th>
<th>$I_{\text{calc}}$ per pore (nA) $^i$ (± x %)</th>
<th>$I_{\text{calc, total}}$ (nA) $^ii$ (± x %)</th>
<th>$I_{\text{exp}}$ (nA) $^iii$ (± x %)</th>
<th>$j_{\text{calc}}$ (mA cm$^{-2}$) $^iv$ (± x %)</th>
<th>$j_{\text{exp}}$ (mA cm$^{-2}$) $^v$ (± x %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>$1.79 \times 10^{-3}$ (± 5)</td>
<td>$1.79 \times 10^{-3}$ (± 5)</td>
<td>$1.22 \times 10^{-3}$ (± 7)</td>
<td>$15$ (± 11)</td>
<td>$10$ (± 12)</td>
</tr>
<tr>
<td>b</td>
<td>$2.32 \times 10^{-3}$ (± 19)</td>
<td>$2.08 \times 10^{-2}$ (± 2)</td>
<td>$1.61 \times 10^{-2}$ (± 2)</td>
<td>$12$ (± 42)</td>
<td>$9$ (± 38)</td>
</tr>
<tr>
<td>e</td>
<td>$1.36 \times 10^{-3}$ (± 13)</td>
<td>$1.36 \times 10^{-1}$ (± 13)</td>
<td>$9.22 \times 10^{-2}$ (± 32)</td>
<td>$20$ (± 29)</td>
<td>$13$ (± 41)</td>
</tr>
<tr>
<td>f</td>
<td>$1.13 \times 10^{-3}$ (± 15)</td>
<td>$4.52 \times 10^{-1}$ (± 15)</td>
<td>$1.75 \times 10^{-1}$ (± 23)</td>
<td>$24$ (± 34)</td>
<td>$9$ (± 39)</td>
</tr>
<tr>
<td>g</td>
<td>$8.97 \times 10^{-4}$ (± 10)</td>
<td>$8.97 \times 10^{-2}$ (± 10)</td>
<td>$1.43 \times 10^{-1}$ (± 11)</td>
<td>$30$ (± 22)</td>
<td>$47$ (± 22)</td>
</tr>
<tr>
<td>h</td>
<td>$8.68 \times 10^{-4}$ (± 3)</td>
<td>$8.68 \times 10^{-2}$ (± 3)</td>
<td>$5.08 \times 10^{-2}$ (± 6)</td>
<td>$31$ (± 7)</td>
<td>$18$ (± 9)</td>
</tr>
</tbody>
</table>

$^i$ $I_{\text{calc}}$ is the calculated current (using Equation 3.3.2) for a given pore radius assuming an inlaid liquid | liquid interface

$^ii$ $I_{\text{calc, total}}$ is obtained by multiplying the current per pore and the number of pores in the array

$^iii$ $I_{\text{exp}}$ is the measured current from cyclic voltammetry experiment

$^iv$ $j_{\text{calc}}$ is the calculated current density (using Equation 3.3.4) employing a diffusion coefficient of the analyte of $7.5 \times 10^{-10}$ m$^2$ s$^{-1}$

$^v$ $j_{\text{exp}}$ is the experimental current density

$^x$: % relative standard deviation (% r.s.d.). All the standard deviations of $I$ and $j$ were calculated using the method of propagation of random errors [176]

3.3.2.4 Influence of the TAA cation types on the transfer process

Figure 3.3.7 shows voltammograms for the transfer of three different tetraalkylammonium cations (TEA$^+$, TPrA$^+$ and TBA$^+$) (concentration of 500 µM) across the nanoITIES array utilising a $10 \times 10$ nanopore array membrane (design (e)). As expected, the cations transferred in the order of their affinity for the organic phase. The more hydrophobic cations, TBA$^+$, transfer at the lowest applied potential ($E_{1/2}$ of 0.40 V), while the more
hydrophilic cations, TEA\(^+\), transfer at the highest applied potential \(E_{1/2}\) of 0.68V, following the sequence TBA\(^+\) < TPrA\(^+\) < TEA\(^+\). The values of the diffusion coefficients, \(D\), for TEA\(^+\), TPrA\(^+\) and TBA\(^+\) from literature are 8.4 x 10\(^{-6}\) cm\(^2\) s\(^{-1}\), 7.5 x 10\(^{-6}\) cm\(^2\) s\(^{-1}\) and 6.0 x 10\(^{-6}\) cm\(^2\) s\(^{-1}\), respectively [180].

The differences observed between the respective TAA\(^+\) limiting currents (as shown in Figure 3.3.7) are due to their diffusion coefficients. The smallest ion in this study, TEA\(^+\), diffused faster than the other two ions since the diffusion rate is higher, and so the corresponding limiting current is higher. In contrast, TBA\(^+\), the biggest ion, diffused slower than the others, and demonstrated a lower limiting current. As the interface formed was assumed to be inlaid, the theoretical current can be estimated employing Equation 3.3.2. The experimental currents were 35 – 50 % lower than those of this inlaid disc model, due to diffusion zone overlap at adjacent interfaces. In addition, the CVs showed that currents in the limiting-current region rose with the applied potential up to the switching potential, irrespective of the analyte.

![Figure 3.3.7](image)

*Figure 3.3.7: Background subtracted CV (forward scan only) recorded for 500 \(\mu\)M of TEA\(^+\), TPrA\(^+\) and TBA\(^+\) at water / 1,6-dichlorohexane interface obtained with nanopore array design (e) \((r_a\) of 47 ± 6 nm\). Dotted line represents the blank voltammogram. The potential is reported with respect to the experimentally-used reference electrodes*
3.3.2.5 Estimation of charging time

Table 3.3.3 presents the geometric and electrical properties of the single and array nanopore membranes studied in this work, in particular the analysis of the resistance, capacitance and charging time. In the ITIES electrochemical cell, the uncompensated resistance is contributed by the ‘bulk solution’ resistance, $R_b$, and the pore resistance, $R_p$. $R_b$ is termed the resistance due to the distance between the tip of the counter/reference electrode and the orifice of the pore, while $R_p$ is termed the resistance within the pore. The low conductivity of the organic electrolyte solution is the main contributor of the uncompensated resistance. Katano and Senda [181] demonstrated a conductivity, $\kappa$, of 49 $\mu$S cm$^{-1}$ for DCH with tetraketammonium tetrakis(4-chlorophenyl)borate as an electrolyte, where the conductivity value was independent of the electrolyte types. The pore resistance, $R_p$, is given by the expression [84, 182]:

$$R_p = l/\pi r_a^2 \kappa$$  \hspace{1cm} (Eq. 3.3.5)

An increase of the pore length and a reduction of the pore radius results in an increase of the pore resistance. Since the pores of the array behave like resistors in parallel, the total pore array inverse resistance is obtained by multiplying the inverse resistance of a single pore by $N_p$ [67, 84, 101]. The individual pore resistance for the single and array nanopores are in the range of $10^8$ to $10^9$ $\Omega$, with the smaller nanopore radius membrane exhibiting higher resistance (in this case, demonstrated by design (h) membrane of $3.61 \times 10^9$ $\Omega$), and will be similarly observed for the three nanopore membrane designs (with pore radii of 75, 50 and 17 nm) employed in Section 4.3.2 of Chapter 4. However, the pore array resistance in this study decreased with an increase in the number of pores, in agreement with a previous report [84]. The pore array resistance decreased by 1, 2 and 3 orders of magnitude with an increase in the number of pores from 1 to 9, 100 and 400, respectively.

On the other hand, the bulk resistance, $R_b$, can be calculated as follows [183]:

$$R_b = 1/4\pi r_a \kappa$$  \hspace{1cm} (Eq. 3.3.6)
The bulk resistance established between each individual pore in an array and the tip of the counter/reference electrode behaves like resistors in parallel. However, the same pore will have this bulk resistance in series with the pore resistance. The total bulk array resistance, $R_{a,b}$, decreased with an increase in $N_p$, a similar trend to that observed with the pore array resistance. In addition, the total or uncompensated resistance decreased by up to 3 orders of magnitude with incorporation of array (from single to $20 \times 20$ array nanopore) in the design.

In this study, the experimental capacitances, $C_{exp}$ of the single and array nanopore membrane systems were also tabulated in Table 3.3.3. The capacitance was determined by the CV responses of a blank electrolyte solution system (i.e. in the absence of TPrA$^+$), as given by the following expression:

$$C_{exp} = \frac{I_c}{2v}$$

(Eq. 3.3.7)

where $I_c$ is the total charging current [184]. Both the single and array nanoITIES exhibited experimental capacitance values in the nF regime, a similar trend to that observed with the nanoITIES array in Chapter 4 (Section 4.3.2).

To investigate the influence of the nanopores on the overall capacitance, the CV responses obtained at a blank membrane (i.e. membrane without pores) and at single and array nanoITIES are compared (as shown in Figure 3.3.8). It was observed that the blank CVs in both conditions are virtually similar (charging current in the pA regime), demonstrating that the overall capacitance has only marginal contribution from the nanopores, in agreement with previous observation [67].
Figure 3.3.8: Comparison of the CV responses for a blank system in the absence of TPrA$^+$ at 50 nm thickness Si$_3$N$_4$ membranes which contain no pores (grey), single nanopore with pore radius of 62 ± 3 nm (medium grey) and array nanopores of 10 × 10 arrays with pore radii of 47 ± 6 nm (black). Scan rate employed was 5 mV s$^{-1}$. The potential is reported with respect to the experimentally-used reference electrodes.

Combining the experimental capacitance and the uncompensated (or total) resistance, the cell time constant was obtained, and is employed to calculate the charging time (Table 3.3.3). The charging time was taken as 5$R_uC_{exp}$, as will be described later in Section 4.3.2 (Chapter 4). The cell time constant and also the charging time, decreased with increasing $N_p$, analogous to the pore and bulk array resistance trends. The resistance has more influence on the charging time than the capacitance, being the broader of its range, as will be similarly observed in Section 4.3.2. An increase in $N_p$ from 1 to 400, results in a decrease of the cell time constant (from 4.6 to 0.02 s), and correspondingly the charging time (from 23 to 0.1 s). The introduction of the concept of array of nanopores so as to increase the number of pores in the membrane was successful to reduce the charging time.

The detail discussion of the resistance, capacitance and charging time will be given in the later section (Section 4.3.2 of Chapter 4).
Table 3.3.3: Geometric and electrical behaviour of the single and array nanopore membranes studied in this work. Pore radius, \( r_a \) values were taken from SEM data listed in Table 3.3.1 and membrane thickness, \( l \) is 50 nm. The standard deviations of \( A \) was calculated using the method of propagation of random errors [176]

<table>
<thead>
<tr>
<th>Design</th>
<th>Single</th>
<th>Array</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Pore radius, ( r_a ) (nm)</td>
<td>62 ± 3</td>
<td>80 ± 15</td>
</tr>
<tr>
<td>No. of pore, ( N_p )</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Total cross-sectional area, ( A ) (m²)</td>
<td>(1.21\times10^{-14})</td>
<td>(1.81\times10^{-13})</td>
</tr>
<tr>
<td>Pore resistance, ( R_p ) (Ω) (^a)</td>
<td>(8.45\times10^8)</td>
<td>(5.08\times10^8)</td>
</tr>
<tr>
<td>Pore array resistance, ( R_{a,p} ) (Ω)</td>
<td>(8.45\times10^8)</td>
<td>(5.64\times10^7)</td>
</tr>
<tr>
<td>Bulk resistance, ( R_b ) (Ω) (^b)</td>
<td>(2.62\times10^8)</td>
<td>(2.03\times10^8)</td>
</tr>
<tr>
<td>Bulk array resistance, ( R_{a,b} ) (Ω)</td>
<td>(2.62\times10^8)</td>
<td>(2.26\times10^7)</td>
</tr>
<tr>
<td>Uncompensated resistance, ( R_u ) (Ω)</td>
<td>(1.11\times10^9)</td>
<td>(7.89\times10^7)</td>
</tr>
<tr>
<td>Experimental capacitance, ( C_{exp} ) (F) (^c)</td>
<td>(4.12\times10^{-9})</td>
<td>(1.94\times10^{-9})</td>
</tr>
<tr>
<td>Cell time constant, ( R_u C_{exp} ) (s)</td>
<td>4.560</td>
<td>0.153</td>
</tr>
<tr>
<td>Charging time, ( 5R_u C_{exp} ) (s)</td>
<td>22.802</td>
<td>0.767</td>
</tr>
</tbody>
</table>

\(^a\) Pore resistance calculated using Equation 3.3.5
\(^b\) Bulk resistance calculated using Equation 3.3.6
\(^c\) Estimated from CV data
3.4 Conclusion

For the first time, nanopores prepared by FIB milling were used to nanostructure the liquid | liquid interface, thereby forming the nanoITIES. Single and array nanoporous SiN membranes were prepared with radii in the range of 30 to 80 nm, and with pore-to-pore separation ratios of 16 to 32 in SiN films of thickness 50 nm. These membranes were electrochemically characterised via the formation of nanoscale-liquid | liquid interfaces and the CV of TPrA$^+$ ion transfer across the interfaces between water and 1,6-DCH. While steady-state voltammograms were achieved, the limiting current region demonstrated an increase of current with applied potential up to the switching potential at all the nanoITIES studied. A single nanoITIES gave excellent agreement between the experimental current and the theoretical current for an inlaid disc interfaces, showing that such FIB-milled nanopores enable the formation of inlaid nanointerfaces that are co-planar with the aqueous side of the membrane. At nanoITIES arrays, experimental currents lower than calculated currents were thus attributed to diffusion zone overlap. NanoITIES array with a large $r_c/r_a$ ratio gave higher current values, all other variables being constant, which is consistent with diffusion zone overlap causing lower currents. Nevertheless, experimental currents depended, as expected, on the nanopore size, the pore-to-pore separation and the numbers of pores in the array used to nanostructure the interface. Besides, the nanoITIES membrane system exhibited prolonged charging time, due to the dominance of uncompensated resistance, hence the measurement of the Faradaic current is predicted only at long times (timescale of seconds). The results show that FIB-milled nanopores can be used for prototyping of the nanoITIES arrays and opens up the development of new chemical and biochemical sensing systems.
Chapter 4

Chronoamperometric Response at Nanoscale Liquid | Liquid Interface Arrays

4.1 Introduction

In this chapter, the characterisation of the behaviour of nanoITIES arrays, formed at geometrically regular silicon nitride nanoporous membranes, by PSCA is presented. The aim of the work was to evaluate the response times of these membrane-supported ITIES so as to determine whether the radial diffusion advantage of nanoITIES was coupled with a fast response. The electrochemical transfer of the tetrapropylammonium cation (TPrA\(^+\)) across the water \(\mid\) 1,6-dichlorohexane (DCH) interface was chosen as a model system. PSCA has not been applied previously to these nanoITIES arrays and can possibly reveal information about mass transport effects as well as the response time of the array, both of which may be useful in designing new arrays and sensors or detection systems based on them.

As discussed in Section 1.3.3, to date, nano-interface between two immiscible electrolyte solutions (nanoITIES) have been prepared in two ways: (a) those supported at the tip of a single [89, 90, 118] or dual [90, 185] nanopipette (producing single or double nanoITIES), and (b) those produced by placing nanoporous materials containing geometrically irregular or regular pore arrays at the ITIES [67], such as track-etched polyester [101, 105], \(\gamma\)-alumina [106] and silicon nitride [67, 109] membranes. NanoITIES can offer
benefits comparable to those experienced at nanoelectrode arrays, such as enhanced mass transport (due to radial diffusion), decreased charging current and decreased impact of solution resistance [67, 85]. NanoITIES studies reported by Rimboud et al. [109], Liu et al. [22] and Shao and Mirkin [89] focussed on ion sensing using cyclic voltammetry as a technique. However, there is little information published on the chronoamperometric response at nano-interface arrays despite numerous studies reporting the chronoamperometric response at microelectrochemical devices, e.g. micropipette-based ITIES [39, 96, 186, 187], microdisc electrodes [132, 188-190], micropore-based ITIES [101] and microarray electrodes [191].

Potential step chronoamperometry (PSCA) involves stepping the potential applied across the interface from a region where no Faradaic process occurs to a potential where a Faradaic process occurs and is mass-transport controlled. The resulting current is monitored as a function of time. This method generates high charging currents at short timescales, which decay exponentially with time. PSCA is widely used in the determination of diffusion coefficients and characterisation of electrodes and electrode reactions [17]. It also enables the determination of response time, a crucial parameter for chemical sensor applications. The response time of a chemical sensor can be defined as the time for the sensor signal to reach 95% of its final value $t_{95\%}$ [192]. For PSCA at a nanoITIES, we can define the response time as the time needed to reach a current that is 95% of the steady-state current.

At electrified interfaces, two types of processes occur, Faradaic and non-Faradaic, and both contribute to the overall current, with Faradaic processes being of primary interest for sensing applications. At the ITIES, the Faradaic process is associated with the transfer of a charged species (ions, electrons or both) between the two liquid phases. Usually, in an amperometric or voltammetric sensing system, the non-Faradaic current associated with the charging of the interfacial capacitance is minimized. In designing or analysing an electrochemical experiment, consideration must be taken of the charging of the double layer at the electrochemical interface and its combination with
the uncompensated resistance of the cell [193]. The Faradaic response for fast electrochemical or chemical reactions is restricted by this charging process [194].

The $RC$ time constant or cell time constant characterizes the timescale for the charging process and is generally modelled on the basis of a resistor and a capacitor in series [1, 193]. This constant (which then leads to calculation of the charging time) has been reported by various researchers [98, 186, 191, 195]. Nirmaier and Henze reported that the cell time constant for establishing the electrode potential at the electrode surface varies linearly with the electrode surface radius for disk-shaped electrodes. Therefore, when a potential step is applied at a microelectrode, the charging process is much faster than for a millimetre-sized electrode [191].

However, this was found not to be the case when dealing with some liquid | liquid microinterface. PSCA experiments by Yuan et al. [186], Beattie et al. [195] and Shao and Mirkin [98] reported that the lower time scale (or charging time) for carrying out potential step experiments at a microITIES is crucially restricted by the large resistance at the narrow microhole of polymer membranes or glass micropipettes used to form such microITIES. Comparison of the charging time of a water | 1,2-dichloroethane (DCE) microinterface with a metal | water microinterface of the same size showed that the charging time of the former was nearly three orders of magnitude greater than that of the latter [186]. For example, a cell time constant $R_uC_{dl}$ value of 80 $\mu$s [186] was reported for a 10 $\mu$m diameter water | DCE interface with an uncompensated resistance, $R_u$ of 10 M$\Omega$ [98, 195] and double-layer capacitance, $C_{dl}$ of 8 pF [196]. Taking $\sim 5R_uC_{dl}$ [1, 186] as the fully established potential step, resulted in a charging time of 400 $\mu$s [186]. The corresponding charging time for a metal | water interface of the same dimensions was 0.85 $\mu$s [1].
Numerous analytical expressions have been developed for analysing PSCA transients. For planar diffusion, the current-time transient is described by the Cottrell equation:

\[
I = \frac{nFAC\sqrt{D}}{\sqrt{\pi t}} \quad \text{(Eq. 4.1.1)}
\]

where \(I\) is the diffusion-limited current, \(n\) is the stoichiometric number of electrons involved in an electrode reaction, \(F\) is Faraday’s constant, \(A\) is the electrode surface area, and \(C\), \(D\) and \(t\) are, respectively, the bulk concentration of the electroactive species, the diffusion coefficient and the time [170].

At a microelectrode where convergent diffusion dominates, current-time expressions have been developed by Shoup and Szabo [172] and by Mahon and Oldham [173, 174]. The latter are the most exact closed-form expressions to describe the current-time transient, with a maximum error of 0.02 %. For short times, the expression is

\[
\frac{I(t)}{nFrdC} = \pi \left( (\pi \tau)^{-1/2} + \frac{1}{2} \left( \frac{\tau}{\pi} \right)^{1/2} - 0.12003\tau + 0.013273\tau^{3/2} \right) \quad \text{(Eq. 4.1.2)}
\]

and for times greater than \(1.281r^2/D\) the expression is

\[
\frac{I(t)}{nFrdC} = \pi \left( \frac{4}{\pi} + 8\pi^{-5/2}\tau^{-1/2} + 8.9542 \times 10^{-3}\tau^{-3/2} - 2.5664 \times 10^{-4}\tau^{-5/2} - 2.2312 \times 10^{-4}\tau^{-7/2} + 2.7628 \times 10^{-5}\tau^{-9/2} \right) \quad \text{(Eq. 4.1.3)}
\]

where \(r\) and \(\tau\) are, respectively, the electrode radius and the dimensionless time parameter.

\[
\tau = \frac{Dt}{r^2} \quad \text{(Eq. 4.1.4)}
\]

Equations 4.1.1, 4.1.2 and 4.1.3 can be readily applied to the ITIES, with \(n\) replaced by the charge number of the transferring ion, \(z\).
Dr. Jorg Strutwolf (University of Tübingen, Tübingen, Germany) provided assistance relating to the interpretation of results in Section 4.3.2 and 4.3.3.

4.2 Experimental methods and materials

4.2.1 Reagents
The chemical reagents used in this chapter are as explained in Section 2.4.1 of Chapter 2. The model analyte studied was the chloride salt of tetrapropylammonium (TPrACl) in 0.01 M LiCl in DCH-saturated water.

4.2.2 Preparation of nano-interface arrays
Arrays of nano-ITIES were formed at a water | DCH interface using silicon nitride (Si₃N₄) nanopore array membranes (100 nm thick), which were fabricated at Tyndall National Institute, University College Cork, Cork, Ireland, as explained in Section 2.1.1 of Chapter 2. Three sizes of nanopore radius were used in this study (rₐ = 75, 50 or 17 nm). Scanning electron microscopy (SEM) micrographs of these nanopore arrays have been reported previously [67, 109, 197].

4.2.3 Experimental procedure
In this chapter, the electrochemical methods PSCA and CV were applied with procedures as outlined in Section 2.4.5 of Chapter 2. A two-electrode electrochemical cell with two Ag | AgCl electrodes was employed as shown in Figure 2.4.4 of Chapter 2. The voltammetric sweep rate was 10 mV s⁻¹ (75 and 50 nm radii pores) or 5 mV s⁻¹ (17 nm radius pores). No automatic positive feedback compensation was applied during PSCA and CV. For PSCA, the steady-state current Iₛₛ was taken as the average of the final ten current points of the 10 s transient, taken at 10 ms intervals, corresponding to the final 100 ms of the measurement.

