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**SMART ELECTROCHEMICAL SENSING  
SYSTEM FOR THE REAL TIME DETECTION  
OF ENDOCRINE DISRUPTING COMPOUNDS  
AND HORMONES**

A thesis presented in partial fulfilment of the  
requirements for the degree of  
Doctor of Philosophy  
in  
Electronics Engineering  
at Massey University, Manawatu,  
New Zealand

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**30<sup>th</sup> October,  
2014**

## **Dedications**

**In the name of Allah the most beneficent and the most merciful**

### **To my parents**

*Mr Sher Afzal Zia*

*Mrs Shamim Akhter*

### **To my wife**

*Mrs Nazia Asif*

&

### **To my boys**

*Muhammad Shaheryar Asif*

*Muhammad Asfandyar Asif*

*Muhammad Abdur-Rehman Bin Asif*

## Abstract

Presented research work has not only provided a real-time tool to perform week-long chemical and bio-chemical assays in minutes yet, it had been operating as a source of community awareness about the said chemicals that we keep ingesting knowing or unknowingly. Phthalates are the most ubiquitous chemicals that pose a grave danger to the human race due to their extraordinary use as plasticizer in consumer product industry. All contemporary detection methods require high level of skills, expensive equipment and long analysis time as compared to the technique presented in this research work that introduces a real time non-invasive assay. A novel type of silicon substrate based smart interdigital sensor fabricated by employing thin film micro-electromechanical system semiconductor device fabrication technology. Electrochemical Impedance Spectroscopy was used in conjunction with the fabricated sensor to detect hormones and phthalates in deionized water. Various concentrations of phthalates as low as 2 parts per billion to a higher level of 2 parts per million in deionized water were detected distinctively using new planar ID sensor based EIS sensing system. The sensor was functionalized by a self-assembled monolayer of 3-aminopropyltriethoxysilane with embedded molecular imprinted polymer to introduce selectivity for the phthalate molecule. Spectrum analysis algorithm interpreted the experimentally obtained impedance spectra by applying complex nonlinear least square curve fitting in order to obtain electrochemical equivalent circuit and corresponding circuit parameters describing the kinetics of the electrochemical cell. Principal component analysis was applied to deduce the effects of surface immobilized molecular imprinted polymer layer on the evaluated circuit parameters and its electrical response. The results obtained by the testing system were validated using commercially available high performance liquid chromatography diode array detector system.



## Research Outputs

The research outputs which have been published are listed below. The research outputs are in conjunction with the author's PhD candidacy:

### Journals

1. **A. I. Zia**, S. C. Mukhopadhyay, P.-L. Yu, I. Al-Bahadly, C. P. Gooneratne, and J. Kosel, "Rapid and molecular selective electrochemical sensing of phthalates in aqueous solution," *Biosensors and Bioelectronics*, **2014**.  
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# Table of Contents

<b>Abstract</b> .....	<b>iii</b>
<b>Acknowledgements</b> .....	<b>ix</b>
<b>Table of Contents</b> .....	<b>xi</b>
<b>Table of Figures</b> .....	<b>xv</b>
<b>List of Tables</b> .....	<b>xix</b>
<b>Abbreviated Terms</b> .....	<b>xx</b>
<b>Chapter 1 Introduction</b> .....	<b>1</b>
1.1 Hormones and Endocrine Disrupting Compounds .....	1
1.2 Receptor–ligand binding assays.....	4
1.2.1 Radio Receptor Assay (RRA).....	5
1.2.2 Scintillation Proximity Assay (SPA) .....	5
1.2.3 Fluorescence Resonance Energy Transfer (FRET).....	6
1.2.4 Fluorescence Polarization (FP) .....	8
1.2.5 Fluorometric Micro volume Assay (FMAT).....	8
1.2.6 AlphaScreen™ .....	9
1.2.7 Flow Cytometry .....	9
1.2.8 Fluorescence Correlation Spectroscopy (FCS).....	10
1.3 Immunoassay .....	10
1.3.1 Surface Plasmon Resonance (SPR).....	12
1.3.2 Total Internal Reflection Fluorescence (TIRF).....	13
1.3.3 Ellipsometry .....	14
1.3.4 Nuclear Magnetic Resonance Spectroscopy .....	14
1.3.5 Amperometric Immunosensors .....	15
1.3.6 Conductimetric Immunosensors.....	15
1.3.7 Surface Acoustic Wave Immunosensors (SAW).....	16
1.3.8 Enzyme-linked Immunosorbent Assay (ELISA).....	16
1.4 Conclusions .....	19
1.5 Research Contributions .....	20
1.6 Organization of the Thesis .....	21
<b>Chapter 2 Impedance Spectroscopy and Experimental Setup</b> .....	<b>23</b>
2.1 Introduction .....	23