The electrochemical cell employed in this study can be summarized as follows:
Ag | AgCl | x mM TPrACl + 0.01 M LiCl<sub>w</sub> | 0.01 M BTPPATPBCl<sub>DCH</sub> | 0.01 M BTPPACl in 0.01 M LiCl<sub>w</sub> | AgCl | Ag

where x is the concentration of TPrACl in the aqueous phase.

4.3 Results and discussion

4.3.1 Cyclic voltammetry of TPrA<sup>+</sup> transfer at the nano-ITIES array
Initially, ion transfer across the nano-ITIES array was characterised using CV so that initial and step potentials could be selected for the PSCA study. TPrA<sup>+</sup> was chosen as the model analyte ion and aqueous phase concentrations between 20 and 100 µM in increments of 20 µM in a background of 0.01 M LiCl were employed. Prior to the addition of analyte, a CV of the background electrolyte solutions was recorded, so that background-subtracted voltammograms could be obtained. Figure 4.3.1 (a) shows a typical set of background-subtracted voltammograms (forward scans only) obtained at a nano-ITIES array with interface radius of 50 nm. This figure corresponds to the transfer of TPrA<sup>+</sup> from aqueous to organic phase. These CVs show that the current rose steadily with applied potential up to the switching potential and that no true steady-state current plateau was reached in the diffusion-limited region, in agreement with previous reports [109] for the transfer of tetraethylammonium cation (TEA<sup>+</sup>) across the water | liquid DCH interface. These results are also in agreement with the voltammograms of TPrA<sup>+</sup> transfer across the water | liquid DCH nano-ITIES array obtained in Figure 3.3.3, and the possible contributing factors have been discussed in Section 3.3.1 of Chapter 3. In this chapter, the nanopore array membranes employed were different from those employed in Chapter 3 in terms of nanopore fabrication technique and geometric characteristics (number of pores, pore radius, pore centre-to-centre separation and pore arrangement) despite the fact the voltammograms shown in Figure 4.3.1 are similar to that of Figure 3.3.3 (d). From Figure 4.3.1 (a), it can be seen that the current in the diffusion-limited region (above 0.6 V) increases with the aqueous phase concentration of TPrA<sup>+</sup>, with a linear relationship as shown by the inset graph.
of current *versus* concentration of transferring ion. In this case, the experimental currents were determined at a potential *ca.* 200 mV positive of the foot of ion transfer wave [109]. Concurrently, CV was used in the determination of the step potentials for chronoamperometric experiments in Section 4.3.3 in which the potential was stepped from 0.2 V to 0.6 V.
Figure 4.3.1: (a) Background-subtracted voltammogram (forward scan) of 20 to 100 μM TPrACl at a water | DCH nanoITIES array. The membrane employed comprised 400 pores of 50 nm pore radius. The foot of the ion transfer wave was at ca. 0.4 V and the experimental limiting current was determined at approximately 0.6 V (200 mV positive of the wave foot) for all concentrations studied. Inset: A calibration graph correlating limiting current and analyte concentration and a diagram (not to scale) showing the cross section of the nanopore membrane filled with the organic phase and in contact with the aqueous phase. (b) Blank CV at the 50 nm pore radius nanoITIES array. The charging current was determined at 0.2 V, and used with Equation 3.3.7 to determine the experimental capacitance at 10 mV s⁻¹.
sweep rate. The potentials are reported with respect to the experimentally-used reference electrodes

### 4.3.2 Estimation of charging time

Determination of the cell time constant, $R_u C_{dl}$ allows in turn the determination of the charging time. Execution of a PSCA measurement at times shorter than this charging time will not produce useful data $[1]$. In fact, the minimum time for full establishment of a potential step requires $\sim 5R_u C_{dl}$ $[1]$ as applied by Yuan $et$ $al.$ $[186]$ in their micropipette-based ITIES study. Normally, the step must last at least $10R_u C_{dl}$ which includes time for recording data beyond the time required for establishment of the potential step $[1]$. The size of the electrode governs the cell time constant and consequently the charging time. In this study, the charging time was taken as $5R_u C_{dl}$ $[1]$. In order to estimate the cell time constant and the charging time, values of the uncompensated resistance and the double layer capacitance are needed.

The main source of uncompensated resistance in ITIES electrochemical cells is the low conductivity of the organic electrolyte solution. Katano and Senda $[181]$ reported a conductivity of $\kappa = 49$ $\mu$S cm$^{-1}$ for DCH with tetraoctylammonium tetrakis(4-chlorophenyl)borate as an electrolyte. It was shown $[23, 84, 198]$ that the conductivity was similar regardless of the electrolyte types, based on a study with a series of electrolytes in DCE. Therefore, it is reasonable to assume a value of 49 $\mu$S cm$^{-1}$ for the conductivity of the organic phase employed here. For comparison, the conductivity of aqueous 0.01 M LiCl, used as the aqueous electrolyte, is 1073 $\mu$S cm$^{-1}$ $[199]$. In the organic phase, the resistance will be due to the distance between the tip of the counter/reference electrode and the orifice of the pore (bulk solution resistance, $R_b$) and to the resistance within the pore (pore resistance, $R_p$). Both, of $R_b$ and $R_p$ will contribute to the uncompensated resistance. The pore resistance, $R_p$, is given by Equation 3.3.5.
Each pore is assumed to be filled by the organic electrolyte solution, resulting in inlaid nanoITIES arrays [67, 109]. The pore resistance increases with an increase in the pore length and reduction in pore radius. The interface cross-sectional region in direct electrical contact with the aqueous or organic phase is defined by the diameter of the nanopore. If the individual pores in the array behave like resistors in parallel, the total inverse resistance value is obtained by multiplying the inverse resistance of a single pore by the number of pores in the array [84, 101]. The individual pore resistances for the 75, 50 and 17 nm pore radii are $1.15 \times 10^9 \, \Omega$, $2.60 \times 10^9 \, \Omega$ and $22.48 \times 10^9 \, \Omega$, respectively, calculated using Equation 3.3.5. The smaller nanopore radius membrane demonstrated higher resistance, similarly observed for the nanopore membranes employed in Section 3.3.2.5 of Chapter 3. The total pore resistance across the three membranes studied are in the range 2.89 to 56.19 MΩ, again the smaller nanopore radius membrane exhibiting larger resistance. These results agree with the resistance estimation by Strutwolf et al. [84] of $103 \times 10^5 \, \Omega$ per pore for 25 μm radius pore and $650 \times 10^5 \, \Omega$ per pore for a 10 μm radius pore. The resistances were higher at nanoscale pores. Additionally, the total pore resistance of the membranes with micron-sized pores ($r_a = 25 \, \mu m$) increased from $9.8 \times 10^4 \, \Omega$ to $3.4 \times 10^6 \, \Omega$ with a decreased number of pores (from 105 to 3 pores) [84].

The dimension of the counter/reference electrode is much larger than the critical dimension of the pore ($r_a$), and the distance between the pore and the tip of the reference/electrode is in the millimetre range and therefore much bigger than $r_a$. Under these conditions, the bulk resistance can be calculated from Equation 3.3.6 [183].

For the 17, 50 and 75 nm radius pores, $R_b$ values of $0.95 \times 10^9 \, \Omega$, $0.32 \times 10^9 \, \Omega$, and $0.22 \times 10^9 \, \Omega$ are calculated for the individual pores in the arrays. The individual bulk resistance demonstrated higher resistance with decreasing pore radius, in agreement with previous observation (Section 3.3.2.5 of Chapter 3). Again, the resistance established between each individual pore
of an array and the tip of the reference/counter electrode acts like a resistor in parallel with the other bulk solution resistors, but in series with the pore resistance of the same pore (given by Equation 3.3.5). So the total bulk resistances for the three arrays are 0.55, 0.81 and 2.37 MΩ with decreasing pore radius. The total or uncompensated resistance is 3.42, 7.30 and 58.57 MΩ for the 75, 50 and 17 nm arrays, respectively (Table 4.3.1). Since the pore resistances scales with \( r_a^{-2} \), while bulk resistance scales with \( r_a^{-1} \), the influence of the pore resistance on the uncompensated resistance is more pronounced the smaller the pore radius. For the 75 nm pore array, the bulk resistance contributes 19 % to the total resistance, while for the 17 nm array the contribution is 4 %.

In this study, the experimental capacitance \( C_{\text{exp}} \) was determined by CV of the electrolyte system in the absence of ion transfer, as obtained by Equation 3.3.7. The experimental capacitance is used here because, as will be apparent below, the double layer capacitance of the ITIES is but one contribution to the overall capacitance measured. The charging current was determined from CVs of the electrolyte solutions at a potential where no ion transfer occurs (Figure 4.3.1 (b)). All membranes studied exhibited experimental capacitance values in the nanoFarad regime, ranging from 1.57 to 4.83 nF (Table 4.3.1), similar regime to that observed with the single and array nanoITIES in Section 3.3.2.5 of Chapter 3.

Assuming for the moment that the experimental capacitance is solely due to the capacitance of the nanoITIES, the specific capacitance or capacitance per unit area \( C_{\text{exp}}^0 \) can be determined. The experimental capacitance was divided by the total pore cross-sectional area, resulting in the experimental specific capacitances \( C_{\text{exp}}^0 \) with the average value ranging between 630 and 4320 F \( \text{m}^{-2} \) \( (0.63 \times 10^5 \text{ and } 4.32 \times 10^5 \text{ μF cm}^{-2}) \) (Table 4.3.1).

For comparison purposes, the capacitance per unit area from the literature \( C_{\text{lit}}^0 \) was obtained by dividing the literature value \( C_{\text{lit}} \) by the nanopore array
cross-sectional area. Trojanek et al. [196] reported a capacitance value of 0.08 F m$^{-2}$ for the interface between 0.1 M LiCl in water and 5 mM BTP-PATPBCl in DCE. Assuming this value as the typical specific interfacial capacitance at the ITIES, and applying it to our system, showed that our experimental values were about four orders of magnitude higher (Table 4.3.1). These results indicate that capacitance effects in addition to that of the ITIES are present. A further source of capacitance is the Si$_3$N$_4$ membrane. In the experimental set-up, the Si$_3$N$_4$ membrane can be considered as a dielectric ($\varepsilon_r = 7.2$) membrane sandwiched between two conductors (electrolyte solutions) whose capacitance can be described by the parallel plate model:

$$C = \frac{\varepsilon_r \varepsilon_0 A}{l}$$  \hspace{1cm} (Eq. 4.3.1)

where $\varepsilon_0 = 8.85 \times 10^{-12}$ F m$^{-1}$ is the vacuum permittivity, $A = 500 \times 500$ µm$^2$ is the area of the membrane (neglecting the pores) and $l = 100$ nm is the membrane thickness [200, 201]. The calculated capacitance is 0.16 nF. This is one order of magnitude lower than the overall capacitance of the systems determined experimentally in this study (Table 4.3.1). The difference between the experimentally-determined capacitances and the calculated capacitance for the silicon nitride membrane suggests that additional capacitive components are present, in addition to that of the membrane material.

Scanlon et al. [67] investigated the capacitance between two electrolyte solutions separated by a Si$_3$N$_4$ membrane without pores and compared this with a nanoITIES array containing 23 nano-interfaces, with $r_a \sim 45$ nm. In both cases, a blank electrolyte solution system was applied (i.e., in the absence of a transferring ion). The blank CVs obtained were virtually the same, showing that the overall capacitance has only a marginal contribution from the nanoITIES. This observation is in agreement with the results obtained in the previous chapter (Section 3.3.2.5).

By combining the experimental capacitance and the uncompensated resistance, the cell time constants for the three membrane designs employed here were obtained. The three membrane array designs exhibited (Table
4.3.1) cell time constants and charging times in the range of 0.017 s to 0.092 s and 0.08 s to 0.46 s, respectively, with the nanoITIES array with the smallest interface radius (17 nm radius) exhibiting the largest pore resistance and consequently the longest charging time. The charging time was observed to be more influenced by the resistance than the capacitance, which can be seen by the resistance range (from 3.42 to 58.57 MΩ) being broader than the capacitance range (1.57 to 4.83 nF), as similarly observed in Section 3.3.2.5. In all experimental current transients reported below, data from times shorter than the charging times were omitted.

Data from this study were compared to data from Yuan et al. [186] at a single microITIES formed at 5 μm radius micropipette, since the total nanoITIES array may behave as microITIES. The principle underlying this assumption was that an array of nanoelectrodes eventually behaves as if the entire array were a single electrode of the same interfacial surface area, with its equivalent properties [67, 109, 135]. If the charging time was taken as $5R_uC_{exp}$, the microITIES exhibited a charging time of 0.4 ms, which was approximately three orders of magnitude faster than at the nanointerface arrays (Table 4.3.1). Similarly, use of the literature value for the liquid | liquid interfacial capacitance [196] together with the estimated uncompensated resistance for the nanoITIES arrays studied here, gives values of charging time between 0.14 ms to 2.34 ms, which are again much shorter than those obtained using the experimentally-determined capacitances. The comparison of our results with literature data indicates that the combination of the large resistance of the nanopore system with the as-yet undetermined capacitance due to the presence of the silicon nitride membrane contributes to long charging times at the nanoITIES array employed here. As a result the application of methods such as differential pulse voltammetry (DPV) and square wave voltammetry (SWV) to characterise and exploit the ion transfer process at the nano-interface arrays may not produce useful data as these methods operate in millisecond and sub-millisecond time domains [202].
Table 4.3.1: Geometric, electrical and temporal behaviour of the three nanoITIES arrays studied in this work by potential step chronoamperometry, and comparison with a single microITIES

<table>
<thead>
<tr>
<th></th>
<th>NanoITIES arrays</th>
<th>Single microITIES [186]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore radius, $r_a$ (nm)</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>No. of pores $N_p$</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Total ITIES cross-sectional area (m²)</td>
<td>$7.07 \times 10^{-12}$</td>
<td>$3.14 \times 10^{-12}$</td>
</tr>
<tr>
<td>Experimental capacitance, $C_{exp}$ (F)</td>
<td>$4.83 \times 10^{-9}$</td>
<td>$4.44 \times 10^{-9}$</td>
</tr>
<tr>
<td>Uncompensated resistance, $R_u$ (MΩ)</td>
<td>3.42</td>
<td>7.30</td>
</tr>
<tr>
<td>Cell time constant, $R_uC_{exp}$ (s)</td>
<td>0.017</td>
<td>0.032</td>
</tr>
<tr>
<td>Charging time, $5R_uC_{exp}$ (s)</td>
<td>0.08</td>
<td>0.16</td>
</tr>
<tr>
<td>Experimental specific capacitance, $C_{exp}^0$ (F/m²)</td>
<td>630</td>
<td>1450</td>
</tr>
<tr>
<td>Response time (s)</td>
<td>$6 \pm 1$</td>
<td>$6 \pm 1$</td>
</tr>
</tbody>
</table>

* Estimated from CV data

b Total pore and bulk resistance for each membrane calculated using Equations 3.3.5 and 3.3.6

4.3.3 Chronoamperometry at the nanoITIES array

The variation of the current response with time under potentiostatic control across the nanopore array-supported water $|$ DCH interface was recorded using TPrA⁺ as the model analyte for these PSCA studies. Chronoamperograms were recorded at five different concentrations of TPrACl ranging from 20 to 100 µM in steps of 20 µM. The initial potential, $E_1$ (+0.2 V), was chosen where no current flows, while the step or transfer potential, $E_2$ (+0.6 V), was chosen where the ion transfer process occurs and was always in the region beyond the lower limits of mass transport control, i.e. within the “sloping” limiting current region of Figure 4.3.1 (a). The
potential was held for 20 s at the initial potential and 10 s at the step potential. By holding at the initial potential for 20 seconds, ions present in the organic phase were back extracted into the aqueous phase and hence regenerating the initial conditions.

Figure 4.3.2 (a) shows the background-subtracted chronoamperograms for 100 μM TPrACl in the aqueous phase, using nanoITIES arrays patterned by 75, 50 and 17 nm radii pore arrays. The data at shorter times than the calculated charging times (Table 4.3.1) were excluded from the figures. It can be seen that the experimental transient obtained at the nanoITIES arrays decayed to a steady-state current as suggested by Equations 4.1.2 and 4.1.3, as opposed to the $t^{-1/2}$ dependence expected for Cottrellian behaviour. Two different time regimes can be observed at the nanoITIES arrays from these PSCA experiments. Firstly, at short times, a rapid decrease of current is seen and, secondly, a steady-state current is observed at long times [203]. However, note that currents at times shorter than the charging times are omitted from Figure 4.3.2 (a). Nevertheless, there is a substantial current decrease and given that the currents were background-subtracted, these decreasing currents must be due to mass transport of the TPrA$^+$ cations to the nanoITIES arrays. Figure 4.3.2 (b) shows examples of background, ion-transfer and background-subtracted ion transfer current transients. The background-subtraction removed the background-charging process from the current transient, and demonstrated that the short-time region is mainly influence by the charging current but this charging current has a minimal impact on the longer-time diffusion-controlled current.
Figure 4.3.2: (a) Background-subtracted chronoamperograms of 100 μM \text{TPrACl} obtained using \text{nanoITIES} arrays formed by nanopore array membranes with three different sizes of nanopores (radii of 75, 50 and 17 nm). (b) Chronoamperograms at the 50 nm pore radius \text{nanoITIES} array of blank, analyte (100 \text{μM TPrACl}) and background-subtracted chronoamperograms, represented by dotted, dashed and solid lines, respectively.

Chronoamperometry allows the determination of the response time, an important parameter in sensor applications, where a fast response is often required. In chemical sensors, the response time may be typically defined as the time taken to reach a signal that is 95% of the final signal value [192].
Similarly, in PSCA, the response time can be defined as the time taken for the current to reach 95% of the steady-state value, given by

\[ I_{95\%} = \frac{1}{(0.95 \times I_{ss})} \]  

(Eq. 4.3.2)

Theoretically, the smallest membrane pore radius (17 nm) should achieve steady-state faster as compared to the larger pore membranes, because of the dominance of radial diffusion [85]. However, the results obtained from Figure 4.3.2 (a) clearly show that the response was dominated by the electrical properties of the membrane. This can be seen in the similar response times for the three nanoITIES arrays studied at 6 ± 1 s (Table 4.3.1). The raw experimental response times were all in the range of 5 to 7 s, irrespective of the pore size used in the experiments and no apparent trend was observed as the pore size was changed. The long response times obtained can be attributed to the highly resistive and capacitive membranes used to pattern the nanoITIES and may render them unfavourable for use in rapid-response chemical and biochemical sensor applications.

In Section 4.3.2, an assumption was made that the interfaces studied were inlaid. Thus, the formula to calculate the steady-state current at a single interface for an ion transferring from the aqueous to the organic phase is given by the Saito equation:

\[ I_{ss} = 4|z|FDCr_a \]  

(Eq. 4.3.3)

where \( I_{ss} \) is the steady-state current (or limiting current) [131]. The steady-state current is a linear function of the concentration and radius [109, 131, 132]. To obtain the total current of the array, the steady-state current calculated for one pore must be multiplied by the number of pores, \( N_p \), in this case, 400. By applying this expression for a fixed analyte concentration (e.g. 100 µM of TPrA\(^+\)), the calculated steady-state currents are 0.80, 0.53, and 0.18 nA for the nanoITIES arrays based on pore radii of 75, 50 and 17 nm, respectively, which varied linearly with the radius. The average of the last 10 current points from the PSCA transient was selected as the experimental steady-state current (i.e. the final 100 ms of the current transient).
Figure 4.3.3 (a) shows a typical set of background-subtracted chronoamperograms of 20 to 100 μM TPrACl obtained at a nanoITIES array with interface radius of 50 nm. As shown in Figure 4.3.3 (b), the experimental steady-state currents were lower than the calculated inlaid disc currents (Equation 4.3.3), in agreement with previous studies [109, 135]. They were lower by 30-50 % of the theoretical currents, while Rimboud et al. [109] reported the experimental limiting currents were ca. 50 % of the calculated currents (Equation 4.3.3). Those studies demonstrated that diffusion zone overlap, which occurred at adjacent interfaces in the array, lead to non-independent diffusion to each interface in the array, and resulted in the difference between experimental and calculated steady-state currents. Radial diffusion dominates the interfaces at the edge of the arrays while linear diffusion governs the interfaces within the arrays [67]. Particularly, the electrode-to-electrode separation (in this study equivalent to the pore-to-pore separation) and the number of nanoelectrodes (equivalent to the number of nanopores or nano-interfaces) are the key parameters that influenced the measured currents. Although the lower currents recorded could be associated with recessed rather than inlaid interfaces, previous studies have indicated that the interfaces are indeed inlaid and thus implicating diffusion zone overlap as the reason for lower currents [67, 109].
Figure 4.3.3: (a) Background-subtracted chronoamperograms of 20 to 100 μM TPrACl at a nanoITIES array based on a 50 nm pore radius membrane. (b) The corresponding calibration curve of the steady-state currents versus the TPrA⁺ concentration. The dashed line represents the theoretical current calculated using Equation 4.3.3 while the solid line is the best linear fit to the experimental data.

Figure 4.3.4 represents the experimental, Cottrellian and Mahon and Oldham current transients for transfer of 100 μM TPrACl across the 75 nm radius nanoITIES array. The Cottrellian current response in the time range was so low that it appears as zero in the figure. However, the inset magnified view of the current transient shows that it approaches zero, but does not reach zero.
In addition, the change in current over the timescale is too small to be seen in the comparison figure. For the Mahon and Oldham currents, the steady-state current is portrayed on the figure and is larger than the experimental current, for the reasons discussed above. Additionally, the transient response is not visible on the current scale displayed, but the inset clearly shows that this current decays rapidly with time to achieve the steady state value. For the Mahon and Oldham current, only the long-time expression (Equation 4.1.3) was applied, as the short time expression was too short ($t < 0.01\, ms$) for the charging times of the experimental arrangements used here. The Mahon and Oldham expression is accurate provided that the uncompensated resistance is negligible [174], in contrast with large resistance results from this study. The large resistance has a retarding effect on the response time, illustrated by the slower decrease of experimental current than this expression (Equation 4.1.3). However, the steady-state current at longer times will not be affected by the large resistance. Note that the long-term expression of Mahon and Oldham gives the same value as the Saito equation for the steady-state current.

Figure 4.3.4: The experimental, Cottrellian and Mahon & Oldham (M&O) current transients for 100 $\mu M$ TPrACl at a nanoTIES array formed by 75 nm pore radius membrane. The inset shows a magnified view of the Cottrellian and Mahon & Oldham current response.
Comparison of the experimental currents with the Cottrell and Mahon and Oldham expressions showed non-agreement with either model, in terms of both timescale of response and magnitude of current. The time-dependent currents will obey the Cottrell equation only if the electroactive interface is subjected to planar diffusion [204]. On the other hand, at microITIES, convergent diffusion dominates. In the case of a nanoITIES array with overlapping diffusion zones at adjacent nanoITIES, as is the situation here, the array may behave like a single microITIES of the same geometric parameters as those of the total nano-array [135]. In such a case, the current transient may be expected to follow the trend of single microITIES. The transition to a steady-state can be observed in Figure 4.3.4. The steady-state current was smaller than expected because of diffusion zone overlap, i.e. the diffusional transport to the individual pores was reduced (compared to the case of a single pore membrane) due to competing diffusion zones around the pores. The time required to achieve the steady-state current was also slower than predicted by the Mahon and Oldham equation (Equation 4.1.3).

The impact of solution resistance of the \( IR \) drop can be considered as follows. If the current at an electrode is under diffusion control, the potential drop is expressed by [205, 206]

\[
I_{ss}R = \frac{nFD(C_b-C_s)}{\kappa}
\]  

(Eq. 4.3.4)

where \( C_b \) and \( C_s \) are the bulk and surface concentrations and the other parameters are as previously defined. Note that the surface concentration is zero in the region of the steady-state limiting current. The number of electrons involved in the electrode reaction, \( n \), is equivalent to \( z \), the charge number of the transferring ion. Equation 4.3.4 was derived under the assumption that the size of the counter electrode and the distance between it and the working electrode (here the ITIES established at the orifice of the nanopore) are much bigger than the size of the working electrode (i.e. the pore radius) and the diffusion layer established during an experiment. Furthermore, an excess of supporting electrolyte is present. These conditions
are met in the present experiments. Using a concentration of 100 µM, a diffusion coefficient of $10^{-6}$ cm² s⁻¹ and a conductivity of 1073 µS cm⁻¹ for the aqueous electrolyte (0.01 M LiCl) [199] results in an $I_{ss}R$ drop of 0.01 mV in the limiting current region, a value which is negligible. However, this approach does not take into account the resistance of the organic phase, which is a combination of the pore resistance and the solution resistance. A rough estimation of the potential drop can be made as follows. Previously, the individual pore resistance was calculated to be $2.6 \times 10^9$ Ω for pores with a radius of 50 nm. The limiting current of the pore array (Figure 4.3.2 (b)) is ca. 0.4 nA which gives a current of approximately 1 pA for an individual pore, resulting in $I_{ss}R \approx 3$ mV. The potential drop is likely to be bigger due to the additional solution resistance. These values of potential drop will interfere with quantitative analysis of transfer kinetics. However, the steady-state limiting current will not be affected and therefore a potential drop of this magnitude might be negligible for electroanalytical purposes based on analysis of limiting currents.

Finally, to study the agreement between currents obtained from the forward scans of cyclic voltammetry experiments with the currents obtained from PSCA, the step potential was increased in increments of 0.05 V. At long times ($t = 10$ s), the PSCA data agreed well with the voltammetry data, as shown in Figure 4.3.5. As discussed in Section 4.3.1, no true limiting current plateau is reached when implementing voltammetry at the nanoITIES arrays. Nevertheless, current data obtained from potential-dependent and time-dependent experiments are in excellent agreement.
Figure 4.3.5: Comparison of the chronoamperometric steady-state current with the voltammetric response at a nanoITIES array formed at a 75 nm pore radius membrane. Voltammetric sweep rate: 10 mV s\(^{-1}\). The potential is reported with respect to the experimentally-used reference electrodes.

4.4 Conclusions

Chronoamperometry of TPrA\(^+\) ion transfer was performed at the water | 1,6-dichlorohexane nano-interface array using three different nanopore array membrane designs (nanopore radii of 75, 50 and 17 nm). An assumption that the interfaces were inlaid was made, in which liquid organic phase filled the silicon nitride nanopores. The nanoITIES arrays exhibited prolonged charging times hence the Faradaic current can be measured only at long times (timescale of seconds). The system demonstrated greater capacitances than predicted using literature values for the capacitance of the ITIES, indicating that capacitances additional to the interfacial liquid | liquid capacitance have an impact on the behaviour of these nanoporous membrane systems, such as the capacitance of the silicon nitride membrane employed. The resistances of the nanopores filled with organic phase electrolyte varied more with nanopore size than the overall system capacitance, indicating that the uncompensated resistances dominated the responses. The experimental current transients were not in agreement with either Cottrell or Mahon and Oldham expressions, and experimental steady-state currents were lower than predicted, indicating overlapping diffusion zones. However, the currents
from chronoamperometry were in excellent agreement with those obtained from cyclic voltammetry. The response times (time to reach 95% of the steady-state current) of the silicon nitride membrane-based nanoITIES arrays to a potential step were in the region of 6 s, indicating a slow response and the impact of nanopore resistance as well as membrane capacitance. The results presented here provide further characterisation of nanoITIES arrays and will be beneficial in the design of chemical and biochemical sensing systems.
Chapter 5

Electrochemical Characterisation of Parylene C Porous Membrane Arrays

5.1 Introduction

This chapter discusses the electrochemical characterisation of parylene C porous membrane arrays (PMA)-modified ITIES, featuring uniform arrays of concave-pores prepared via conformal vapour deposition. The electrochemical transfer of tetrapropylammonium cation (TPrA\(^+\)) across the water | 1,6-dichlorohexane (1,6-DCH) interface was chosen as the experimental system. In addition, a simulation study was also conducted by the finite element method by Dr. Jorg Strutwolf (University of Tübingen, Tübingen, Germany). The comparison of the experimental and simulated voltammograms will allow the estimation of the operating parameters such as the location of the liquid | liquid interface, which in turn is beneficial for array design optimisation.

Micro- and nano-pore membranes have gained great interest for applications in biosensing, molecular separation, molecular electronics and device fabrication [143, 145]. The fabrication of single and multiple micro- and nanopores in inorganic membranes can be achieved routinely using advanced fabrication methods from the semiconductor processing industry, with silicon nitride (Si\(_3\)N\(_4\)) [139, 146, 147] and silicon dioxide (SiO\(_2\)) [141, 152] reported as the most common fabrication materials since they have been extremely well developed and studied over 50 years [107, 140]. Focused ion beam (FIB) milling [139, 161], direct drilling by high-energy focused electron beams
[147, 153] and electron beam lithography (EBL) [67, 150] have been reported to prepare micro- and nano-pores in inorganic membranes. On the other hand, the preparation and characterisation of micro- and nano- pores of organic membranes, namely cellulose [28, 102] and track-etched polyester and polyethylene terephthalate (PET) [100, 101, 105] have been investigated too. The formation of organic membrane with controlled pore properties has recently been reported [164].

A polymer, poly(chloro-para-xylylene), also known as parylene C, from the parylene (poly(p-xylylene)) family, is gaining interest in membrane fabrication processes due to its intrinsic properties such as chemical inertness to organic and inorganic solvents, mechanical flexibility (Young’s modulus of 2 - 4 GPa, and high ductility up to 200 % elongation), insulation (dielectric constant of 5.6), biocompatibility (FDA approved USP Class VI grade), and manufacturing advantages [164, 207-210]. Parylene C has been reported as a material for micro-electro-mechanical-system (MEMS) devices, and for biomedical applications [208, 209, 211]. The chemical vapour deposition (CVD) process enabled parylene C layers to be deposited on solid substrates [164, 210-212] or grown onto liquid surfaces [207]. Conformal vapour coating of parylene C onto microstructured materials, (e.g. electron microscopy grids) was reported for the fabrication of single- or highly ordered pore array membranes, with micro- and nano- scale dimensions [164]. This membrane contained concave-shape pores in a cubic close-packed (CCP) arrangement. Each individual pore measured 8.3 µm at the top and bottom openings, 0.7 µm in the central constriction diameter, 11.0 µm in length (membrane thickness) and 16.5 µm in the pore centre-to-centre distance [164].

Voltammetry at the ITIES has been previously applied to characterise inorganic and organic membranes such as γ-alumina [106], silicon [66], Si₃N₄ [67, 109], cellulose [28, 102], polyester [105] and PET [100, 101]. Cellulose was observed to be less suitable as it showed a tendency to swell (to a thickness of over 100 µm) when in contact with the organic solvent [28], while polyester, which are chemically inert in the aqueous and organic phases,
exhibited better stability [105]. To the best of our knowledge, no studies have reported on parylene C organic membrane characterisation as the basis for ITIES formation and electrochemical sensing, which is the purpose of this study. However, parylene C coated microelectrodes have been reported [211, 213, 214].

5.2 Experimental methods and materials

5.2.1 Reagents
The chemical reagents used in this study are as detailed in Section 2.4.1 of Chapter 2. The organic phase was prepared as a gel at 1 % w/v solution for the micro- or submicro-ITIES study. PVC composition in the organic phase at millimetre-scale ITIES study was varied from 5 to 12 % w/v. The procedure to prepare the organogel is as explained in Section 2.4.1 too. The model analyte species investigated was the tetrapropylammonium cation (TPrA+), used as its chloride salt in 10 mM LiCl in 1,6-DCH-saturated water.

5.2.2 Preparation of ITIES
Arrays of micro- or submicro-scale ITIES were formed at a water | 1,6-DCH interface using parylene C membrane fabricated at Indiana University, USA (Figure 5.2.1). The procedures to attach parylene C membrane onto cylindrical borosilicate glass tube, and the micropore- or submicropore-supported ITIES assembly have been described in Section 2.4.2 of Chapter 2.
5.2.3 Experimental procedure

The voltammetric experiments at parylene C PMA-supported liquid | organogel interface arrays were executed following the procedures outlined in Section 2.4.5 of Chapter 2. The membrane-modified water | 1,6-DCH interface was polarised using a four-electrode electrochemical cell to compensate the resistance associated with the organic phase and the membrane [105]. This cell comprises two Pt counter electrodes and two Ag | AgCl reference electrodes, one in each phase, as outlined in Section 2.4.3 (Chapter 2). Initially, the membrane-modified water | 1,6-DCH interface was polarised utilising a two-electrode electrochemical cell. However, the high resistance experienced, results the electrochemical cell to be changed to a four-electrode system and used throughout this chapter. Thus, no comparable data is available for this reason.

Approximately 100 μL of the gelled-organic phase and 200 μL of the organic reference solution were placed using a glass pipette into the borosilicate glass tube with the parylene C membrane sealed to the bottom, or without the membrane in the case of pure liquid | organogel interface. The
The electrochemical cell can be represented as follows, in which x is the concentration of TPrACl in the aqueous phase:

\[
\text{Ag} \mid \text{AgCl} \mid x \, \mu\text{M TPrACl} + 10 \, \text{mM LiCl}_w \mid 10 \, \text{mM BTPPATPBCl}_{DCH} \mid 10 \, \text{mM BTPPACl in 10 mM LiCl}_w \mid \text{AgCl} \mid \text{Ag}
\]

where the double bar denotes the interface to be polarised, either with or without the parylene C membrane.

### 5.2.4 Contact angle

Contact angle was performed to investigate parylene C membrane hydrophobicity. The instrument and procedure are as explained in Section 2.4.6 of Chapter 2.

### 5.2.5 Computational simulations

Simulation study was performed utilising the finite element method (FEM) program package COMSOL Multiphysics. This study was performed by Dr. Jorg Strutwolf.

### 5.3 Results and discussion

#### 5.3.1 Cyclic voltammetry of TPrA\(^+\) transfer at parylene C porous membrane arrays

Ion transfer voltammetry of TPrA\(^+\) at PMA-supported ITIES was undertaken. Prior to the addition of the model analyte ion, a sequence of CVs of the background electrolyte solution was recorded so that background-subtracted voltammograms could be prepared. Aqueous phase concentrations of TPrACl were varied between 20 and 100 \(\mu\text{M}\) in increments of 20 \(\mu\text{M}\) to produce calibration curves correlating currents (in the forward and reverse scans) with the concentration of transferring ions, TPrA\(^+\).

In the following, an assumption was made that transport was by diffusion only. The shape of the voltammetric responses and the magnitude of the
current observed are influenced by several parameters. These parameters include: (i) the geometric properties of the pores, namely the pore radius, $r_a$, the pore-to-pore separation, $r_c$, the pore shape, and the number of pores, $N_p$; (ii) the properties of the electrolyte solutions and the transferring ion species; (iii) the recess depth or the liquid | liquid interface location within the pore channel, $l$; (iv) the diffusion regime shape, and (v) the magnitude of the diffusion zone extension, $\delta$ [67, 81, 84, 86].

The CV response of the PMA to increasing TPrA$^+$ concentration in the aqueous phase, shown in Figure 5.3.1 (a) and (b), exhibited asymmetric behaviour. The voltammogram on the forward scan corresponded to the transfer of TPrA$^+$ from aqueous to organic phase. This showed a combination of steady-state and peak behaviour, while the voltammogram on the reverse scan, where the ions were transferred back from the organic phase to aqueous phase, exhibited peak behaviour. The experimental voltammogram in this study appeared to have a contribution of radial and linear diffusion control on the forward scan, while a linear diffusion control dominated on the reverse scan. A linear relationship exists between the experimental currents (forward and reverse scans) and TPrA$^+$ concentrations, as illustrated in Figure 5.3.1 (c). A discussion of the possible causes of the observed voltammogram behaviour is next presented.
Figure 5.3.1: (a) Cyclic voltammograms of 20 to 100 µM TPrA\(^+\) transfer in steps of 20 µM and (b) its corresponding background-subtracted scans across water / 1,6-dichlorohexane (DCH) PMA formed with parylene
membrane at 5 mV s\(^{-1}\) sweep rate. Dotted and solid lines represent blank and analyte voltammograms, respectively, while arrows indicated increasing analyte concentrations (c) The corresponding calibration curves of the forward and reverse experimental currents (background-subtracted) against TPrA\(^+\) concentrations. The potentials in (a) and (b) are reported with respect to the experimentally-used reference electrodes.

The effect of the diffusion regime shape and the diffusion zone overlap is first addressed with an assumption that the interface formed is inlaid. An inlaid liquid | liquid interface array with the pore orifice at the aqueous side was established if the organic phase electrolyte solution entirely fills the pores. At the array of micro- and nano-ITIES, the possibility of diffusion zone overlap exists. In the case where radial diffusion dominates at the edge of the arrays despite linear diffusion within the arrays (where overlap of diffusion zones is present), steady-state characteristics are still achieved, as demonstrated via a recent computer simulation [135]. However, the diffusion zone overlap resulted in the lower experimental current observed. If the diffusion zones are heavily overlapped, a purely linear diffusion will be observed, resulting in the appearance of a peak-shaped voltammogram [81, 106]. A combination of steady-state and peak behaviour observed on the forward scan has been reported previously by Amatore et al. [215]. At partially blocked surfaces, charge transfer process occurs by both radial and linear diffusion. The voltammetric response change from steady-state to peak behaviour was due to transition from a radial diffusion (individual diffusion fields at each interface) to a linear diffusion (diffusion fields overlap) [105, 215].

Many research investigations conducted previously reported the linear relationship between the size of the diffusion zone, \(\delta\), and the electrode radius, \(r\) (in this case analogous to pore radius). If two electrodes are sufficiently close to each other, their diffusion zones will interact. The often-applied condition for non-interacting diffusion zones at the micrometer-scale pore is \(r_c > 20r_a\) [137]. A study by Scanlon et al. [67] demonstrated that micro-arrays with greater pore centre-to-centre separations had lower
diffusion zone overlap at neighbouring interfaces in the array, and thus greater flux and current values. If $r_c < 20r_a$, diffusion zone overlap will lead to non-independent diffusion to each interface. In this study, the radius of the pore orifice is approximately 4.2 µm, hence the pore centre-to-centre separations should at least 83.0 µm to avoid diffusion zone overlap. Since the interpore distance is only 16.5 µm, diffusion zone overlap is expected to occur and contribute to the voltammogram shape observed on the forward scan. In fact, for inlaid interfaces of this magnitude and separation, heavily overlapped diffusion zones and hence peaked voltammograms, can be expected.

The liquid | liquid interface location within the pore is another possible cause for the observed voltammogram behaviour. The interface location which primarily depends on the hydrophobicity or wetting properties of the membrane material will determine the supply of the transferring ions to the interface. In inlaid configuration, the interface formed is planar at the pore orifice on the aqueous side of the membrane, with no shielding effect from the surrounding pore wall. On the other hand, a recessed pore can vary from 0 % (organic phase fills the pores, interface at the pore mouth on the aqueous side) to 100 % (aqueous phase fills the pores, interface at the pore mouth on the organic side). Previously, a simulation study of ITIES located at a micro-cylindrical pore [84] demonstrated steady-state current-potential curve for an inlaid interface, with the dominance of steady-state behaviour on the forward scan, and peak behaviour on the reverse scan. However, the experimental results from this study exhibited the dominance of peak current on the reverse scan over the peak observed on the forward scan (Figure 5.3.1 (a) and (b)). The presence of a current peak on the reverse transfer may be a consequence of the linear diffusion control.

In the case of recessed interface, simulation demonstrated the shielding of transferring species transport to the interface due to the surrounding pore wall results in a lower current, compared to an inlaid interface. If the interface location is not in the centre of the pore length, the forward and reverse ion
transfer processes will experience different wall shielding effects and produce non-symmetrical transfer [84], as observed in the experimental voltammogram, and will be apparent in the discussion of the simulation section. Platt et al. [106], and Kralj and Dryfe [101] reported a number of possible diffusion fields for the transfer of TEA⁺ ion across ITIES located within nano-cylindrical pore of γ-alumina, and micro- and nano-cylindrical pore of polyethylene terephthalate membrane, respectively. Where the liquid | liquid interface location is inside the pore channel, the diffusion fields were retained within the pore, so that linear diffusion of ions was observed. When the interface is inlaid and the individual diffusion fields are heavily overlapped, linear diffusion of ions is also observed. Another possibility for the inlaid interface is that the individual diffusion fields traverse the membrane but do not overlap, hence radial diffusion dominates. In our study, the situation for a recessed interface is complicated by the concave pore geometry, as compared to cylindrical pore geometry.

The pore shape has also been reported to influence the voltammetric response shape and the current magnitude. Britz and Strutwolf [216] reported a simulation of electrochemistry at a conically recessed ultramicrodisk electrode (CRUMDE), where the ultramicroelectrode (in this case equivalent to the liquid | liquid interface) is situated at the bottom of a conical well of height, \( h \), and the side walls being inclined from vertical by an angle, \( \alpha \). For the lowest recess (nearly inlaid) interface, no distinguishable difference was noticed in the voltammogram shape and current magnitude, at \( \alpha = 0^\circ \) (vertical wall) and \( \alpha = 20^\circ \) (inclined). However, with increasing recess, the voltammogram current decayed for vertical walls since radial diffusion is suppressed, in contrast to inclined walls which allow a certain amount of radial diffusion. In this thesis, the concave pore geometry exhibited an inclined wall angle of approximately 45°, measured at the joint between the tapered part of the pore and the straight narrow region (Figure 5.2.1) [164].
The ratio of the diffusion coefficient, $\gamma$, in the two phases can also influence the experimental voltammogram. In this study, the organic phase was prepared as a gel (1 % w/v of PVC). The incorporation of PVC in the organic phase increases the viscosity, producing a slower rate of diffusion for the ion transfer process [84, 87, 109]. The difference in the diffusion coefficient of TPrA$^+$ ions in the gellified organic phase, $D^\beta$ and aqueous phase, $D^\alpha$ may contribute to the non-symmetrical transfer behaviour ($\gamma = D^\beta / D^\alpha$). The $\gamma$ in this study is lower than the liquid aqueous | liquid organic system [109], but higher than the liquid aqueous | organogel system which utilised 10 % w/v of PVC. A previous simulation study demonstrated the peak current in the reverse scan increased with decreasing values of $\gamma$. During the transfer, if $D^\beta < D^\alpha$ the ion accumulates near the interface within the pore on the organic phase side. Linear diffusion controls the reverse transfer and the diffusion layer is thinner for small $\gamma$ values, hence the peak current increases when $\gamma$ decreases [84].

As discussed earlier in this section, the diffusion regime shape and the diffusion zone overlap, the liquid | liquid interface location within the pore, the pore shape, and the ratio of the diffusion coefficient in the two phases are the factors that contribute to the observed asymmetric voltammogram behaviour. These parameters are equally important towards the shape of the voltammetric responses and the magnitude of the current observed, as will be apparent in the discussion of the simulation study in the later section (Section 5.3.5) and Appendix E.

Figure 5.3.2 (a) illustrates the Ohmic drop (or $IR$ drop) problem observed in this study. The Ohmic distortion behaviour is evident from the experimental CVs where the peak-to-peak separation (the difference in peak potentials for the forward and reverse scans) increases with an increase in TPrA$^+$ concentration in the aqueous phase. The peak potential in the forward and reverse scans progressively shifted to a more positive and negative applied potential value, respectively. The Ohmic distortion was also reported due to
the effect of the pore resistance [84], which might be greater for the concave pore geometry applied in this study, and will be apparent later in the simulation section. The plot of the normalised CVs in Figure 5.3.2 (b) also demonstrated the IR drop problem through the voltammogram shift.

Figure 5.3.2: (a) Plot of peak-to-peak separation versus analyte concentration to illustrate the Ohmic drop behaviour. Plot of normalised cyclic voltammograms (20 µM (light gray) to 100 µM (black) TPrA⁺ transfer in increment of 20 µM) to the (b) maxima current in the forward scan of that CV. The potential in (b) is reported with respect to the experimentally-used reference electrodes.
5.3.2 CV of TPrA⁺ transfer at pure ITIES

At pure liquid | liquid interface (millimetre-scale ITIES), linear (one-dimensional) diffusion occurred, resulting in peak-shaped voltammograms, and can be expressed by the Randles-Sevcik equation [1]:

\[ I_p = (2.69 \times 10^5) z^{3/2} AD^{1/2} C v^{1/2} \]  
(Eq. 5.3.1)

where \( I_p \) is peak current and \( A \) is the total cross sectional area of the interfaces. \( z \) and \( C \) are the charge and bulk concentration, of the transferring ions in the aqueous phase, respectively.