2.2	Electrochemical Impedance Spectroscopy.....	23
2.2.1	AC bridges .....	24
2.2.2	Lissajous Curves .....	24
2.2.3	Fast Fourier Transforms (FFT) .....	25
2.2.4	Phase Sensitive Detections (PSD).....	25
2.2.5	Frequency Response Analysis (FRA).....	25
2.2.6	Electrochemical Impedance Spectroscopy; Theory and Analyses .....	27
2.2.7	‘Nyquist’ and ‘Bode’ plots for Impedance Data Analysis.....	30
2.2.8	Randle’s Electrochemical Cell Equivalent Circuit Model.....	31
2.3	Experimental Setup.....	36
2.3.1	Equipment and Instrumentations .....	36
2.3.2	Fixture and Test Probe Connections .....	38
2.3.3	RS-232C Interface for 3522-50/3532-50 LCR Hi Tester .....	39
2.4	Conclusions.....	40
<b>Chapter 3</b>	<b>Novel Interdigital Sensors’ Development .....</b>	<b>41</b>
3.1	Introduction to Interdigital Sensors.....	41
3.2	Novel Planar Interdigital Sensors .....	44
3.3	Finite Element Modelling using COMSOL Multiphysics® .....	45
3.4	Sensors’ Fabrication.....	56
3.5	Sensors’ Profiling and Problem Definition.....	60
3.5.1	Connection Pads Soldering .....	61
3.6	Performance Evaluation.....	62
3.6.1	Experimental Evaluation.....	62
3.7	Achieving Stability in Sensors’ Performance .....	64
3.7.1	Post-fabrication Anneal of ID Sensor .....	65
3.7.2	Results’ Validation.....	67
3.7.3	Complex Nonlinear Least Squares Curve Fitting .....	69
3.7.4	Principal Component Analysis (PCA) .....	72
3.7.5	PCA analysis – ECI (30°C – 90°C) anneal .....	72
3.7.6	PCA analysis – EC2 (91°C – 150°C) anneal.....	74
3.7.7	PCA analysis – EC3 (151°C – 210°C) anneal.....	76
3.8	Conclusions.....	79
<b>Chapter 4</b>	<b>Electrochemical Detection of Hormones.....</b>	<b>81</b>

4.1	Introduction .....	81
4.2	Detection of Ovarian Hormone Estrone Glucuronide (E1G).....	82
4.2.1	Motivation .....	82
4.2.2	Point-of-care Methods.....	83
4.2.3	Basal Body Temperature method (BBT) .....	83
4.2.4	Billings Ovulation Method.....	83
4.2.5	Symptothermal Method (STM).....	83
4.2.6	Ovarian Monitor.....	84
4.2.7	Materials and Methods to Detect E1G .....	84
4.2.8	Results and Discussions .....	86
4.2.9	Electrochemical Impedance Spectroscopy Analyses for E1G .....	88
4.2.10	E1G Sensitivity Analysis .....	90
4.3	Electrochemical detection of Progesterone Hormone.....	92
4.3.1	Motivation .....	92
4.3.2	Materials and Methods for Progesterone Detection.....	94
4.3.3	Electrochemical Impedance Analyses for Progesterone .....	95
4.4	Conclusions .....	99
<b>Chapter 5</b>	<b>Detection of Endocrine Disrupting Compounds .....</b>	<b>101</b>
5.1	Introduction .....	101
5.2	Impedimetric Detection of DEHP and DINP.....	103
5.2.1	Motivation .....	104
5.2.2	Materials and Methods.....	105
5.2.3	DEHP Detection Test in Deionized Water.....	106
5.2.4	Experimental Data Analyses by CNLS Curve Fitting .....	108
5.2.5	Sensitivity Analysis – DEHP .....	110
5.2.6	DEHP Detection in Commercially Sold Energy Drink.....	111
5.2.7	Impedance Measurements of DINP-spiked Ethanol Samples .....	113
5.2.8	Impedance Measurements of DINP-Spiked Orange Juice.....	115
5.2.9	Conclusions .....	118
<b>Chapter 6</b>	<b>Inducing Analyte Selectivity in the Sensing System .....</b>	<b>119</b>
6.1	Introduction .....	119
6.1.1	Materials and Methods.....	121
6.1.2	Synthesis of DEHP imprinted polymer .....	122