In order to calculate \( A \), an assumption was made that pure liquid | liquid interface area is equivalent to the cylindrical borosilicate glass tube inner area (inner diameter of 1.4 mm). Comparison of the experimental current at parylene C PMA (from Figure 5.3.1(c)) with calculated current for a pure liquid | liquid interface employing Equation 5.3.1 showed a 48 % lower current, indicating that incorporation of membrane into the liquid | liquid interface system lowered the ion transfer current (Figure 5.3.3), similar to a previous observation [105]. Modification of the ITIES with porous membrane material affects the ion transfer process through the pore, from the aqueous to organic phase and vice versa, and leads to a few possible diffusion fields, as described earlier.
Figure 5.3.3: Comparison of the experimental current at parylene C PMA (solid) and calculated current at pure ITIES (dashed) in the forward scan. The current calculated according to Equation 5.3.1

The pure ITIES was mechanically stabilised by partial solidification of the organic phase. This gelification approach was first introduced by Senda and co-workers [122, 123] where the organic phase was gelified with PVC-nitrobenzene (PVC-NB). The gelification of either phase used to form the ITIES (typically the organic phase) was to overcome the problem of mechanical instability at the liquid | liquid interface, however, this approach increases the system resistance. Miniaturisation of the ITIES has been observed to minimise the problem of high interfacial resistance.

Attempts to prepare an organogel with lower PVC composition (1 % w/v, similarly employed at parylene C PMA) were unsuccessful as the organogel leaked through the millimetre-scale ITIES. Hence, no direct comparison on the effect of modifying the ITIES with porous membrane material can be made. The PVC composition was increased so as to stabilise the interface, and the influence of PVC composition in an organogel on CV at pure ITIES was investigated. The low molecular weight PVC composition dissolved in the organic phase solution was varied in the range of 5 to 12 % w/v.
Figure 5.3.4 (a) to (f) show CVs and the background-subtracted scans of 20 to 100 µM TPrA⁺ ion transfer across the pure ITIES at different PVC compositions, while the corresponding calibration curves are given in Figure 5.3.5 (a) to (c). The voltammogram on the forward and reverse scans showed peak behaviour, since diffusion to both sides of the millimetre-scale interface would be linear. The tailing-off observed at higher applied potentials (0.9 to 1.0 V) might be due to an artefact associated with the background subtraction procedure employed (Figure 5.3.4 (b), (d) and (f)) [84]. The incorporation of PVC (from 5 to 12 % w/v) in the organic phase decreased the ratio of the diffusion coefficient between the organic and aqueous phases, $\gamma$. The peak current in the forward potential scan decreased with increasing values of PVC compositions, contrary to previous report [84], where the limiting current for the forward scan in the case of a micro-ITIES array remained constant with variation in the ratio of the diffusion coefficient in the two phases. However, the available potential window is practically independent of the composition of PVC in the organic phase, as reported previously [97]. The random errors in the values for the slope (and intercept) of the regression line (plot of forward experimental current against TPrA⁺ concentration) at different PVC compositions, at 95 % confidence level are summarised in Table 5.3.1.
Figure 5.3.4: (a), (c) and (e) CV of 20 to 100 µM TPrA\(^+\) transfer in steps of 20 µM, and (b), (d) and (f) the corresponding background-subtracted scans across millimetre-scale water / 1,6-DCH interface at different PVC compositions. (a) and (b) 5 % w/v PVC, (c) and (d) 10 % w/v PVC, and (e) and (f) 12 % w/v PVC. Dotted and solid lines represent blank and analyte voltammograms, respectively, while arrows represent increment in analyte...
concentrations. Sweep rate used was 5 mV s$^{-1}$. The potentials are reported with respect to the experimentally-used reference electrodes.

**Figure 5.3.5:** Calibration curves of the forward and reverse experimental lines against TPrA$^+$ concentrations at different PVC compositions: (a) 5 % w/v PVC, (b) 10 % w/v PVC, and (c) 12 % w/v PVC to illustrate the influence of
organogel composition on CV signal. All the experimental data were background-subtracted.

Table 5.3.1: Summary of slope and intercept for the plot of current versus TPrA⁺ concentration (forward scan only) at 5, 10 and 12 % w/v PVC, and the corresponding standard deviations

<table>
<thead>
<tr>
<th>PVC composition / % w/v</th>
<th>Slope / nA µM⁻¹</th>
<th>Standard deviation of slope, S_B / nA µM⁻¹</th>
<th>Intercept / nA</th>
<th>Standard deviation of intercept, S_A / nA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.93</td>
<td>0.04</td>
<td>3.79</td>
<td>2.96</td>
</tr>
<tr>
<td>10</td>
<td>0.63</td>
<td>0.03</td>
<td>-0.06</td>
<td>2.10</td>
</tr>
<tr>
<td>12</td>
<td>0.58</td>
<td>0.03</td>
<td>-3.26</td>
<td>2.14</td>
</tr>
</tbody>
</table>

Comparison of slope of the forward peak current against TPrA⁺ concentration at different PVC compositions is illustrated in Figure 5.3.6. In order to estimate the slope of the peak current versus TPrA⁺ concentration at 1 % w/v PVC, the calibration line was extrapolated, assuming a linear relationship between the two parameters. The slope for a 1 % w/v PVC composition was estimated 1.13 nA µM⁻¹.

As described in the earlier part of this section (Figure 5.3.3), modification of the liquid | liquid interface with a membrane lowered the ion transfer signal as compared to the theoretical current. This behaviour is again evident from the experimental current where the experimental parylene C membrane slope was lower than all slopes at the millimetre-scale ITIES (with variation in PVC compositions) (Figure 5.3.6). The presence of parylene C PMA lowered the ion transfer current obtained from both calculation (employing Equation 5.3.1) and experiment. A possible explanation for the reduction in current observed as reported by Dryfe and Kralj [105] was modification of the diffusion field through the pores of the membrane, as compared to an inlaid interface response expected. In addition, the theoretical slope falls within the range of experimental slopes for differing organic phase compositions (Figure 5.3.6).
Figure 5.3.6: Plot of the slope (of forward peak current against TPrA$^+$ concentration) (as shown in Table 5.3.1) versus PVC composition. Solid horizontal line represents slope from experimental parylene C membrane-modified ITIES, while dashed horizontal line represents calculated pure ITIES slope. Dotted line demonstrated extrapolation to determine slope for a 1 % w/v PVC composition.

Due to the fact that the diffusion coefficient of the TPrA$^+$ ion in the gellified organic phase is lower than in the aqueous phase, the ion transferred from the aqueous phase to the organic phase did not diffuse into the bulk organic phase, but instead accumulated near the interface on the organic phase side, within the millimetre-scale ITIES. Linear diffusion dominates the reverse transfer and the diffusion layer is thinner for small $\gamma$ value. The peak current observed on the reverse transfer was enhanced as compared to the forward peak current for all PVC compositions studied. This behaviour is evident from the ratio of forward peak current ($I_{p,W\rightarrow O}$) to reverse peak current ($I_{p,O\rightarrow W}$) at various PVC compositions (as summarised in Table 5.3.2). The ratio changed with PVC composition, nevertheless the values were always lower than 1. However, the peak current for the reverse transfer did not increase with decreasing value of $\gamma$, as simulated by Strutwolf and co-workers [84], possibly due to the different simulation system. In the simulation study, the amount of ion transferred from aqueous to organic phase was constant,
contrary to this study, where the amount of ion transferred in the forward scan decreased with increasing PVC composition.

Table 5.3.2: The influence of PVC composition on the ratio of forward peak current to reverse peak current to illustrate the mass transport effect

<table>
<thead>
<tr>
<th>[TPrA⁺] / µM</th>
<th>5 % w/v PVC</th>
<th>10 % w/v PVC</th>
<th>12 % w/v PVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.87</td>
<td>0.54</td>
<td>0.40</td>
</tr>
<tr>
<td>40</td>
<td>0.80</td>
<td>0.54</td>
<td>0.59</td>
</tr>
<tr>
<td>60</td>
<td>0.80</td>
<td>0.54</td>
<td>0.56</td>
</tr>
<tr>
<td>80</td>
<td>0.77</td>
<td>0.61</td>
<td>0.64</td>
</tr>
<tr>
<td>100</td>
<td>0.72</td>
<td>0.60</td>
<td>0.67</td>
</tr>
<tr>
<td>Average</td>
<td>0.79</td>
<td>0.57</td>
<td>0.57</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.05</td>
<td>0.03</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table 5.3.3 presents the influences of varying PVC composition in the organic phase of the pure ITIES towards the separation of the peak potentials of the forward and reverse scans, ΔEₚ, to illustrate the resistance effect (or Ohmic distortion). The resistance behaviour is evident from the experimental CVs where the peak-to-peak separation increases with an increase in PVC composition in the organic phase (from 5 to 12 % w/v) for all concentrations studied. The forward and reverse peak potential progressively shifted to a more positive and negative applied potential value, respectively. In addition, increasing TPrA⁺ concentration in the aqueous phase increased ΔEₚ for all PVC compositions studied, as observed at the parylene C membrane-modified ITIES. The lowest experimental ΔEₚ at parylene C PMA (160 mV) was larger than at the millimetre-scale ITIES (110 mV at 5 % w/v PVC), suggesting that modifying the interface with parylene C membrane increased the resistance effects. This result was in agreement with the previous report by Dryfe and Kralj [105], where a considerable broadening of the voltammetric response was observed when the ITIES was modified with a membrane, reported as due to residual uncompensated Ohmic drop. The
decrease in forward peak current with an increase in PVC composition, as demonstrated in Figures 5.3.4 and 5.3.5, might be due to Ohmic drop.

Table 5.3.3: The influence of PVC composition on the peak-to-peak separation to illustrate the resistance effect

<table>
<thead>
<tr>
<th>[TPrA⁺] / µM</th>
<th>ΔEₚ (Eₚ,w→o - Eₚ,o→w) / V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 % w/v PVC</td>
</tr>
<tr>
<td>20</td>
<td>0.11</td>
</tr>
<tr>
<td>40</td>
<td>0.16</td>
</tr>
<tr>
<td>60</td>
<td>0.20</td>
</tr>
<tr>
<td>80</td>
<td>0.24</td>
</tr>
<tr>
<td>100</td>
<td>0.28</td>
</tr>
</tbody>
</table>

5.3.3 Membrane porosity

The membrane macroscopic coverage of pores, θ, or membrane porosity, is one of the parameters influencing the voltammetric response resulting from ion transfer across the ITIES. It is defined as the fractional pore coverage of the whole membrane surface, given by the equation [86]:

\[
\theta = \frac{N_p A_{pore}}{A_{total}} \tag{Eq. 5.3.2}
\]

where \(A_{pore}\) is the cross-sectional area of the individual pore, and \(A_{total}\) is the total surface area of the membrane (in this case equivalent to cylindrical borosilicate glass tube inner area of \(1.50 \times 10^{-6}\) m²). In order to calculate \(N_p\) for the parylene C membrane, an assumption was made that the parylene C membrane area is equivalent to cylindrical borosilicate glass tube inner area onto which the membrane was sealed. Taking into account the pore centre-to-centre distance of 16.5 µm, and that the pores occupied the whole membrane area, \(N_p\) is approximated to 5500. To obtain the total cross-sectional area of the pores, the cross-sectional area of the individual pore was multiplied by \(N_p\). Employing Equation 5.3.2, and if the pore surface area is taken at the pore orifice (total pore surface area of \(3.79 \times 10^{-7}\) m²), \(\theta\) is 0.25. If the pore surface area is taken at the central constriction (total pore
surface area of $2.69 \times 10^{-9}$ m$^2$), $\theta$ is 0.0018. These values demonstrate that small fraction of the membrane surface is occupied with the pores.

On the other hand, the voltammetric response resulting from ion transfer across the ITIES can be applied to the measurement of the membrane porosity. This technique has been applied previously to $\gamma$-alumina membranes [106]. A few possible diffusion zones arose from ion transferring from the aqueous phase to the organic phase since the ion has to travel via the membrane pore. In the case where the diffusion profile remains one dimensional due to overlap of diffusion zones formed at adjacent interfaces, Equation 5.3.1 holds (with $A$ represents the total membrane area). In the case where linear diffusion zone is retained within the pore, Equation 5.3.1 is replaced by the following expression [106]:

$$I_p = (2.69 \times 10^5) \theta z^{3/2} AD^{1/2} C v^{1/2}$$

(Eq. 5.3.3)

where $\theta$ is the membrane porosity and represents the fraction of the membrane surface that is porous. The membrane porosity can be determined from comparison of the slopes of the calculated and experimental Randles-Sevcik plot, as long as the linear diffusion model applies and Equations 5.3.1 and 5.3.3 hold [106]. A Randles-Sevcik plot of the $I_p$ dependence on the square root of scan rate was employed to validate these equations.

In this study, the sweep rate was varied between 5 and 100 mV s$^{-1}$ in the presence of 100 $\mu$M TPrA$^+$. A blank CV was recorded in the absence of analyte, hence background-subtracted CV could be obtained by subtracting the blank response from that of the analyte at each sweep rate. The resulting CVs are shown in Figure 5.3.7 (a), demonstrating the forward peak shifts to more positive potential with increasing sweep rate. Plot of the peak current of the background-subtracted forward scan versus the square root of the scan rate is shown in Figure 5.3.7 (b). The linear relationship between the peak current and the square root of the scan rate indicated a linear diffusion-controlled electrochemical process, as described by the Randles-Sevcik.
expression, and allows membrane porosity to be determined. At lower sweep rates, a more clearly-defined peak shape was observed, in agreement with peak distortion by cell resistance and capacitance at higher sweep rates [84]. A nonzero current axis intercept demonstrated in Figure 5.3.7 (b), was similarly observed at the γ-alumina membrane [106], caused by current from the transfer of adsorbed ions on the internal surface of the membrane. A nonzero intercept has been reported for various cations (e.g. tetramethylammonium and tetraethylammonium) and anions (e.g. nitrate and iodide), suggesting non-specific dependent of this effect on the chemical species [106].

Figure 5.3.7: (a) Background-subtracted CVs of 100 µM TPrA⁺ at increasing potential sweep rates of 5 (light gray), 10, 25, 50, 75 and 100 (black) mV s⁻¹, as indicated by the arrow. (b) Peak currents versus the square root of the
sweep rate on the forward and reverse sweeps. The potential in (a) is reported with respect to the experimentally-used reference electrodes.

In this study, θ was calculated from comparison of the Randles-Sevcik plot slopes between parylene C membrane-modified ITIES and pure ITIES, both at 1 % w/v PVC. The slope value (1.13 nA µM⁻¹) of the pure ITIES was from extrapolation (Figure 5.3.6), as discussed earlier. The porosity value measured from the voltammetric experiment was 0.36. If the slope of the pure ITIES is taken from calculated slope employing Equation 5.3.1 (as shown in Figure 5.3.6), θ is 0.52. These values were higher than 0.25 measured from the pore geometry (with the total pore surface area of 3.79 × 10⁻⁷ m² taken at the pore orifice). As a comparison, Platt et al. [106] reported the porosity values of highly porous γ-alumina ultrafiltration membranes in the range of 0.13 to 0.30, determined from voltammetric experiments, which was in agreement with various techniques such as Hg porosimetry, gas penetration and SEM combined with computerised imaging analysis (CIA) with the porosity values in the range of 0.20 to 0.56.

5.3.4 Hydrophobicity and stability study

The hydrophobicity of the parylene C membranes before and after electrochemical study was investigated via contact angle measurement. Contact angle (C.A.) is defined as quantitative measure of the wetting of the parylene C membrane (solid) by the deionised water (liquid). Hydrophilic surfaces exhibit C.A. < 65 ° while hydrophobic surfaces exhibit C.A. > 65 ° [217]. The results demonstrated that the fresh parylene C membrane surface was hydrophobic, in agreement with previous reports [164, 210, 212, 218], even though the measured value (C.A. of 83 ± 3 °) was lower than literature reported value (C.A. of 95 °) [218]. However, upon usage, the hydrophobicity of parylene C membrane decreased (in this study, C.A. of 70 ± 5 °).

In order to study the stability of the organic membrane in these electrochemical conditions, scanning electron microscopy (SEM) images of the parylene C membranes were taken before and after the electrochemical
study (Figure 5.3.8). After electrochemical measurements, SEM images indicated the presence of some solid within the pores. These may be due to residue from the electrolyte salts improperly rinsed from the membrane. In addition, the pore opening geometry demonstrated an apparent size reduction, which might be due to electrolyte salt residue, and the possibility of a swollen membrane when in contact with the aqueous or organic phases.

![SEM images of fresh (a) and used (b) parylene C membranes. Solid within the pores after electrochemical study (b) might be due to residue from electrolyte solutions](image)

**Figure 5.3.8: SEM images of fresh (a) and used (b) parylene C membranes. Solid within the pores after electrochemical study (b) might be due to residue from electrolyte solutions**

### 5.3.5 Simulations of membrane pore within an array

The simulation study presented in this section was provided by Dr. Jorg Strutwolf. The pore geometries and the diffusion domain approximation for a regular array of disk interfaces in a cubic close-packed arrangement applied in the simulation study are discussed in detail in Appendix E. In this simulation study, the following parameters are applied: potential scan rate of 10 mV s⁻¹, initial and final potential of 0.0 V, switching potential of 1.0 V, and
standard ion transfer potential, $E^0$ of 0.5 V. The details of the simulation parameters are given in Appendix E. Since the geometric properties were of the normalised values, the simulated CVs demonstrate the current in dimensionless form. Hence, to obtain the current in unit of A, the dimensionless current function has to be multiplied by the Saito expression (Equation 1.3.1 in Chapter 1).

Emphasis in this discussion is given to the location of the liquid | liquid interface, the diffusion zone overlap and the pore geometry. Three different situations are considered in the simulation study:

a) The interface is located at the orifice of the pore on the aqueous side of the membrane. The pore is filled with the organic phase (inlaid interface, inset of Figure 5.3.9 (a))

b) The interface is located at the orifice of the pore on the organic side of the membrane. The pore is filled with the aqueous phase (recessed interface, inset of Figure 5.3.9 (b))

c) The interface is located at the centre of the pore. The pore is half-filled with the organic and aqueous phases (recessed interface, inset of Figure 5.3.9 (c))

In situation (a), the transfer of model cations from the aqueous phase to the organic phase exhibited a strong diffusion zone overlap, indicated by the appearance of a peak current (Figure 5.3.9 (a)). The presence of a peak current in the forward scan is due to overlap of diffusion zones between the pores. This results in a strong decrease of the radial diffusion component and an increase of the linear diffusion.

In situation (b) (Figure 5.3.9 (b)), the transfer of model cations from the aqueous phase to the organic phase is made difficult due to the recessed interface. Therefore, the forward scan current is smaller than in Figure 5.3.9 (a). Simulation demonstrated that diffusion zone overlap still occurred even though the diffusion zone interactions do not extend as far into the radial direction as in the case of Figure 5.3.9 (a). This situation is indicated by the
slight decrease of the current on the forward scan, and by emergence of a peak current. During the reverse scan, a peak current is observed, indicating linear diffusion dominance.

In situation (c) (Figure 5.3.9 (c)), both the organic and aqueous phases meet at the centre of the pore. The forward scan current is higher than in Figure 5.3.9 (b), demonstrating that the interface is more accessible for the transferring ions, although it is less accessible compared to Figure 5.3.9 (a). The diffusion zone overlap is higher than in Figure 5.3.9 (b), shown by the more pronounced peak shape. The shape of the CV in the forward and reverse scans is symmetric due to the symmetrical geometry at the location of the interface.
Figure 5.3.9: Simulated voltammograms illustrating the effect of the liquid / liquid interface locations, shown as inset in each figure for a pore within an array. The interface is located at the (a) pore orifice on the aqueous side of the membrane (b) pore orifice on the organic side of the membrane, and (c) centre of the pore channel.
As discussed in Section 5.3.1, the CV shape obtained experimentally showed a combination of steady-state and peak behaviour in the forward scan, while strong peak behaviour was observed on the reverse scan. Comparison of the experimental CV shape (Figure 5.3.1) with the three situations examined by simulation (Figure 5.3.9) does not present a perfect match. It is very likely that the liquid | liquid interface is not located at the pore orifice in either phase since peaks appeared in both the forward and reverse scans. It is most likely that the liquid | liquid interface is located somewhere within the pore channel. Since the experimental peak present on the reverse scan is much stronger than the one on the forward scan, the liquid | liquid interface is most probably located towards the organic phase side, where the tapered part of the pore joins the straight narrow region. Figure 5.3.10 shows simulation results obtained when the liquid | liquid interface is slightly moved from the middle of the pore towards the organic phase side. This small movement provides a better match to the experimental CVs in which strong peak behaviour was observed on the reverse scan, eventhough the peak behaviour in the forward scan diminished to steady-state behaviour when the interface was moved from the middle of the pore towards the organic phase orifice.
In addition, the effect of the pore geometry on the shape of the voltammetric response and the magnitude of the current observed was investigated by comparing the current of the concave wall pore to the current of the straight wall pore (with similar radius as the orifice of the concave pore) (Figure E.2, E.3 and E.4 in Appendix E). In the case where the interface is located at the pore orifice on the aqueous side, both the concave and straight wall pores exhibited identical limiting current magnitudes. However, the concave pore imposes a diffusive resistance towards the ionic flux, hence the CV shifted towards more positive potential. In the case where the interface is located at the pore orifice on the organic side, the shielding effect diminished by approximately 75% the limiting current in straight wall pore. The shielding effect increased with the concave pore geometry, resulting in further current decrease. In the case where the interface is located in the centre of the pore, the limiting current magnitude is somehow between the two discussed situations.
5.4 Conclusion

Cyclic voltammetry of TPrA⁺ ion transfer was performed at the water | 1,6-dichlorohexane interface supported by parylene C PMA, featuring uniform arrays of concave-shaped pores. This organic membrane exhibited good performance in modifying a liquid | liquid interface and was stable under the electrochemical conditions. The membrane/interface system can be polarised and ions transferred electrochemically through the membrane, allowing in situ measurement of ionic flux. The experimental voltammograms appeared to have contributions from both radial and linear diffusion on the forward scan (producing a combination of steady-state and peak behaviour), while a linear diffusion control on the reverse scan (producing peak behaviour). Lower current with incorporation of membrane into the interface system was observed compared to the pure liquid | liquid interface. The membrane porosity determined from voltammetric experiments was higher than the one measured geometrically. A simulation study predicted the liquid | liquid interface is located most probably towards the organic phase side inside the concave wall pore. The results presented show that organic membrane grown by chemical vapour deposition (CVD) process may be used to influence the behaviour of the ITIES. Further improvements in membrane preparation may enable controlled location of the ITIES within the pore structure.
Chapter 6

Electrochemical Detection of Ractopamine at Arrays of Micro-Liquid | Liquid Interfaces

6.1 Introduction

In this chapter, emphasis is placed on the electrochemical behaviour of protonated ractopamine (RacH⁺) at the micro-ITIES array due to the issue of insufficient potential window for the RacH⁺ transfer process. However, RacH⁺ detection at the micro-ITIES array will provide a basis for future studies at nano-ITIES array, which is briefly presented in this chapter. Quantitative methods that involve the detection of RacH⁺ by simple ion transfer at the water | 1,6-dichlorohexane (DCH) micro-interface array are presented, using CV and linear sweep stripping voltammetry (LSSV). Stripping analysis at micro-liquid | liquid interface arrays is appropriate for analyte detection in media such as biological fluids, soil extracts and water [169], thus this technique is examined in this study. This report briefly discussed the oxidation behaviour of ractopamine at the solid | liquid interface too. The thermodynamic parameters for the transfer of ionisable ractopamine are discussed. In addition to the analytical parameters, the influence of the interfering substances, including serum protein, towards RacH⁺ detection are also reported.

Ractopamine (Rac) (Figure 6.1.1) is a phenyl β-ethanolamine, with β-adrenergic agonist properties [219-222]. It is primarily used as a therapeutic
drug for treatment of pulmonary diseases such as asthma in human and veterinary medicine [219, 220]. Unfortunately, this substance is also illegally applied in the livestock industry as a nutrient repartitioning agent. Research shows that β-agonists divert fat deposition to the production of muscle tissues by increasing nitrogen retention, protein synthesis and lipolysis [219, 220, 222-224]. It also improves growth rate and feed conversion when fed to livestock (such as calves, poultry etc.) [221, 222, 225]. Recently, veterinary drug residues have become a public food safety concern where ractopamine-treated animals may pose adverse effects on human health, especially in the cardiovascular and central nervous systems [219, 220, 222, 225, 226]. Thus, it is banned in many countries, including within the European Union and China, although it is approved by the United States’ Food and Drug Administration (U.S. FDA) [225, 227-229]. As a result, rapid, simple and sensitive analytical methods for the detection of ractopamine residues are required.

Figure 6.1.1: Molecular structure of ractopamine

To date, various analytical methods have been reported for the detection of ractopamine, such as immunoassays [222, 223, 230], electrochemical methods [219, 220, 226, 229], gas chromatography-mass spectrometry [224], liquid chromatography tandem mass spectrometry [225, 231] and high performance liquid chromatography [221, 227, 228]. Electrochemical methods offer the advantages of low instrumental cost and fast analysis, and thus may be the preferred methods in ractopamine detection [220]. Despite the fact that many electrochemical methods have been developed, those studies focused on solid | liquid interfaces, using primarily cyclic voltammetry (CV) [219, 220, 229]. The two phenolic groups in ractopamine are easily
oxidised [219, 220, 226]. Differential pulse voltammetry (DPV) has also been employed for the detection of ractopamine [219, 220, 226].

As far as can be determined, no studies have been reported on the electrochemical detection of ionised ractopamine based on transfer across the ITIES. Thus, this study opens up the possibilities for the detection of ractopamine based on charge transfer across micro-ITIES. However, the detection of other drugs at the ITIES via various electrochemical methods has been reported in the literature, namely the anticancer drug daunorubicin at a microporous polyethylene terephthalate (PET) membrane-supported ITIES [34], catamphiphilic drugs at a solvent polymeric membrane [33], and β-blocker drugs (propranolol, timolol and sotalol) at a microporous silicon membrane-supported ITIES [35]. Besides analytical studies, the ability of the ITIES to mimic the drug transfer across biological membranes has offered insight into mechanisms of drug action [34]. Voltammetry at the ITIES has been used to investigate the transfer characteristics of charged drug molecules, for example, the Galvani potential difference $\Delta \phi$ for the ion transfer and the Gibbs energy of transfer, which is directly related [41].

Previous studies by Girault and co-workers [232, 233] have shown that the ITIES is a suitable platform for the determination of the partition coefficient of the ionised species, which in turn defines the drug’s lipophilicity in biological systems [33, 34, 41].

Direct drug detection in physiological matrices, such as blood and blood-derived samples, is important because it offers information regarding circulating levels. Yet, this can be hindered due to drug-protein binding [234]. The drug-protein interaction in blood plays an important role in determining drug transportation, absorption, distribution, metabolism and excretion [235-237]. Serum albumins are present at the highest abundance in blood (ca. 60% of the total albumin) [238, 239], and these proteins exhibit high affinity towards drugs [235-237, 240]. In addition, the pharmacological activity of drugs relates to their free concentration in blood [234]. The measurement of drug-protein interactions has seen the emergence of a number of novel label-
free strategies [241]. In this study, albumin from bovine serum (BSA) is employed due to the fact that human and bovine serum albumins are homologous proteins [238, 242, 243]. BSA is a highly water-soluble globular protein, which has a molar mass of 69,000 amu and a hydrodynamic radius of ca. 3.25 nm [234, 244]. BSA also has a low isoelectric point (pI of 5.4) and high negative net charge at neutral pH [245].

Preliminary results on protonated ractopamine and salbutamol transfer detection at the nano- and micro-ITIES array, respectively, are also presented. The molecular structure of salbutamol (Sal) is presented in Figure 6.1.2.

![Molecular structure of salbutamol](image)

**Figure 6.1.2: Molecular structure of salbutamol**

### 6.2 Experimental methods and materials

#### 6.2.1 Reagents

All reagents used were purchased from Sigma-Aldrich Pty. Ltd., Australia and used without further purification, unless stated otherwise. D-Glucose and sodium chloride (NaCl) were purchased from Ajax Finechem Pty. Ltd., Australia, L-ascorbic acid and potassium phosphate monobasic (KH$_2$PO$_4$) from BDH Laboratory Supplies, Australia, and sodium sulfate (Na$_2$SO$_4$) from Chem-Supply Pty. Ltd, Australia.

The descriptions of the aqueous phase, organic phase and organic reference solutions are as detailed in Section 2.4.1 of Chapter 2. The organic phase
was prepared as a gel at 10 % w/v, and the organogel preparation procedure is as explained in the same section.

Ractopamine hydrochloride and tetrapropylammonium chloride (TPrACl) served as the primary drug and model analyte species studied, respectively. A stock solution of TPrACl was prepared in 10 mM LiCl while the stock solution of ractopamine hydrochloride was prepared in methanol (MeOH) due to its low solubility in water [228]. The stock solution of salbutamol was also prepared in MeOH.

In the study of the oxidation of ractopamine, the supporting electrolyte solution of 0.1 M phosphate buffered saline (PBS) (pH 7.4) was prepared in ultrapure water.

In the interfering substances study, the interferents were prepared in 1 mM PBS solution as the supporting electrolyte, which contained 1 mM phosphate buffer, 0.27 mM potassium chloride (KCl) and 13.7 mM NaCl. D-Glucose, L-ascorbic acid, KCl, NaCl, Na₂SO₄, glycine, urea and uric acid were the interfering substances studied, and the concentration of each of these was fixed at 5.0 mM. The effect of artificial serum matrix [234, 246, 247] on the detection of RacH⁺ was also investigated. The artificial serum used was composed of 1.5 mM KCl, 5.0 mM calcium chloride (CaCl₂), 1.6 mM magnesium chloride (MgCl₂), 4.7 mM D-glucose, 1.0 mM sodium phosphate monobasic (NaH₂PO₄), 1.0 mM KH₂PO₄, 2.5 mM urea and 0.6 mM bovine serum albumin, prepared in ultrapure water.

### 6.2.2 Preparation of micro- and nano-interface arrays

The micropore arrays used for micro-ITIES patterning were 11.09 ± 0.12 μm radius, $r_a$, 30 pores in a hexagonal close-packed arrangement, and with pore centre-to-centre separation, $r_c$, of 18.4 ± 2.1 times the pore radius, $r_a$ (i.e. $r_c = 18.4r_a$) as have been outlined in Section 2.1.1 and depicted in Figures 2.1.4 and 2.1.5 of Chapter 2.
The nanopore arrays used for nano-ITIES patterning were fabricated in 50 nm thick silicon nitride membrane by FIB milling methods, as described in Section 3.2.2 of Chapter 3. The nanopores are $39 \pm 6$ nm in $r_a$, 400 pores in cubic closed-packed arrangement, and with $r_c$ of $20 \pm 3$ times $r_a$ (design (f) in Table 3.3.1 (Chapter 3)). $r_c$ was measured between two adjacent pores in horizontal or vertical direction.

The micropore and nanopore membranes were sealed onto the lower orifice of a cylindrical glass tube as outlined in Section 2.4.2 (Chapter 2). The micropore- and nanopore-supported ITIES assemblies are as outlined in the same section.

### 6.2.3 Experimental procedure at the micro-ITIES array

The electrochemical techniques (CV and LSSV) were performed following the procedures outlined in Section 2.4.5 of Chapter 2. A two-electrode electrochemical cell was employed in this study since the $IR$ drop effect at a micro-ITIES system is not too high as compared to a macro-ITIES system [248]. Both Ag|$|$AgCl electrodes served as reference and counter electrodes in either phase, as outlined in Section 2.4.4.

The electrochemical cells employed in this study can be schematically summarized as follows:

- **Cell 1**
  - Ag|$|$AgCl|$|$x M ractopamine HCl + 10 mM LiCl|$|$W|$|$10 mM BTPPATPBCl$_{DCH}$|$|$10 mM BTPPACl in 10 mM LiCl|$|$AgCl|$|$Ag

- **Cell 2**
  - Ag|$|$AgCl|$|$5 mM interfering substance + 10 mM LiCl|$|$W|$|$10 mM BTPPATPBCl$_{DCH}$|$|$10 mM BTPPACl in 10 mM LiCl|$|$AgCl|$|$Ag

- **Cell 3**
  - Ag|$|$AgCl|$|$x M ractopamine HCl + artificial serum$_w$|$|$W|$|$10 mM BTPPATPBCl$_{DCH}$|$|$10 mM BTPPACl in 10 mM LiCl|$|$AgCl|$|$Ag
where $x$ is the concentration of ractopamine in the aqueous phase. In every electrochemical cell, TPrA$^+$ was spiked into the aqueous phase, normally after the final RacH$^+$ or interferent injection, as a control of the potential axis.

In LSSV, the parameters pre-concentration potential, pre-concentration time and sweep rate were explored to determine the optimum values, and these were implemented in subsequent experiments.

### 6.2.4 Experimental procedure at the solid electrode

A conventional three electrode system was employed. The working electrode was a glassy carbon electrode (GCE) (CH Instruments Inc., Austin, USA), the reference electrode was a commercial Ag | AgCl electrode (CH Instruments Inc., Austin, USA), and the counter electrode was a platinum wire. The surface of the GCE ($d = 3.0$ mm) was freshly polished with 3.0, 1.0 and 0.05 µm alumina slurries, then sonicated in high-purity water for approximately 10 minutes to obtain a clean surface, prior to each scan. CV of 20 to 100 µM ractopamine was recorded from 0.2 to 0.9 V, and the sweep rate, $\nu$ was 50 mV s$^{-1}$. In the sweep rate study, CV of 20 µM ractopamine was recorded while varying $\nu$ between 5 to 100 mV s$^{-1}$.

### 6.3 Results and discussion

#### 6.3.1 CV of RacH$^+$ transfer at the micro-ITIES array

CV profiles of 20 to 100 µM RacH$^+$ at the gelled micro-ITIES arrays (Cell 1) are presented in Figure 6.3.1 (a) and (b), in the form of experimental data and background-subtracted voltammograms, respectively. The $pK_a$ of the amine group in ractopamine is 9.4 [221], so that in the aqueous phase used in this work, 10 mM LiCl (pH ~ 6) [34, 35], the drug is cationic. The CVs show that RacH$^+$ ions, which are initially present in the aqueous phase, are transferred into the gelled organic phase under potential control, on the forward CV sweep. These ions are transferred back from the organic into the aqueous phase, during the reverse scan.
The shape and magnitude of the voltammetric response are highly dependent on factors such as the diffusion regime shape, the position of the ITIES within the pores, the properties of the electrolyte solutions and of the transferring ion species, and the magnitude of the diffusion zone extension (\(\delta\)) [84, 86]. The interfaces employed here were inlaid so that each pore was filled with the organic electrolyte solution and the interface was at the aqueous side of the membrane [84]. Generally, for an inlaid micro-ITIES array, steady-state voltammograms are obtained in the forward CV sweep, indicating establishment of radial diffusion fields, while peak-shaped voltammograms are obtained on the reverse scans, attributed to linear diffusion control.

However, in this study, the CVs on the forward scan show that the foot of the ion transfer wave was at a very positive applied potential difference (0.8 V), close to the upper limit of potential window (Figure 6.3.1 (a)). The current increased steadily with applied potential up to the switching potential, so that a fully-developed steady-state wave shape was not obtained. On the reverse scan, a peak-shaped feature was observed, due to the transfer of RacH\(^+\) ions which had been retained within the micropores. The incorporation of PVC in the organic phase increases the viscosity, and hence slows down rate of diffusion of ions in the organogel [84], so that the diffusion coefficient of a target analyte in the gelled organic phase may be ca. nine-times lower than in the aqueous phase [84].

The background-subtracted currents on the forward and reverse scans (Figure 6.3.1 (b)) increased linearly with RacH\(^+\) concentration in the aqueous phase, with the maximum currents chosen to construct the calibration graphs of the forward scan (Figure 6.3.1 (c)). Theoretically, the current of the forward scan can be modelled by the Saito equation [131] if a steady-state current (radial diffusion control) had been achieved. At the ITIES, the potential window is restricted by the transfer of background electrolyte ions [80]. However, in this study it was found that RacH\(^+\) transfer occurred at a
potential just below the transfer of the background electrolytes and steady-state current for RacH$^+$ transfer was not achieved.

Following the final RacH$^+$ experiment, 100 µM TPrA$^+$ was spiked into the aqueous phase (Figure 6.3.1 (a) inset – top, and (b) inset) as a model ion and a potential axis reference ion [34, 36]. The resulting CV showed a steady-state behaviour on the forward scan, while the reverse scan demonstrated a peak shape, in agreement with previous experiments and computer simulations [84]. This micro-ITIES array thus displayed the expected mass-transport behaviour. The foot of the ion transfer wave, and the half-wave potential were observed at approximately 0.45 V and 0.52 V, respectively, in agreement with previous studies [234]. The formula to calculate the steady-state current (limiting current) for ion transferring from the aqueous to the organic phase is given by the Saito equation modified for the number of micropores in the membrane [131]:

$$I_{ss} = 4zFDCAr_oN_p$$  \hspace{1cm} (Eq. 6.3.1)

where $I_{ss}$ is the steady-state current, $z$, $D$ and $C$ are the charge, diffusion coefficient and bulk concentration, of the transferring ions in the aqueous phase, correspondingly. $F$ is the Faraday constant (96,487 C mol$^{-1}$) and $N_p$ is the number of micropores. The steady-state experimental current was within ca. 10 % of the current obtained from Equation 6.3.1. This may indicate that the interfaces formed at the micropore mouths were not flat, in turn enhancing the radial diffusion to the interfaces so that the current is increased. The steady-state current for radial diffusion to an array of hemispherical micro-ITIES is given by [204, 249]:

$$I_{ss} = 2\pi zFDCAr_oN_p$$  \hspace{1cm} (Eq. 6.3.2)

However, in this case the experimental current was ca. 30 % lower than that calculated from Equation 6.3.2. This indicates that the µITIES were closest to inlaid microinterface behaviour.
As described earlier, the RacH\(^+\) transfer at the gelled micro-ITIES arrays did not demonstrate a fully-developed steady-state wave shape. In order to estimate the steady-state current, Equation 6.3.1 was employed. There is a lack of information in the literature on the diffusion coefficient of ractopamine. Therefore, an assumption of the diffusion coefficient value was made based on the published data for salbutamol sulfate \(D = 3.3 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}\) [250], since ractopamine and salbutamol are a group of \(\beta\)-adrenergic agonist which possess similar chemical structures and functions [229]. Use of this value in Equation 6.3.1 produced a steady-state current for the transfer of RacH\(^+\) across microITIES array that was lower than the maximum current in the forward scan, suggesting the need for an accurate diffusion coefficient value. The diffusion coefficient depends on factors such as chain length (related to molecular weight, MW), branching and polarity [251]. In addition, Malcolm et al. [252] reported a linear relationship between the log of the diffusion coefficient and the drug molecular weight, in silicone medium. In this case, the molecular weight of ractopamine \((\text{C}_{18}\text{H}_{23}\text{NO}_3; \text{MW of 301.38 g mol}^{-1})\) was higher as compared to salbutamol \((\text{C}_{13}\text{H}_{21}\text{NO}_3; \text{MW of 239.31 g mol}^{-1})\), thus its diffusion coefficient was expected to be lower.

A final control experiment was the impact of methanol on the CV, because the ractopamine stock solution was prepared in MeOH (see lower inset in Figure 6.3.1 (a)). The same injection volume used to prepare 20 to 100 \(\mu\)M of analyte was applied. It was observed that the addition of MeOH had no observable effect on the analyte CV.
Figure 6.3.1: Cyclic voltammograms of 20 (light gray) to 100 µM (black) RacH⁺ transfer, in increment of 20 µM (a) and its corresponding background-
subtracted CV (b) at 5 mV s⁻¹ sweep rate. Inset (a – upper) and (b): voltammograms of blank (dotted line) and 100 µM RacH⁺ with (dashed line) and without (solid black line) the addition of TPrA⁺. Inset (a – lower) is a control experiment with 100 µM MeOH (solid gray line) added to the aqueous phase (c) The corresponding calibration curve of the maximum currents (forward scan) and peak currents (reverse scan) against RacH⁺ concentrations. The potentials in (a) and (b) are reported with respect to the experimentally-used reference electrodes

6.3.2 Oxidation response of ractopamine
This study was carried out to determine the diffusion coefficient of ractopamine, and to employ this value in the calculation of the steady-state current for RacH⁺ transfer since the approximation using the diffusion coefficient of salbutamol, as described in Section 6.3.1 was not accurate.

The oxidation of ractopamine was investigated using CV of 20 to 100 µM ractopamine in 0.1 M PBS as the supporting electrolyte (Figure 6.3.2 (a) and (b)). During the anodic sweep from 0.2 to 0.9 V, an oxidation peak was observed at 0.65 V, however no reduction peak was observed on the reverse scan, suggesting an irreversible oxidation of ractopamine [219, 220, 253]. Ractopamine contains two phenolic hydroxyl groups where the oxidation reaction occurred, involving two protons and two electrons [219, 220, 253, 254]. As the concentration increased, the oxidation peak current increased linearly (Figure 6.3.2 (c)). The CV response of GCE in the absence of ractopamine was recorded as a control experiment (shown as inset in Figure 6.3.2 (a)), showing a flat curve demonstrating the oxidation peak observed in Figure 6.3.2 (a) and (b) is caused by ractopamine. In addition, it was observed that ractopamine oxidation peak current decreased significantly during the second anodic scan, thus for the analysis of ractopamine, the oxidation peak current in the first anodic scan was employed. Strong adsorption of the ractopamine oxidation product may contribute to the significant peak current decrease [219]. Previous studies reported a low
ractopamine oxidation peak on GCE, thus the GCE surface was modified to increase the detection sensitivity [219, 220, 226, 254].

The drug’s diffusion coefficient was estimated from the plot of peak current versus concentration, employing the Randles-Sevcik equation [1]:

\[ I_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} C v^{1/2} \]  

(Eq. 6.3.3)

where \( I_p \) is the peak current, \( n \) is the number of electrons transferred, \( A \) is the cross-sectional area of the electrode, and \( v \) is the sweep rate. The value of \( n \) and \( A \) were two and \( 7.07 \times 10^{-6} \text{ m}^2 \), respectively. The slope of peak current versus concentration was employed in the calculation, and substituting all other parameters into Equation 6.3.3, \( D \) is obtained. The diffusion coefficient of ractopamine was estimated \( 1.54 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} \), too high for a drug with molecular weight of 301.38 g mol\(^{-1}\). Several publications have reported the diffusion coefficients of drug molecules, for example salbutamol sulfate \((D = 3.3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1})\) [250], protonated dopamine \((D = 6.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1})\) [46], and daunomycin \((D = 8.8 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1})\) [255], supporting the fact that the experimentally-determined value for ractopamine was too high. Hence, in the case of ractopamine, \( D \) was estimated in the range of \( 10^{-6} \) to \( 10^{-7} \text{ cm}^2 \text{ s}^{-1} \).

Attempts to determine \( D \) from the oxidation response at GCE were not successful, which may be due to the problems associated with fouling of the surface, probably by the adsorption of either the drug or its oxidation products. The oxidation mechanism was not a simple diffusion-controlled process since a pre-wave was observed (at potential of 0.3 to 0.5 V, shown as current increases prior to the oxidation peak) at higher electroactive species concentration (from 60 to 100 µM ractopamine) (Figure 6.3.2 (a) and (b)). Besides, at higher potential (0.7 to 0.9 V) and higher concentration (60 to 100 µM ractopamine), current continued to increased, suggesting an adsorption at the electrode surface which enhanced the observed current. A similar fouling problem was reported previously for salbutamol oxidation at GCE [256].
Figure 6.3.2: Cyclic voltammograms of 20 (light gray) to 100 µM (black) ractopamine oxidation in 0.1 M PBS, in step of 20 µM (a), and the corresponding background-subtracted CV (b). Dotted line represents blank voltammogram ((a) and inset (a)), while arrows indicated increasing analyte concentrations. Sweep rate applied was 50 mV s⁻¹. (c) The corresponding calibration curve of the peak currents (background-subtracted) versus
ractopamine concentrations. The potentials in (a) and (b) are reported relative to the commercial Ag/AgCl reference electrode.