6.1.3	EIS for detection of DEHP in MilliQ.....	125
6.1.4	Results and Discussions .....	127
6.1.5	Adsorption studies of DEHP to MIP.....	127
6.1.6	Static Adsorption of DEHP to MIP.....	128
6.1.7	Uptake Kinetics of MIP Coated Sensor to DEHP.....	129
6.1.8	Data Analyses Using Non-linear Least Square Curve fitting .....	130
6.1.9	Results validation by HPLC.....	132
6.1.10	DEHP in Energy Drink – MIP Coated Sensor .....	134
6.1.11	Conclusions.....	136
<b>Chapter 7</b>	<b>Conclusions and Future Research.....</b>	<b>139</b>
7.1	Conclusions.....	139
7.2	Future Work.....	144
7.2.1	Sensitivity and selectivity Improvement.....	144
7.2.2	Thick Film Electrodes .....	144
7.2.3	Substrate Type.....	145
<b>Bibliography</b>	<b>.....</b>	<b>147</b>

## Table of Figures

Figure 1.1 The endocrine system in human body	1
Figure 1.2 Leaching of DEHP and DEP from tetra packaging within expiry date of orange packaged orange juice[11]	3
Figure 1.3 Types of phthalates used in industrial sector as plasticizers	4
Figure 1.4 Basic concept of FRET as photophysical process. (A) The plot shows the dependence of the FRET efficiency on the proximity of the donor-acceptor pair. (B) shows the effect of angle between donor fluorochrome and acceptor molecule on FRET [25]	7
Figure 1.5 Detection principle of SPR technique[38]	13
Figure 1.6 Schematics of a SAW immunosensor [57]	16
Figure 2.1 Formation of Lissajous Figure	25
Figure 2.2 I-V curve for a non-linear system. Pseudo-linearity of the system is achieved by considering a small part of the curve	27
Figure 2.3 Phase shift in current $I_t$ as a response to excitation potential $E_t$ in a linear system	28
Figure 2.4 Flow chart for the measurement and characterization of a material-electrode system by EIS [77]	31
Figure 2.5 Kinetic processes taking place at electrode-electrolyte interface (Randle's cell model)	32
Figure 2.6 Randle's electrochemical cell equivalent circuit model	32
Figure 2.7 Nyquist plot for Randle's electrochemical cell model [79]	34
Figure 2.8 Bode plot for Randle's electrochemical cell model[79]	35
Figure 2.9 Extraction of component parameters from Bode plot[79]	35
Figure 2.10 Laboratory Test bench with Hioki Hi Precision LCR and data acquisition system.	36
Figure 2.11 Hioki (Japan) 3522-50 LCR Hi-tester	37
Figure 2.12 Connecting LCR3522-50/3532-50 to the 9262 Test fixture and developed interdigital sensing system	38
Figure 3.1 Planar interdigital sensor geometry	42
Figure 3.2 Concept of transformation (a) parallel plate capacitor (b) transformation to planar geometry (c) coplanar structure.	43