Figure 6.3.3 (a) shows CV of 20 μM ractopamine at different sweep rates (5, 10, 25, 50 and 100 mV s⁻¹). The peak current (background subtracted) was plotted against the square root of sweep rate (Figure 6.3.3 (b)), and the sweep rate (Figure 6.3.3 (c)). The former exhibited a higher linear correlation coefficient of R = 0.9971, as compared to R = 0.9915 for the latter, suggesting a 1-dimensional diffusion-controlled reaction. However, the pre-wave (at potential of 0.2 to 0.5 V, shown as current increases prior to the oxidation peak) observed on the voltammograms at faster sweep rate (and higher electroactive species concentration) suggested that the electrochemical behaviour of ractopamine at the GCE was dominated by an adsorption process.
Figure 6.3.3: (a) Cyclic voltammograms (background-subtracted) of 20 µM ractopamine oxidation in 0.1 M PBS at different sweep rates, 5 (gray), 10, 25, 50 and 100 (black) mV s⁻¹. Plots of the peak currents (background-subtracted) versus the square root of the sweep rate (b), or the sweep rate (c). The potential in (a) is reported relative to the commercial Ag/AgCl reference electrode.

6.3.3 Thermodynamic parameters of RacH⁺ transfer at the water | DCH interface
A number of thermodynamic parameters for the drug transfer were determined from the CV data. These are listed in Table 6.3.1.
Table 6.3.1: Thermodynamic data for the transfer of cationic β-agonist drug, protonated ractopamine (RacH⁺) at the micro- water / DCH interface arrays

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$pK_a$</td>
<td>9.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>$\Delta_u^w \phi^0$ (drug)</td>
<td>~0.29 V (in DCH)</td>
</tr>
<tr>
<td>$\Delta G_{transfer}^{0', w \rightarrow DCH}$</td>
<td>27.88 kJ mol⁻¹</td>
</tr>
<tr>
<td>log $P_{DCH}^{0}$ (ionised)</td>
<td>-4.89</td>
</tr>
<tr>
<td>log $P_{n-oct}^{0}$</td>
<td>2.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Data obtained from reference [221]
<sup>b</sup>: Data obtained from reference [257]

The value of the formal transfer potential of an ionised drug is obtained by transposing the measured experimental value to the Galvani potential scale, as given by the following expression [258-262]:

$$E_{1/2}^{(drug)} - \Delta_u^w \phi^0 (drug) = E_{1/2}^{(TPrA^+)} - \Delta_u^w \phi^0 (TPrA^+) \quad (Eq. 6.3.4)$$

where $\Delta_u^w \phi^0 (TPrA^+)$ is the formal transfer potential of the TPrA⁺ ion. $E_{1/2}^{(drug)}$ and $E_{1/2}^{(TPrA^+)}$ are the experimental half-wave potentials of the drug and TPrA⁺ transfer, respectively. In this study, a value of -0.086 V was used for $\Delta_u^w \phi^0 (TPrA^+)$ [181]. TPrA⁺ ion was selected as a model ion since the transfer potential differs from that of RacH⁺ and there is no mutual interference.

The Gibbs energy of transfer is directly related to the formal potential of the ion transfer [263]. The ability to measure this value depends on the condition that the transferring ion has a lower magnitude of the Gibbs transfer energy than the ions of the supporting electrolytes [262, 264]. However, for RacH⁺, the ion of interest transfers at the upper limit of the potential window so that the experimental measurement of the half-wave potential ($E_{1/2}^{(drug)}$) was not possible. A similar observation was reported by Osborne and Girault [262] for the transfer of ammonium ions across a water | 1,2-dichloroethane (DCE) interface and by Cacote et al. [265] for the direct transfer of Ag⁺ ions.
across a water | DCE interface. A method to determine the \( E_{1/2} \) of the species limiting the potential window was developed by Shao et al. [264], which was based on a set of simulated CVs in which the switching potential is set prior to the forward peak potential. The half-wave potential of the species limiting the potential window was then determined from the resultant working curve, which was a plot of \( I_{eos}/I_{rp} \) versus \( E_{rp} - E_{1/2} \), where \( I_{eos} \) and \( I_{rp} \) are the current at the end of the forward scan and the return peak current, respectively, \( E_{rp} \) is the return peak potential and \( E_{1/2} \) is the half-wave potential. However, the published working curve applied was developed for an ITIES subjected to linear diffusion on both forward and reverse transfer processes and hence is not applicable to the present situation. Our approach was to compare the shape of the experimental CV for RacH\(^+\) transfer to that for TPrA\(^+\) transfer recorded with different switching potentials [52, 266].

Finding a shape for the model ion transfer process which matched to that for RacH\(^+\), by visual comparison, allowed the \( E_{1/2} \) for the ionised drug transfer to be determined by assuming that similar current ratios and potential ratios apply to both ions (i.e. that they have similar transfer kinetics and are not distorted by experimental variables such as cell resistance and capacitance). The \( E_{1/2} \) of the RacH\(^+\) ion was estimated in this way to be 0.9 V. Taking into account the assumption that Walden’s rule \( (D^o/D^w = \eta^w/\eta^o) \) applies to the transfers of RacH\(^+\) and TPrA\(^+\), the Galvani transfer potential of RacH\(^+\) is obtained using Equation 6.3.4 to be 0.29 V (Table 6.3.1) [1, 262]. \( \eta \) represents viscosity.

The formal Gibbs energy of transfer of any ionic species is expressed as [233, 248, 262]:

\[
\Delta^w \phi^0' = \frac{\Delta G_{transfer}^{w \rightarrow o}}{zF} \quad \text{(Eq. 6.3.5)}
\]

where \( \Delta G_{transfer}^{w \rightarrow o} \) is the formal Gibbs transfer energy of ion from the aqueous (w) to the organic (o) phase. The calculated value is presented in Table 6.3.1. The formal Gibbs energy of transfer of RacH\(^+\) was 27.9 kJ mol\(^{-1}\) across
a water | DCH interface. Values of 18.3 kJ mol\(^{-1}\) and 7.7 kJ mol\(^{-1}\) have been reported previously for protonated pyridine (PyH\(^{+}\)) transfer across a water | 2-nitrophenyl octyl ether (NPOE) interface, and protonated quinidine (QH\(^{+}\)) transfer across a water | DCE interface, respectively.

Another important thermodynamic parameter is the partition coefficient (log \(P\)) of a given solute (i.e. drug) between two immiscible solvents. This is a measurement of its relative affinity for the two phases, and is related to the free energy of transfer of the solute between the two solvents [233, 267]. The measurement of the ionised drug partition coefficient is vital since most drugs (>70 %) are ionisable under physiological conditions [248]. In this case, the partition coefficient of RacH\(^{+}\) between water and DCH is obtained from Equation 6.3.6 [34, 248, 263]:

\[
\log P_{\text{DCCH}}(\text{ionised}) = -\frac{\Delta \mu_{\text{transfer}}^{\text{w-o}}}{2RT} \tag{Eq. 6.3.6}
\]

where \(R\) is the gas constant (\(R = 8.31 \text{ J K}^{-1} \text{ mol}^{-1}\)) and \(T\) is the temperature in kelvin. The partition coefficient of the RacH\(^{+}\) was -4.9 (Table 6.3.1), while the value for protonated daunorubicin (DNRH\(^{+}\)) was -8.0 [34], both for the water | DCH system. Comparison of the partition coefficients of the neutral form of both drugs in \(n\)-octanol-water systems shows that the \(\log P_{\text{n-oct}}\) value for daunorubicin was higher than that for ractopamine, meaning that ractopamine is more hydrophilic, since a lower partition coefficient value indicates a more hydrophilic property [34]. A review of the electrochemical investigations made on the transfer of ionisable drugs at ITIES to determine their partition coefficients employing voltammetric methods for water | nitrobenzene (NB), water | NPOE, and water | DCE systems does not include any data for the water | DCH system [41], as this has been introduced only relatively recently [181].

6.3.4 Optimisation of the LSSV parameters for the detection of RacH\(^{+}\)
In order to detect lower concentrations, LSSV was employed, as this entails a pre-concentration step that enhances sensitivity. In the pre-concentration
step, RacH\(^+\) was extracted from the aqueous to the organogel phase under potential control. Then, the pre-concentrated analyte was stripped out of the organogel using a voltammetric scan [35, 169]. The pre-concentration step is important in LSSV as it enables the detection of lower concentrations relative to CV. This stripping analysis method is reported to offer greater sensitivity and lower detection limits, down to nanomolar and parts per billion levels [169]. As a result, the optimisation of LSSV parameters, namely the sweep rate, the pre-concentration potential and the pre-concentration time, was explored, employing Cell 1. Voltammetric scans were conducted in the negative direction, from 1.0 to 0.4 V.

To assess the impact of sweep rate on the results (Figure 6.3.4), the sweep rate value was varied between 5 and 100 mV s\(^{-1}\), using 100 µM RacH\(^+\). The pre-concentration potential was fixed at 1.0 V while the pre-concentration time was set at 60 s. A blank LSSV was recorded in the absence of analyte, so that background-subtracted LSSV could be obtained by subtracting the blank response from that of the analyte. The stripping peak current was found to be linearly-dependent on the square root of the sweep rate, indicating a linear diffusion-controlled organic phase to aqueous phase transfer process [66]. Despite this result, it was found that lower sweep rates produced a more clearly-defined peak shape, consistent with peak distortion by cell resistance and capacitance at the higher sweep rates. Consequently, all subsequent experiments employed a sweep rate of 5 mV s\(^{-1}\) since this sweep rate produced a well-defined peak shape with less sensitivity to these distortions.
Figure 6.3.4: Background-subtracted LSSV of 100 µM RacH⁺ showing the influence of sweep rate of 5 (light gray), 7.5, 10, 15, 20, 25, 50, 75 and 100 mV s⁻¹ (black). The pre-concentration potential and time were fixed at 1.0 V and 60 s, respectively. Inset: calibration curve of peak current versus square root of sweep rate. The potential is reported with respect to the experimentally-used reference electrodes.

The influence of the pre-concentration potential was studied by employing 20 µM RacH⁺ with a 60 s pre-concentration time, while varying the pre-concentration potential from 0.60 to 1.00 V in increment of 0.05 V (Figure 6.3.5). From the inset graph, it can be seen that the stripping peak current only appeared when the pre-concentration potential was higher than 0.85 V. As observed earlier by CV, RacH⁺ transfers at an applied potential very close to upper limit of the potential window. The maximum stripping peak current was observed when the pre-concentration potential was 1.00 V, thus this potential was applied for all subsequent experiments.
Figure 6.3.5: Influence of pre-concentration potentials from 0.6 V (light gray) to 1.0 V (black) in increment of 0.05 V on the LSSV (background-subtracted) of 20 µM RacH⁺. The pre-concentration time and sweep rate were fixed at 60 s and 5 mV s⁻¹, respectively. Inset: graph of current at selected pre-concentration potentials of 0.80, 0.85, 0.90, 0.95 and 1.00 V. The potential is reported with respect to the experimentally-used reference electrodes.

The influence of pre-concentration time was studied at 1.00 V pre-concentration potential using 100 µM RacH⁺, with variations in the pre-concentration time from 15 s to 180 s. Over the time range of 0-1 min, the increment was 15 s, while in the range 1-3 min, the time increment was increased to 30 s (Figure 6.3.6). The stripping peak current increased as the pre-concentration time increased, eventually reaching a constant value, as illustrated in the inset graph. This effect was reported previously for the detection of propranolol [35] and oligopeptides [38] at the micro-ITIES array, and was attributed the diffusion of analyte away from the interface to the bulk organic phase during the longer pre-concentration times. Therefore, during stripping step, these analytes are not stripped back to the aqueous phase and do not contribute to the stripping peak [169]. The optimum pre-concentration time of 120 s was selected and applied in all subsequent experiments.
Figure 6.3.6: Influence of pre-concentration times on the LSSV (background-subtracted) of 100 µM RacH⁺. The pre-concentration potential and sweep rate were fixed at 1.0 V and 5 mV s⁻¹, respectively. LSSV responses of 15 (light gray), 30, 45, 60, 90, 120, 150 and 180 s (black) pre-concentration times. Inset: calibration graph of peak currents versus pre-concentration times. The potential is reported with respect to the experimentally-used reference electrodes

6.3.5 LSSV analysis of RacH⁺ transfer at the micro-ITIES array

Employing all of the optimised parameters (5 mV s⁻¹ sweep rate, 1.00 V pre-concentration potential, 120 s pre-concentration time), low concentrations of RacH⁺ in the range 0.1-1.0 µM were analysed and a calibration graph was plotted of stripping peak current versus concentration of RacH⁺ (Figure 6.3.7 (inset)). The increase in RacH⁺ concentration resulted in an increase of the stripping peak current, with a linear concentration dependence observed within the range studied, $I_p = -0.2718 \text{ (nA \, \mu M}^{-1}\text{) (concentration)} - 0.0974 \text{ (nA)}, R = 0.9964, n = 6)$. The calculated limit of detection (LOD) is 0.1 µM (34 ng mL⁻¹), based on 3 times the standard deviation of the blank ($3s_b$) [176]. The procedure for calculating the LOD is as outlined in Appendix C. This compares well with previously published values. LODs of 1.17 µM (CV and electrochemical impedance spectroscopy at a modified electrode) [268], 0.06 µM (DPV at a carbon nanotube-modified glassy carbon electrode (GCE)) [219], 0.05 µM (DPV at a graphene oxide-modified GCE) [226], and
0.06 µM (DPV at an ordered mesoporous carbon-modified GCE) [220] have been reported for ractopamine.

![Graph showing LSSV scans of increasing RacH⁺ concentrations](image)

**Figure 6.3.7**: Background-subtracted LSSV scans of increasing RacH⁺ concentrations in the aqueous phase (indicated by arrow) at the micro-ITIES arrays at 5 mV s⁻¹ sweep rate. LSSV response of 0.1 (light gray), 0.2, 0.4, 0.6, 0.8 and 1.0 µM (black) drug concentrations. Inset is the calibration curve of stripping peak current versus concentration. The potential is reported with respect to the experimentally-used reference electrodes.

### 6.3.6 Influence of the potentially interfering substances on RacH⁺ detection

Possible interferences in the detection of RacH⁺ were investigated using substances such as a sugar, ascorbic acid, metal ions, an amino acid, urea and uric acid [34, 219, 220, 269]. These substances were selected since they are all models of compounds likely to be present in a biological sample. This study was conducted using CV. The electrochemical cell (setup as in Cell 2) was spiked in the aqueous phase with the individual interference substances at a concentration of 5.0 mM. In addition, TPrA⁺ was spiked to every aqueous test phase as a model ion and a potential axis reference ion. The results obtained are summarised in Figure 6.3.8.
In this study, glucose was chosen as a model of possible sugar interference. Since it is an uncharged molecule that and hence does not provide a current for transfer across the water | DCH interface, it did not impact on the detection of RacH$^+$ since no ion transfer signal was detected (Figure 6.3.8 (a)). The potential interference of ascorbic acid was also investigated, and no transfer of ascorbate was observed at the water | DCH interface (Figure 6.3.8 (b)). Ascorbic acid ($pK_a$ of 4.17 [34]) was de-protonated in the aqueous phase (which had pH in the range of 5 to 6). The possible interference of metal ions such as K$^+$ and Na$^+$ were also evaluated. Metal ions, in particular K$^+$, are expected to transfer at the positive end of the potential window, which results in a decrease of the potential window [34, 270]. In this study, additions of KCl to the aqueous phase (Figure 6.3.8 (c)) did alter the available potential window slightly, in agreement with previous reports [34, 270]. However, NaCl (Figure 6.3.8 (d)) and Na$_2$SO$_4$ (Figure 6.3.8 (e)) did not alter the potential window limit. Glycine was used as a model amino acid interferent and is widely reported in interference studies [34, 219, 220]. At physiological pH, glycine is a net neutral molecule hence it should not show any ion transfer behaviour at the interface (Figure 6.3.8 (f)), as was found and in agreement with another study [34]. Note that although amino acids can be transferred across the ITIES [40], a low pH (pH $\leq$ 1.0) to ensure complete protonation of the amino acid, an ionophore must be used to facilitate the transfer process. The possible interference of urea and uric acid were also investigated (Figure 6.3.8 (g) and (h), respectively). Urea did not show a transfer signal at the interface, since it is a neutral compound [271]; neither was transfer of uric acid observed at the interface investigated.

Based on these observations, the interfering substances investigated were not seen to significantly decrease the available potential window nor to introduce new peaks in the voltammograms. The interferents can be tolerated even at what can be regarded as relatively high concentrations (5.0 mM).
Figure 6.3.8: Electrochemistry of the individual interfering substances at the micro-ITIES arrays. Cyclic voltammograms of the blank aqueous phase solution (10 mM LiCl) (black line), the aqueous phase spiked with the interfering substances (dark gray line), and the aqueous phase spiked with
the interfering substances and 100 µM TPrA⁺ (light gray line). The interfering substances studied: (a) D-glucose (b) L-ascorbic acid (c) KCl (d) NaCl (e) Na₂SO₄ (f) glycine (g) urea and (h) uric acid. The interfering substance concentration in all cases was 5.0 mM, and run at 5 mV s⁻¹ sweep rate. The potentials are reported with respect to the experimentally-used reference electrodes

6.3.7 Determination of RacH⁺ in artificial serum

The determination of RacH⁺ in artificial serum as the aqueous phase electrolyte solution was conducted using Cell 3. In this study, two types of artificial serum solutions were prepared; the first contained no BSA, while the second contained BSA at physiological concentration. These solutions were utilised to investigate the possibility that BSA can impede the detection of RacH⁺, as reported previously for other ionised drugs at the ITIES [234, 244].

Several ways have been identified for BSA to hinder the detection process: potential window shortening, drug-protein binding [234, 272] or protein adsorption at the interface [234, 244]. BSA is a water-soluble globular protein which has a net charge of ~−17 at physiological pH (of 7.4) [245], while RacH⁺ has a charge of +1. These opposite charges contribute to ractopamine-albumin binding, although the binding effect will reduce through shielding from the electrolyte ions. Zhang et al. [272] reported that ractopamine bound to BSA mainly by electrostatic and hydrophobic interactions. The formation of the ractopamine-albumin complex was spontaneous and the strong interactions indicated that the drug has a long residence time in blood plasma. Additionally, BSA may adsorb to the ITIES. Collins et al. reported that BSA diminished the signal of ionised propranolol in artificial serum, although detection was still possible [234]. Vanysek and Sun demonstrated that the transfer of Cs⁺ ion across a water | NB interface was inhibited by the adsorption of BSA on the interface [244]. Plasma proteins such as albumin have been reported to be a major source of interference in plasma measurement at the ITIES [34, 48].
CV of the two artificial serum matrices was carried out with the addition of
100 µM RacH⁺. Prior to that, a background CV was run over a wide potential
range to establish the limits of the available potential window (Figure 6.3.9 (a)
and (b)). Incorporation of BSA in the artificial serum decreased the width of
the potential window to 850 mV, from 950 mV in its absence. Since BSA
shortened the potential window at the upper limit (Figure 6.3.9 (b)), the
detection of ractopamine might be hindered as RacH⁺ transfers at a very
positive applied potential difference, close to the upper limit of the potential
window, in the LiCl solution. It was observed that the RacH⁺ transfer was
possible in artificial serum without BSA. The protonated drug transferred
close to the positive limit of the potential window on the forward scan, while
on the reverse scan, a peak-shaped feature was obtained (Figure 6.3.9 (c)).
A calibration curve in artificial serum solution without BSA was obtained by
plotting the background-subtracted current (both forward and reverse scans)
versus the RacH⁺ concentration. The background-subtracted currents on
both the forward and reverse scans increased proportionally with RacH⁺
concentrations over the range studied (Figure 6.3.9 (c) inset). This is in
agreement with the result obtained in aqueous LiCl electrolyte.

In contrast, no RacH⁺ transfer was observed in artificial serum containing
BSA (0.6 mM). A small current increment was observed on the forward scan,
however no peak-shape feature was observed on the reverse scan (Figure
6.3.9 (d)). Since the current signal varied linearly with the transferring analyte
concentration, these results showed that the concentration of free RacH⁺ in
the aqueous phase was extremely low. This result is in agreement with
previous studies which demonstrated that the presence of 1 mM BSA in
artificial serum decreased the propranolol transfer current signal to ~6.4 nA,
from ~9.7 nA in its absence [234].

In addition, TPrA⁺ was also spiked into each artificial serum solution (Figure
6.3.9 (e) and (f)). No significant shift of the half-wave potential for the TPrA⁺
ion was observed (E_{1/2} of ~0.52 V) [234]. However, the steady-state current
for TPrA⁺ in the presence of BSA decreased from ~12.5 (no BSA present) to
~8.5 nA. A similar observation was also made on the reverse scan, where the peak current decreased from ~12.9 to ~7.9 nA in the absence and presence of BSA, respectively. This indicated that BSA adsorbed at the interface and inhibited ion transfer.

The results presented in this section demonstrate that reduction of the available potential window, complexation of the RacH⁺ by BSA and adsorption of the BSA to the liquid | liquid interface, are the possible interference mechanisms for the detection of ractopamine in serum via ion-transfer voltammetry. Hence, deproteinisation of samples is necessary for this voltammetric analysis to be viable.
Figure 6.3.9: Cyclic voltammetry at the micro-ITIES array of an artificial serum solution in the aqueous phase ((a) and (b)), 100 µM RacH⁺ in an artificial serum solution ((c) and (d)), and an artificial serum solution of 100 µM TPPrA⁺ and 100 µM RacH⁺ ((e) and (f)). Figures (a), (c) and (e) represent cyclic voltammetry of an artificial serum without BSA, while figure (b), (d) and (f) represent cyclic voltammetry in the presence of 0.6 mM BSA. Sweep rate applied was 5 mV s⁻¹. Inset in (c) is the corresponding calibration curve.
obtained for RacH⁺ transfer on the forward and reverse scans. The potentials are reported with respect to the experimentally-used reference electrodes.

6.3.8 CV of RacH⁺ transfer at the nano-ITIES array

The aim of this study is to investigate the possibility of RacH⁺ detection at the nano-ITIES array via CV, and its performance in improving the diffusional mass transport flux.

The CV profile of 100 µM RacH⁺ transfer at the nano-ITIES array (setup as in Cell 1) is presented in Figure 6.3.10. 100 µM TPrA⁺ was spiked into the aqueous phase before RacH⁺ injection, which serves as a model ion and a potential axis reference ion [36]. The resulting CV for RacH⁺ transfer on the forward scan showed a similar behaviour as observed at the micro-ITIES array, in which the current increased gradually with applied potential up to the switching potential. Similar foot of the ion transfer wave potential (0.8 V) was observed in both cases. On the other hand, on the reverse scan, no peak behaviour was observed as at the micro-ITIES array. The organic phase at the nano-ITIES array presents as a liquid where the diffusion coefficient of a target analyte in both the aqueous and organic phases are similar. No peak-shaped feature due to slower diffusion rate of ions in the organic phase was observed, as occurs at the micro-ITIES with gelled organic phase [66]. Also the aspect ratio of the pores (the ratio of the pore radius to the pore depth = \( r_a/l \)) is different, with the nano-ITIES array employed here exhibiting a higher ratio (1.28) as compared to the micro-ITIES array employed throughout this chapter (0.11). Use of relatively deep pores (i.e. a low pore aspect ratio) enhanced the reverse peak current, as observed at the micro-ITIES array discussed in this chapter (Section 6.3.1).

Steady-state voltammetry was observed for the forward and reverse transfers of model ion TPrA⁺ from the aqueous to the organic phase and vice versa, respectively. However, no true steady-state (or limiting) current plateau was reached in the diffusion-limited region and the current rose steadily with applied potential up to the switching potential, in agreement with the previous
study by Rimboud et al. for TEA⁺ transfer at nano-ITIES array confined within silicon nitride (Si₃N₄) membrane [109]. This phenomenon might be influenced by background electrolyte ion transfer at higher potential [109], reversible expansion of the interface during the ion transfer process [178] or artefacts introduced by the background subtraction procedure employed. Thus, the experimental limiting current was determined at a potential ca. 200 mV positive of the foot of ion transfer wave [109]. The foot of the ion transfer wave, and the half-wave potential were respectively approximated at 0.45 V and 0.56 V, within the range observed at the micro-ITIES array. An assumption that the interfaces were inlaid (liquid organic phase filled the Si₃N₄ nanopores) was made, thus the Saito equation (Equation 6.3.1) was employed to calculate the limiting current. The experimental limiting current was 0.16 nA, ca. 65 % lower of the calculated current (0.45 nA), primarily due to diffusion zone overlap as reported previously [67, 109].

Figure 6.3.10: Cyclic voltammograms of 100 µM RacH⁺ transfer spiked after TPrA⁺ injection at the nano-ITIES arrays. Dashed, solid and dotted lines represent the blank aqueous phase solution (a), 100 µM TPrA⁺ (b), and 100 µM TPrA⁺ and 100 µM RacH⁺ (c), respectively. Inset: The forward scan voltammogram of 100 µM RacH⁺ transfer only (d) attained by subtracting the CV of TPrA⁺ (b) from the CV of TPrA⁺ and RacH⁺ mixture (c). Sweep rate employed was 5 mV s⁻¹. The potential is reported with respect to the experimentally-used reference electrodes.
Miniaturisation of the liquid | liquid interfaces from a micro-scale ITIES to a nano-scale ITIES significantly reduce the interfacial area, hence a lower signal in the form of the current, \( i \) is obtained [22, 67, 80]. However, reducing the size of the ITIES increases the flux of diffusional mass transport, thus giving a greater current density, \( j \). The current density for the array is defined by the ratio of the current, \( I \) (A) and the total geometric interfacial area, \( A \) (m\(^2\)). The normalisation of the current to the electrified interfacial area allows direct comparison of responses from interfaces of different length scales [109]. The limiting current determined from the forward scan of CV was employed to calculate the respective current density. Comparison of the current density signal (background-subtracted; maximum current density at the end of scan) generated at the micro- and nano-ITIES arrays, showed a 5-times higher signal for the latter than the former, respectively (Figure 6.3.11 (a) and (b)). A previous study reported that the current density for ion transfer voltammetry increased with decreasing pore size for pore radii in the range of 25 to 230 nm [67]. The current density improvement with this nanointerface array detection system serves as a basis for sensitivity and detection limit measurement, which remains part of future investigations.
Figure 6.3.11: (a) The plot of current density versus potential, and (b) the corresponding background-subtracted signal (forward scan), comparing signal generated for 100 µM RacH⁺ transfer at the micro- and nano-ITIES arrays. Solid and dotted lines represent the micro- and nano-ITIES, respectively. The current density at the nano-ITIES array was adjusted to 0 at the y-axis since the background-subtracted signal was higher than 0, hence direct comparison of signal generated at the micro- and nano-ITIES could be obtained. The potentials are reported with respect to the experimentally-used reference electrodes.

6.3.9 CV of protonated salbutamol at the micro-ITIES array
This study was carried out to investigate the possibility of protonated salbutamol (SalH⁺) detection by simple ion transfer at the micro-ITIES array via CV. Cyclic voltammograms of 20 to 100 µM SalH⁺ transfer across the
micro-water | organogel interface array (setup as in Cell 1 with salbutamol replacing ractopamine) are presented in Figure 6.3.12 (a) and (b). The resulting voltammograms for SalH⁺ transfer on the forward and reverse scans exhibited similar behaviour as observed for RacH⁺ ion transfer at the micro- and nano-ITIES array presented in this chapter. On the forward scan, the foot of the ion transfer wave was at a potential just below the transfer of the background electrolytes (0.85 V), slightly higher than that observed for RacH⁺ (0.80 V). The voltammograms on the reverse scan showed a peak-shaped feature, as discussed earlier in Section 6.3.1 for RacH⁺ ion transfer at the micro-ITIES array. The background-subtracted currents on the forward (the maximum currents at the end of scan) and reverse (peak) scans increased linearly with SalH⁺ concentration in the aqueous phase, as depicted in inset of Figure 6.3.12 (b). However, the magnitude of current detected was lower than that of RacH⁺, hence the investigation in this chapter focused on ractopamine signal detection.
Figure 6.3.12: Cyclic voltammograms of 20 (light gray) to 100 µM (black) \( \text{SalH}^+ \) transfer, in increment of 20 µM (a) and its corresponding background-subtracted CV (reverse scan only) (b) at 5 mV s\(^{-1}\) sweep rate. Inset (b): The corresponding calibration curve of the maximum currents (forward scan) and peak currents (reverse scan) against \( \text{SalH}^+ \) concentrations. The potentials are reported with respect to the experimentally-used reference electrodes.

### 6.4 Conclusion

The voltammetric behaviour of the \( \beta \)-agonist drug, protonated ractopamine, at a water | DCH micro-interface array was investigated. The results show that protonated ractopamine can be detected via CV and LSSV. However, this drug transfers at a very positive potential, close to the positive limit of the available potential window; nevertheless, estimation of the half-wave potential enabled determination of some thermodynamic parameters for this
drug, such as the Gibbs energy of transfer and the partition coefficient. As a comparison, an irreversible oxidation of ractopamine was observed at the solid | liquid interface. In addition, a stripping voltammetry approach was employed successfully to detect lower concentrations of the drug. The LOD was calculated to be 0.1 µM, which is suitable for applications to drug detection in real samples. A range of potentially interfering substances was found not to alter the potential window nor to introduce new ion-transfer processes. However, it was found that BSA diminished the ractopamine signal via potential window reduction, drug-protein complexation and protein adsorption at the ITIES. In addition, RacH⁺ and SalH⁺ transfer at the nano- and micro-ITIES arrays, respectively, was feasible. The results presented here indicate that voltammetry at the micro-ITIES array can be used for detection and characterisation of ionised drugs that transfer at high applied potentials but that analysis of biological samples will require deproteinisation. In the future, an improved performance, such as a lower detection limit, might be achieved with an electrochemical cell that provides a wider potential window, which will enable a more efficient pre-concentration reaction to be implemented. Future investigations may also focus on nanointerface arrays which may serve as a platform for improved sensitivity and detection limits.
Chapter 7

General Conclusions and Suggestions for Future Studies

7.1 General conclusions
The aim of this thesis was to explore the electrochemical performances of nano- and micro-scale ITIES localised within nano- and micro-porous membranes (with a focus on the nanopore array membrane). Such ITIES may serve as a platform for new sensor technologies.

The majority of work presented in this thesis is based on electrochemical processes of model ion (TPrA\(^+\)) or drug molecule (RaCH\(^+\)) at the polarised interface between two immiscible electrolyte solutions. The two categories of ITIES investigated in this thesis were: (a) an array of nano-scale ITIES confined within pores in silicon nitride membranes, and (b) an array of submicro-/micro-scale ITIES confined within the pores of parylene C, and pores in silicon membranes. In this study, water | 1,6-dichlorohexane was generally the polarised liquid | liquid interface investigated. The organic phase in the submicro-/micro-scale ITIES of parylene C and Si membranes was partially solidified with incorporation of low molecular weight PVC, 1 and 10 (% w/v), respectively, to increase the mechanical stability of the interface. Various electrochemical techniques, namely cyclic voltammetry, linear sweep stripping voltammetry, and chronoamperometry were used throughout this thesis.
For the first time, FIB-milled nanopores were used to form nanoITIES. FIB milling was successful in the direct preparation of single and array nanopore membranes in 50 nm thick SiN membranes. Pore radii, \( r_a \), in the range of 30 to 80 nm, and pore centre-to-centre separation, \( r_c \), to pore radius ratios \( (r_c/r_a) \) of 16 to 32 were achieved. Electrochemical characterisation of the single and array nano-scale ITIES confined within these SiN membranes by CV of TPrA\(^+\) ion transfer was achieved. The forward scan voltammogram was best approximated as ‘sloping steady-state’ behaviour since no steady-state current plateau was reached in the diffusion-limited region. Radial diffusion zone overlap was observed as the primary reason for the lower experimental current as compared to the calculated current in the array of nanopores. Importantly, the single nanoITIES current was in excellent agreement with calculated current for an inlaid disc.

Three EBL-fabricated nanopore array membranes (\( r_a \) of 75, 50 and 17 nm, and 400 pores, \( N_p \) each) were electrochemically characterised by the voltammetric and amperometric transfer of TPrA\(^+\) ions, and has generated important information on resistance, capacitance, charging time and response time. The nanoITIES arrays exhibited prolonged charging times, in the range of 0.08 s to 0.46 s, with the smallest pore configuration giving the longest time. The uncompensated resistance dominated this response as compared to the overall system capacitance. The experimental steady-state currents were 30 - 50 % lower than of the calculated inlaid disc model currents, demonstrating diffusion zone overlap. Slow response times of 6 ± 1 s indicated the impact of nanopore resistance and membrane capacitance.

CV was successfully used to characterise an array of uniform submicro- /micro-scale ITIES formed within the concave-shape pores of organic parylene C membranes. The voltammetric response in the forward scan indicated a contribution of radial and linear diffusion control, while the reverse scan exhibited linear diffusion control. A simulation analysis provided by Dr. Jorg Strutwolf supported this and predicted the liquid | liquid interface location was inside the pore, most probably towards the organic phase side. This
organic membrane exhibited good performance in modifying the liquid | liquid interface and was stable under the electrochemical conditions employed.

The final part of the thesis demonstrated that the electrochemical detection of protonated ractopamine drug (RacH⁺) could be achieved by means of CV and LSSV at the micro-scale ITIES. RacH⁺ transferred close to the upper limit of the potential window so that the ion transfer wave shape was not achieved. The determination of drug’s thermodynamic parameters was possible via CV based on estimation of its $E_{1/2}$. LSSV with optimised conditions enabled a limit of detection of 0.1 µM to be achieved. The potential window was not interfered significantly by substances such as a sugar, ascorbic acid, metals, an amino acid, urea and uric acid. Serum protein present in an artificial serum solution impeded the ractopamine detection signal via potential window reduction, drug-protein complexation and adsorption at the ITIES, hence a deproteinisation step is suggested before bio-sample analyses.

The microscale ITIES offers many advantages, and these can be expected to be achieved to a greater extent with nanoscale ITIES. Reducing the size of the ITIES enhanced the diffusional mass transport rate and decreased charging current, which in turn enhanced the sensitivity of the analytical response. Use of nano-ITIES should enable nano-spatial resolution in SECM where micro-ITIES has had a certain degree of success, for example, imaging and activity measurements of biological function at the individual molecule level. In addition, the extreme smallness of the nano-ITIES allows more nano-ITIES to be constructed within a given area of a sensor system which is beneficial to amplify the electroanalytical current signal. However, fabrication and handling then become challenges in the nano-ITIES development [85].

The results presented throughout this thesis illustrate the good possibilities of employing electrochemistry at nano- and submicro-/micro-scale ITIES formed within inorganic and organic membranes for chemical sensing and detection.
7.2 Suggestions for future studies

Future work on the preparation of nano-scale pores (in single nanometre scale) via direct drilling by transmission electron microscopy (TEM) is suggested. It may be beneficial to investigate the three dimensional structure of the nanopores utilising TEM tomography, since the TEM-drilled nanopores prepared in this study (as reported in Appendix D) exhibited poor reproducibility when characterised electrochemically. Incomplete pore drilling might be one of the factors leading to the poor electrochemical behaviour.

Another interesting avenue to explore is to fabricate nanopores with greater $r_c/r_a$ ratios (of 30, 40, 50 or 60) to see if the diffusion zone overlap is reduced. In this PhD study, the maximum $r_c/r_a$ ratio that has been implemented was $\sim 30$.

In the electrochemical analysis of a drug substance discussed in this thesis, the available potential window range was not sufficient for efficient protonated ractopamine transfer. The issue of insufficient width of the polarisation window of a liquid | liquid interface may be solved by systematically investigating different electrolyte and solvent combinations in both phases.

A wide range of the potential window is best provided by highly hydrophobic organic ions, among which are well established ions such as bis(triphenylphosphoranylidene)ammonium and tetrakis(pentafluorophenyl)borate [273]. The choice of inorganic electrolyte for the aqueous phase, on the other hand, should be based on maximising the hydrophilicity. It may be useful to study the bulky organic electrolyte bis(triphenylphosphoranylidene)ammonium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, and inorganic electrolyte, LiF, which possess high free energies of transfer between the organic and aqueous phases [68].
In addition, the potential window limit can be increased by changing the solvent [270, 274], which greatly affect the solvation energy of an ion. However, the organic solvent must be immiscible with water and must dissolve organic salts, excluding very non-polar solvents, namely alkanes since they will not dissolve organic salts. Mixed solvents of 1,2-dichloroethane (1,2-DCE) and cyclohexane was reported to increase by 30% the potential window provided by 1,2-DCE alone [68]. On the other hand, \( \alpha,\alpha,\alpha \)-trifluorotoluene (TFT) has been reported as an alternative solvent to replace 1,2-DCE and nitrobenzene (NB) since it offers a large potential window and is less toxic [275]. However, the potential window range that TFT offers is approximately similar to the one produced by 1,6-DCH employed in this study. Hence, future work is suggested to find alternative organic solvents and solvent mixtures which can maximise the size of the ITIES polarisation window. Once the potential window limit has been maximised, further work on ractopamine detection at the nanoITIES array is proposed to improve the sensitivity and detection limit.

In this thesis, cyclic voltammetry and Equations 3.3.5 to 3.3.7 were employed to estimate the resistance and capacitance of the nanopore membrane systems. Further study to develop a better understanding of the electrical properties of the nano-scale liquid|liquid interface arrays as well as verification system, with emphasis on the system resistance and capacitance, is suggested to be conducted using electrochemical impedance spectroscopy (EIS).
References


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Appendix A

List of chemicals

- Chemicals used in this study are classified in alphabetical order and listed in Table A.1
- All aqueous phase solutions were prepared in ultrapure water with a resistivity of 18 MΩ cm from Milli-Q water purification system (Millipore Pty. Ltd., North Ryde, NSW, Australia)

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<th>Mol. wt. (g mol(^{-1}))</th>
<th>Purity / Comment</th>
<th>Product number</th>
<th>Brand</th>
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<td>Aldrich</td>
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<td>Bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl) borate</td>
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<td>996.00</td>
<td>Metathesis reaction</td>
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<td>Chloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetrapropylammonium</td>
<td>TPrACl</td>
<td>221.81</td>
<td>98.0 %</td>
<td>438243</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
<td>60.06</td>
<td>Meets USP testing specifications</td>
<td>U4884</td>
<td>Sigma</td>
</tr>
<tr>
<td>Uric acid</td>
<td>-</td>
<td>168.11</td>
<td>≥ 99.0 %</td>
<td>U2625</td>
<td>Sigma</td>
</tr>
</tbody>
</table>
Appendix B

Preparation of the organic electrolyte salt: (BTPPA)(TPBCl)

Chemicals:
Potassium tetrakis(4-chlorophenylborate), (K)(TPBCl)
Bis(triphenylphosphoranylidene)ammonium chloride, (BTPPA)(Cl)
H₂O:MeOH solution (1:2 v/v)
Acetone solution
Milli-Q water

Metathesis reaction:

\[(\text{BTPPA}^+)(\text{Cl}^-) + (\text{K}^+)(\text{TPBCl}^-) \rightarrow (\text{BTPPA}^+)(\text{TPBCl}^-) + (\text{K}^+)(\text{Cl}^-) \]  \hspace{1cm} (Eq. B.1)

The reaction between (BTPPA⁺)(Cl⁻) and (K⁺)(TPBCl⁻) is 1:1, i.e. 1 mole (BTPPA⁺)(Cl⁻): 1 mole (K⁺)(TPBCl⁻)

Procedure:

a) To produce a final product of 1.0 g of (BTPPA)(TPBCl), 498.6 mg of (K)(TPBCl) was dissolved in 20 mL of H₂O:MeOH (1:2 v/v) solution in Erlenmeyer flask

b) 577.0 mg of (BTPPA)(Cl) was also dissolved in 10 mL of H₂O:MeOH (1:2 v/v) solution in Erlenmeyer flask and added drop-wise to the solution in (a)

c) The formed solution is then filtered under vacuum using a Buchner funnel. KCl solution separated in the flask was removed, while the (BTPPA)(TPBCl) precipitate in the filtering cylinder was collected for the next procedure
d) The product was re-crystallized by its dissolution in acetone. The acetone was then filtered under vacuum into Buchner funnel, then transferred into round-bottom flask.

e) The round-bottom flask containing the electrolyte was covered with Parafilm®, holes were pierced in the Parafilm®, and the container placed in the fume hood to allow the acetone to evaporate (generally 1 day).

f) The resulting precipitate was washed with Milli-Q water, and filtered under vacuum using Buchner funnel.

g) The final pure (BTPPA)(TPBCl) product must be stored in a capped vial covered in aluminium foil to prevent exposure to light.
Appendix C

Procedure for calculating the limit of detection (LOD)

In analytical chemistry, the limit of detection (LOD) [176] is the analyte concentration which gives a signal (in this case is current response) equal to the blank signal, $y_B$, plus three standard deviations of the blank, $S_B$.

$$y_{LOD} = y_B + 3S_B$$  \hspace{1cm} (Eq. C.1)

where $y_{LOD}$ is the current signal equivalent to the current signal of the blank plus three times the deviation of the blank.

An assumption that each point on the calibration graph (comprising the point representing the blank or background) has a normally distributed variation (in the y-direction only) with a standard deviation of $S_{y/x}$, thus $S_{y/x}$ replace $S_B$.

The value of the calculated intercept, $a$, can be used as an estimate of $y_B$. As a result, the equation for $y_{LOD}$ can be rewritten with the new equation given by Equation C.2. LOD now can be calculated from the line of best fit in Equation C.3, or read off the graph in Figure C.1.

$$y_{LOD} = a + 3S_{y/x}$$ \hspace{1cm} (Eq. C.2)

$$LOD = \frac{y_{LOD} - a}{b}$$ \hspace{1cm} (Eq. C.3)
Figure C.1: Calibration plot of analyte concentration (x-axis) versus current response (y-axis) for the determination of the limit of detection

\[ Y_{LOD} = 3S_b + y_0 \]

Fitted linear regression, \( R = 0.9992 \)

\[ y_0 = a \]
\[ S_b = S_{y/x} \]
Appendix D

Preparation of nanopores by transmission electron microscopy (TEM) and the characterisation

D.1 Introduction
The study on transfer of molecules such as DNA and protein in biological nanopore membranes has triggered interest in the development of solid state nanopores in insulating membranes, which also demonstrate more advantages than the biological membranes. Direct drilling by high-energy focused electron beams of transmission electron microscopy (TEM) [147, 153, 154, 162] has been reported in the fabrication of solid-state nanodimension pores in insulating membranes. Several studies have reported the possibility of using TEM-drilled nanopore membranes for DNA detection [276, 277], however, these nanopore membranes have not been investigated as the basis for nano-ITIES formation and nanoelectrochemical sensing, which serve as platform for this study.

In this study, the preparation of single and low-number nanopore arrays in the Si₃N₄ membranes via TEM drilling and the physical characterisation work is presented. Electrochemical characterisation study of selected nanopore membrane is briefly discussed.

D.2 Experimental

D.2.1 Materials and reagents
The membranes for pore preparation work, DuraSiN™ films, were purchased from Electron Microscopy Sciences, Pennsylvania, USA. These Si₃N₄
membranes were of 50, 100 and 200 nm thickness films, supported on 2.65 mm × 2.65 mm × 300 μm (length × width × height) silicon frames. The reagents for electrochemical characterisation study are as described in Section 3.2.1 of Chapter 3.

D.2.2 Nanopores preparation by TEM drilling

The DuraSiN™ membranes for TEM-drilled process were placed within a field emission gun transmission electron microscopy (FEGTEM) by securing the membranes on TEM sample holder. The membranes were not coated with a conductive layer, a typical sample preparation technique as the membranes being scheduled for electrochemical characterisation study, following the nanopore preparation work.

Single and low-number nanopore arrays were drilled in the 500 μm × 500 μm Si₃N₄ film window using JEOL 3000F FEGTEM (JEOL Ltd., Tokyo, Japan) available at the Centre for Microscopy Characterisation and Analysis (CMCA), The University of Western Australia (UWA), Australia. Prof. Dr. Martin Saunders (UWA, Australia) provided assistance in the TEM-drilled nanopore membranes preparation process. In JEOL 3000F instrument, the field emission gun provides the main source of electrons with an electron beam acceleration voltage up to 300 kV, where the highest acceleration voltage is employed in this study. In the nanopore formation process, convergent beam diffraction (CBD) mode with an electron beam probe/spot size of 2.4 nm (the biggest spot size) and a beam divergence angle, α of 9 (the biggest α), were implemented. Prior to the drilling process, the membrane is allowed to stabilize for 5 minutes to minimise the effect of drifting which could be checked using Fast Fourier Transform (FFT) analysis.

The physical characteristics of the prepared nanopore membranes were attained from TEM imaging. Nanopore geometry measurement from the transmission electron micrographs were conducted utilising ImageJ software (National Institutes of Health, Maryland, USA).
D.2.3 Preparation of nanopore-supported ITIES
The single and array nano-ITIES were formed at a water | 1,6-dichlorohexane (DCH) interface utilising the 50, 100 and 200 nm thick Si₃N₄ membranes. The fixation of membrane onto borosilicate glass tube, cell set up and electrochemical cell notation are as explained in Section 3.2.4 of Chapter 3.

D.2.4 Experimental procedure
The electrochemical technique, cyclic voltammetry was applied using an Autolab PGSTAT 302N (Metrohm Autolab B. V., Utrecht, The Netherlands) running the Nova software. Background voltammogram was run over the available potential window prior the injection of TPrA⁺ solution into the aqueous phase. The voltammetric sweep rate implemented was 5 mV s⁻¹.

D.3 Results and discussion

D.3.1 Single and array nanopore patterned by direct electron beam writing of TEM and the physical characterisation
In this study, high-energy focused electron beams of TEM was utilised in the preparation of single and array nanometre-sized pores in the 50, 100 and 200 nm thick Si₃N₄ membranes. Nanopores with radii, 𝑟_{avg}, in the range of 1.1 to 15.3 nm were directly drilled in the 500 µm × 500 µm Si₃N₄ film window. The smallest nanopore radius prepared in this study, is in agreement with previously reported values, in the range of 1 to 4 nm radius for Si₃N₄ and SiO₂ membranes [141, 153, 154]. Nanopores with radius in the range of 2 to 3 nm can be utilised to study the characterisation of DNA molecules [147].

In the nanopore formation process, the electron beam was tightly focused on the Si₃N₄ film area, later the high intensity of the electron beam breaks the covalent bonds result in sputtering of the Si and N atoms into the vacuum [141, 147, 153, 154]. Since the pore formation time can differ significantly depending on the beam parameters, where higher acceleration voltage and higher intensity of the electron beam results in faster drilling time [154], therefore the highest acceleration voltage produced by this JEOL 3000F
FEGTEM instrument is employed in this study. Krapf et al. [154] reported that for a 300 kV beam (at $10^9$ e nm$^{-2}$ s$^{-1}$), the pore formation time was less than 10 s, while 5 minutes drilling time was reported for a 200 kV beam (at $10^8$ e nm$^{-2}$ s$^{-1}$).

The sputtering process can occur from both membrane sides. Due to the intensity dissemination around the central intense point, the sputtering is angled, $\theta$, on both sides of the membrane which depends on the membrane thickness. An ‘hour-glass’ shape pore is created as sputtering continues, where the narrow part of this shape determine the width/size [147]. Kim et al. [147] in their study utilising 50 nm thick Si$_3$N$_4$ membrane observed the narrowest width of the nanopore is positioned $22 \pm 6$ nm above the bottom plane of the membrane. The narrow shape of the ‘hour-glass’ can flatten, producing ‘cylindrical’ shape pore, as a consequence of further expansion.

Figure D.1 represents the transmission electron micrographs of the prepared nanopore membranes (selected) of ca. 4.07 to 9.37 nm pore radii. Figure D.1 (a), (c) and (e) represent single nanopore membranes, while Figure D.1 (b), (d) and (f) represent array nanopore membrane comprised $2 \times 2$ pore arrays. Drifting tails at pore edges were observed in all the designs. Nanopores formed via direct TEM-drilled process exhibited lack reproducibility of the pore size and shape, besides the time consuming procedure (will be apparent in the discussion of the later section). As a consequence, only single or low-number nanopore arrays ($2 \times 2$, and $3 \times 3$ arrays) membranes were prepared. The lack of pore precision was partly due to lack understanding of the pore formation mechanisms, and the geometries have not been fully characterised nor controlled [141, 146, 147].
Figure D.1: Transmission electron micrographs of nanopores drilled in 50 nm (a and b), 100 nm (c and d), and 200 nm (e and f) thick Si$_3$N$_4$ films. (a), (c) and (e) represent single nanopore membranes, while (b), (d) and (f) represent low-number nanopore array membranes, in this case $2 \times 2$ arrays.

The physical characteristics of the single and array nanopore membranes prepared by direct drilling of TEM are tabulated in Table D.1. Since the shape of pore formed was triangular, an assumption was made that the triangular area is equivalent to a circular area hence the radius could be approximated. Design (f) membrane featured the expected cubic close-packed (CCP) arrangement as expected hence the measurement of the ratio between the pore-to-pore separation, $r_c$ and the pore radius, $r_a$ ($r_c/r_a$ ratio) was accurate, as compared to design (b) and (d) membranes. The commonly used
approximation to avoid diffusion zones overlapping of neighbouring nanopores is $r_c/r_a > 20$ as formulated by Fletcher and Horne [86, 137] which will be beneficial in analysing the shape and magnitude of the voltammetric responses in electrochemical characterisation study.

Table D.1: Characteristics of the six nanopore array membranes prepared utilising direct drilling by high-energy focused electron beams of TEM

<table>
<thead>
<tr>
<th>Design</th>
<th>Film thickness (nm)</th>
<th>Number of pores, $N_p$</th>
<th>Pore radius, $r_a$ (nm)</th>
<th>Pore-to-pore separation, $r_c$ (nm)</th>
<th>$r_c/r_a$ $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>50</td>
<td>1</td>
<td>5.41 ± 0.08</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>b</td>
<td>50</td>
<td>4</td>
<td>4.07 ± 1.16</td>
<td>250.54 ± 98.32</td>
<td>62 ± 30</td>
</tr>
<tr>
<td>c</td>
<td>100</td>
<td>1</td>
<td>5.06 ± 0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>d</td>
<td>100</td>
<td>4</td>
<td>7.39 ± 2.26</td>
<td>354.19 ± 216.01</td>
<td>48 ± 33</td>
</tr>
<tr>
<td>e</td>
<td>200</td>
<td>1</td>
<td>6.63 ± 0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>f</td>
<td>200</td>
<td>4</td>
<td>9.37 ± 0.61</td>
<td>250.78 ± 51.11</td>
<td>27 ± 6</td>
</tr>
</tbody>
</table>

$a$ Pore radius (for each individual pore), and pore centre-to-centre separation are average values over 3 and 6 measurements, respectively

$b$ The standard deviation of $r_c/r_a$ ratio was calculated using the method of propagation of random errors [176]

Comparisons of the live fast Fourier transform (FFT) images of Si$_3$N$_4$ membranes without (left) and with (right) TEM-drilled pore are presented in Figure D.2.
Figure D.2: Live fast Fourier transform (FFT) of 200 nm thick Si$_3$N$_4$ film without (left) and with (right) TEM-drilled pore. In this case, the pore is 9.12 nm in radius (design (f) in Table D.1, pore is positioned at top left of the micrograph)

D.3.2 Influence of the Si$_3$N$_4$ film thickness on drilling time via TEM

The pore formation time varied between 3 to 18 min depending on the Si$_3$N$_4$ film thickness (50, 100 or 200 nm) (as shown in Figure D.3), where thicker membrane requires longer drilling time [147]. The 50 nm and 100 nm thick films, required approximately 3.5 min and 9.1 min drilling time per pore, respectively, in agreement with previous reports [147, 153]. Individual pore drilling times for nanopore in array were 7 to 22 % less than that of single nanopore membrane. TEM-drilled process is time consuming as compared to FIB-milled process where individual pore milling by FIB only requires 0.5 (as discussed in Section 3.3.1 of Chapter 3).
Figure D.3: Nanopore formation times for a range of $\text{Si}_3\text{N}_4$ film thickness (50 to 200 nm) and two types of nanopore array. S represents single nanopore while M represents multiple nanopores in array.

D.3.3 Electrochemical characterisation of selected nanopore membrane

Electrochemical characterisation study of selected nanopore membrane (design (d) in Table D.1) was conducted employing cyclic voltammetry (CV) of TPrA$^+$ ion transfer across nano-water | DCH interface array. TPrA$^+$ ion concentrations were varied between 1.0 and 3.0 mM in increment of 1.0 mM, in a background of 10 mM LiCl solution. CV of the background electrolyte solution was recorded prior to addition of the analyte hence background-subtracted voltammogram could be attained.

CV in Figure D.4 corresponds to the transfer of TPrA$^+$ ion from the aqueous to the organic phase. This CV showed the current rose steadily with applied potential up to the switching potential, in agreement with previous study [109]. Since no steady-state (or limiting) current plateau was reached in the diffusion-limited region, the experimental limiting current was determined at a potential ca. 200 mV positive of the foot of ion transfer wave [109]. An
assumption was made that the interfaces formed were inlaid [109]. Comparison of the experimental and theoretical currents exhibited the earlier value was 60% lower than the calculated inlaid disc model current, given by the Saito equation [131].

Due to the pore arrangement as in Figure D.1 (d), diffusion zone overlapping is expected to occur between the two top and the two bottom pores as their pore-to-pore separation, $r_c$, was less than 20 times the pore radius, $r_a$, resulting in lower experimental current. This is in agreement with earlier studies [109].

![Figure D.4](image)

**Figure D.4:** (a) CVs of blank and analyte (3.0 mM TPrACl) represented by solid and dotted lines, respectively, and (b) the corresponding background-subtracted scan at 5 mV s$^{-1}$ sweep rate. Nano-interface arrays were formed by 2 × 2 pore arrays (design (d) in Table D.1) prepared by FEGTEM. The potentials are reported with respect to the experimentally-used reference electrodes.

### D.4 Conclusions

The preparation of nano-scale pore membranes via direct drilling by high-energy focused electron beams of TEM, and the physical and electrochemical characterisation studies have been performed. Single and array nanopore membranes with pore radii, $r_a$, in the range of 1.1 to 15.3 nm have been prepared in the 50, 100 and 200 nm thick Si$_3$N$_4$ membranes. Due to lack pore precision and time consuming process, the preparation of only
single or low-number nanopore arrays is suggested by TEM. These membranes were later electrochemically characterised via the formation of nano-scale liquid | liquid interfaces with cyclic voltammetry of TPrA⁺ ion transfer across a water | DCH interfaces chosen as a model system. However, the electrochemical characterisation study exhibited poor reproducibility hence the results were not presented in the main body of this thesis.

D.5 Suggestions for future progress
The poor reproducibility of the electrochemical characterisation study obtained, suggested the need for TEM tomography which has been reported as an informative tool to investigate the three dimensional structure of the TEM-drilled pore. Possibilities of incomplete pore drilling and closed pore due to rapid pore contraction which might contribute to the poor electrochemical behaviour, are expected to be verified via TEM tomography. The pore contraction is driven by the fluidized atoms surface tension and occurred when the electron beam intensity is reduced. The fluidized atoms migrate to a flat region of the pore to minimise surface energy, results in a reduction of the size, which might eventually cause complete closure of the pore [141].
Appendix E

Simulations of parylene C PMA

E.1 Pore geometry in the simulation
The simulation study presented in this appendix was provided by Dr. Jorg Strutwolf (University of Tübingen, Tübingen, Germany). The pore geometries applied in the simulation study are exhibited in Figure E.1. In the case of isolated pores, the solution reservoirs on both sides of the pores were greater than the maximal expansion of the diffusion layer. However, the diffusion layers developed around neighbouring pores in membrane pore within an array might overlap. In the perspective of modelling study, this behaviour would involve a three-dimensional simulation of the whole array, which is with regard to computer time and memory too expensive. The overall array size in the present case is of macro dimension (membrane size of 3 mm). Therefore, the three-dimensional square geometry was transferred to a cylindrical geometry, so as to reduce the three-dimensional geometry to a two-dimensional axial symmetric system by the so-called diffusion domain approach. The validity of this concept was investigated in detail by Davies and Compton [86]. Later, the space coordinates are normalised by the radius of the pore opening.
Figure E.1: Schematic diagram of (a) the cross section of the pore, (b) the reduction of the three-dimensional geometry to a two-dimensional axial symmetry system, and (c) the normalisation of the space coordinates by the radius of the pore opening. The numbers in (a) and (b) have units of µm

E.2 Simulations of isolated membrane pores

In this simulation study, the following parameters are applied: potential scan rate of 10 mV s⁻¹, initial and final potential of 0.0 V, switching potential of 1.0 V, and standard ion transfer potential, $E^0$ of 0.5 V. The standard potential for the respective analyte transfer was obtained by comparing the half-wave potentials, $E_{1/2}$ of the simulated and experimental voltammograms. The kinetics of the interfacial ion transfer is defined by a Butler-Volmer type equation [1] and the interfacial standard charge transfer rate constant is fixed to a value where the reversible limit is reached. The transferring ion (charge of +1) is initially present only in the aqueous phase. The geometric properties $(r_a, r_c, l)$ applied in the simulations were of the normalised values (as illustrated in Figure E.1). The simulated CVs demonstrate the current in dimensionless form. Hence, to obtain the current in unit of A, the dimensionless current function has to be multiplied by the Saito expression.

Three different situations are considered, depending on the location of the liquid | liquid interface:

d) The interface is located at the orifice of the pore on the aqueous side of the membrane. The pore is filled with the organic phase (inlaid interface, shown in inset of Figure E.2)
e) The interface is located at the orifice of the pore on the organic side of the membrane. The pore is filled with the aqueous phase (recessed interface, shown in inset of Figure E.3)

f) The interface is located at the centre of the pore. The pore is half-filled with the organic and aqueous phases (recessed interface, shown in inset of Figure E.4)

E.2.1 The effect of pore geometry on voltammogram

The effect of the pore geometry on the shape of the voltammetric response and the magnitude of the current observed was investigated by comparing the current of the concave wall pore to the current of the straight wall pore (with similar radius as the orifice of the concave pore).

In situation (a), the interface is located at the pore orifice on the aqueous side. Both the concave and straight wall pores exhibited identical limiting current magnitudes, and are identical to the limiting current at an inlaid disk electrode (value of 1). However, the concave pore geometry imposes a diffusive resistance towards the ionic flux in the organic phase, once the ions have crossed the interface. Hence, more force is required to transfer ions from the aqueous to the organic phase, resulting CV shift towards more positive potential (Figure E.2).
Figure E.2: Simulated voltammograms (bottom) illustrating the effect of the pore geometry (top) for an isolated pore. The interface is located at the pore orifice on the aqueous side of the membrane.

In situation (b), the pore is filled with the aqueous phase and the interface is shielded by the pore walls. Shielding effect diminished 75% of the limiting current (Figure E.3, design A) as compared to situation (a) (Figure E.2, design A). The concave wall enhanced the shielding of the interface, and extreme current decreased is observed (Figure E.3, design B). However, the half-wave potential shifted to a lower potential due to the presence of ions in the pore at initial. These trapped ions are more easily present for transfer, thus current flow starts at lower potential.
Figure E.3: Simulated voltammograms (bottom) illustrating the effect of the pore geometry (top) for an isolated pore. The interface is located at the pore orifice on the organic side of the membrane.

In situation (c), the interface is located in the centre of the pore, and the limiting current magnitude is somehow between the two earlier discussed situations (Figure E.4).
Figure E.4: Simulated voltammograms (bottom) illustrating the effect of the pore geometry (top) for an isolated pore. The interface is located at the centre of the pore channel.

E.3 Simulations of membrane pores within an array

E.3.1 Diffusion domain approximation
Figure E.5 illustrates the diffusion domain approximation for a regular array of disk interfaces in a cubic close-packed (CCP) arrangement. The diffusion domain around the disc interface is approximated by a cylinder with a radius,
This radius is used so that the base of the cylinder has the same area as the highlighted square (as shown in Figure E.5):

\[ r_d = \sqrt{\frac{d^2}{\pi}} \quad \text{(Eq. E.1)} \]

where \( r_d \) is slightly bigger than \( d/2 \). In our case, the centre-to-centre separation of the pore, \( r_c \) is equivalent to \( d \), which is 16.5 µm, and \( r_d \) will be 9.31 µm. Normalisation of \( r_d \) value by division by the pore opening radius, provides a value of 2.24. The total current of the array is obtained by multiplying the current at the unit cell geometry by the number of pores in the array, \( N_p \). This approach neglects the variation of diffusion zone (and hence current) at the edge of the arrays. This error can be ignored since the number of pores within the arrays is much higher than the edge pores.

![Figure E.5: The top view of an array of disk electrodes in a CCP arrangement](image)

E.3.2 Simulations

The simulation parameters are similar to those employed for the isolated pore in Section E.2. Three different situations are considered:

- g) The interface is located at the orifice of the pore on the aqueous side of the membrane. The pore is filled with the organic phase (inlaid interface, shown in inset of Figure E.6 (a))

- h) The interface is located at the orifice of the pore on the organic side of the membrane. The pore is filled with the aqueous phase (recessed interface, shown in inset of Figure E.6 (b))
i) The interface is located at the centre of the pore. The pore is half-filled with the organic and aqueous phases (recessed interface, shown in inset of Figure E.6 (c))

To study the effect of interacting diffusion zones on the voltammogram shape and magnitude, CV of isolated pores (with undisturbed diffusion zones) is compared to CV for a pore within an array (with the possibility of interacting diffusion zones).

In situation (a), the transfer of model cations from the aqueous phase to the organic phase exhibited a strong diffusion zone overlap, indicated by a decrease of the current and the appearance of a peak current (as compared to CV of isolated pore) (Figure E.6 (a)). The presence of a peak current in the forward scan is due to exclusion zones between the pores where the possible transferable ions are vanished. This results in a strong decrease of the radial diffusion component and an increase of the linear diffusion.

In situation (b), the transfer of model cations from the aqueous phase to the organic phase is made difficult due to the recessed interface. Therefore, the forward scan current is smaller than in Figure E.6 (a). Simulation study demonstrated that diffusion zone overlap still occurred even though the diffusion zone interactions do not extend as far into the radial direction as in the case of Figure E.6 (a). This situation is indicated by the slight decrease of the current on the forward scan, and by emergence of a peak current (green line CV). During the reverse scan, a peak current is observed, indicating linear diffusion dominance.

The condition for radial diffusion depend on two length scales: (i) the extension of the diffusion layer \((Dt)^{1/2}\), and (ii) the characteristic size of the electrode (in this case equivalent to pore radius, \(r_a\)). Radial diffusion (and steady-state current) can be established if \((Dt)^{1/2} \gg r_a\). On the other hand, if the radius of the electrode is much bigger than the diffusion layer (in the case of macroelectrode), then linear diffusion will dominate. In this study, despite the fact \(r_a\) is 4.15 µm, the extension of the diffusion layer into the organic
phase is even smaller, thus the diffusion is dominantly linear, and a peak current is established.

In situation (c) (Figure E.6 (c)), both the organic and aqueous phases meet at the centre of the pore. The forward scan current is higher than in Figure E.6 (b), demonstrating that the interface is more accessible for the transferring ions, although it is less accessible compared to Figure E.6 (a). The diffusion zone overlap is higher than in Figure E.6 (b), shown by the more pronounced peak shape. The shape of the CV in the forward and reverse scans is symmetric due to the symmetrical geometry at the location of the interface.
Figure E.6: Simulated voltammograms illustrating the effect of interacting diffusion zones. Red and green lines represent CV for an isolated pore and CV for a pore within an array, respectively. Inset in each figure shows the liquid / liquid interface locations for a pore within an array. (a) Pore orifice on the aqueous side of the membrane, (b) pore orifice on the organic side of the membrane, and (c) centre of the pore channel.

As discussed in Section 5.3.1, the CV shape obtained experimentally showed a combination of steady-state and peak behaviour in the forward scan, while strong peak behaviour was observed on the reverse scan. Comparison of the experimental CV shape with the three situations examined by simulation does not present a perfect match. It is very likely that the liquid / liquid interface is not located at the pore orifice in either phase since peaks appeared in both the forward and reverse scans. It is most likely that the liquid / liquid interface is located somewhere along the pore channel. Since the peak present on the reverse scan is much stronger than the one on the forward scan, the liquid / liquid interface is most probably located towards the organic phase side, where the tapered part of the pore joints the straight narrow region. Figure E.7 shows simulation results obtained when the liquid / liquid interface is slightly moved from the middle of the pore towards the organic phase side. This small movement provides a clear match to the experimental CVs.
Figure E.7: Simulated voltammograms illustrating the effect of moving the liquid / liquid interface locations from the centre of the pore channel towards the organic side (where the tapered and straight parts joint)
Appendix F

Journal publications and conference presentations

Journal publication (published):

Journal publication (in preparation):

Oral presentations:
1) Y. Liu, **M. Sairi**, M. Rimboud and D.W.M. Arrigan, Electrochemical study of ion transfer at nanoscale liquid-liquid interface arrays, 65th Annual ISE Meeting, Lausanne, Switzerland, 31 Aug. - 5 Sept. 2014 *(presented by Y. Liu)*
pore membranes, 9th World Congress Of Chemical Engineering (WCCE9), Seoul, Korea, 18-23 Aug. 2013

3) D.W.M. Arrigan, M. Sairi, R.A. Mitchell, J. Strutwolf and D.S. Silvester,
Sensing at the nanoscale: Properties of nanoscale interfaces between immiscible liquids formed at the mouths of nanopore array, 223rd ECS Meeting, Toronto, Canada, 12-16 May 2013 (presented by D.W.M Arrigan)

Poster presentations:

1) M. Sairi, R. Thakar, A. Saha, L.A. Baker and D.W.M Arrigan,
Electrochemical characterisation of parylene C porous membrane arrays, 8th International Membrane Science & Technology Conference (IMSTEC 2013), Melbourne, Australia, 25-29 Nov. 2013


3) M. Sairi, S. Abdul Aziz, D.S. Silvester, D.W.M Arrigan,
Chronoamperometric study of ion transfer at nano- and micro-ITIES arrays, 18th Australian Electrochemistry Symposium, Perth, Australia, 15 Apr. 2012