Figure 3.3 The penetration depth of electric field lines is proportional to the electrode spatial period $\lambda$	44
Figure 3.4 Excitation pattern for multi sensing electrode ID sensor geometry	45
Figure 3.5 COMSOL geometric models of ID sensors with 25 $\mu\text{m}$ pitch length	47
Figure 3.6 COMSOL geometric models of ID sensors with 50 $\mu\text{m}$ pitch length	48
Figure 3.7 1-5-50 Sensor structure; Silicon substrate (bottom) MUT (top)	48
Figure 3.8 Potential distribution modelling in COMSOL for (a) 1-5-25, (b) 1-5-50	50
Figure 3.9 Potential distribution modelling in COMSOL for (c) 1-11-25 and (d) 1-11-50 ID sensors	51
Figure 3.10 Modelled Capacitance for ID sensors	52
Figure 3.11 Modelled Capacitance of single ID capacitor feature on each sensor	53
Figure 3.12 Electric field and penetration depth simulation using COMSOL (a) 1-5-25, (b) 1-5-50	54
Figure 3.13 Electric field and penetration depth simulation using COMSOL (c) 1-11-25 and (d) 1-11-50 ID sensors	55
Figure 3.14 1-5-25 sensor: basic design geometry and dimensions	57
Figure 3.15 Interdigital electrode fabrication configurations	57
Figure 3.16 Fabrication process for all four types of sensors	58
Figure 3.17 Steps involved in coating 1 $\mu\text{m}$ passivation layer of Parylene C	59
Figure 3.18 (a) 36 Workable sensors fabricated on one wafer (b) Individual sensor shown against scale	60
Figure 3.19 Impedance characteristics using same sensor before (a) and after (b) soldering	62
Figure 3.20 Cole-Cole plot for 1-1-50B ‘as is’ fabricated sensor’s test in air at 44% humidity and 23.3°C temperature at different times	63
Figure 3.21 Cole-Cole plot for 1-1-50A sensor’s test in air at 44% humidity and 23.3°C temperature at different times	63
Figure 3.22 SEM image of the fabricated electrode shows roughness of electrodes due to DC magnetron sputtering process	65
Figure 3.23 Annealing sensors in vacuum oven in Micro-Suit	67
Figure 3.24 Nyquist plot for EIS during annealing process at 30, 60, 90, 120, 150, 180, and 210 ° C	68
Figure 3.25 Nyquist plot for improved performance annealed sensors at initial temperature and humidity conditions	68

Figure 3.26 SEM image of electrode before (a) and after (b) anneal	69
Figure 3.27 EC1 CNLS equivalent circuit for 30-90°C	71
Figure 3.28 EC1 fitted curve (30-90°C)	71
Figure 3.29 EC2 CNLS equivalent circuit for 91-150°C	71
Figure 3.30 EC2 fitted curve (91-150°C)	71
Figure 3.31 EC3 CNLS equivalent circuit for 151-210°C	71
Figure 3.32 EC3 fitted curve (151-210°C)	71
Figure 3.33 Component Plot ECI (30°C – 90°C) anneal	73
Figure 3.34 Scree Plot ECI (30°C – 90°C) anneal	74
Figure 3.35 Component Plot EC2 (91°C – 150°C) anneal	75
Figure 3.36 Scree Plot EC2 (91°C – 150°C) anneal	76
Figure 3.37 Component Plot EC3 (151°C – 210°C) anneal	77
Figure 3.38 Scree Plot EC3 (151°C – 210°C) anneal	78
Figure 4.1 Mean hormonal values of estrone glucuronide (EIG) (-•-), LH (-□-), and pregnanediol glucuronide (PG) (-△-) by cycle day throughout 78 ovulatory cycles from 25 women [107]	82
Figure 4.2 Dip test method for EIG testing	85
Figure 4.3 Laboratory test bench for EIG testing	86
Figure 4.4 Real and imaginary parts of the measured impedance for EIG	87
Figure 4.5 Nyquist (Cole-Cole) plot for the impedance measurements of EIG	88
Figure 4.6 Electrochemical equivalent circuit extracted by CNLS curve fitting	89
Figure 4.7 CNLS curve fitting of Nyquist plot by spectrum analyser for the highest concentration of EIG 33.33nmol/L.	89
Figure 4.8 %age sensitivity of the real part of impedance (Re).	91
Figure 4.9 %age sensitivity of capacitive reactance (X).	91
Figure 4.10 Progesterone concentration level during 21 days reproductive cycle of dairy cows [12]	92
Figure 4.11 Real and Imaginary Impedance (reactance) characteristics	95
Figure 4.12 Bode plot for progesterone detection in deionized water	96
Figure 4.13 Nyquist plot for progesterone detection in DI water	97
Figure 4.14 $Z_{imag}(X)$ %Sensitivity	98
Figure 4.15 $Z_{real}(R_s)$ %Sensitivity	98
Figure 5.1 Molecular structure DEHP	104
Figure 5.2 Molecular structure DINP	104

Figure 5.3 Test bench setup	105
Figure 5.4 Dip-test (bulk)	105
Figure 5.5 Imaginary and real parts of impedance measurements for DEHP dip-test	106
Figure 5.6 Nyquist plot for detection of DEHP in DI water	107
Figure 5.7 Equivalent circuit proposed by CNLS curve fitting	108
Figure 5.8 CNLS curve fitting plot for absolute value of Impedance.	109
Figure 5.9 CNLS curve fitting plot for imaginary value of Impedance.	109
Figure 5.10 CNLS curve fitting plot for phase shift ( $\theta$ )	109
Figure 5.11 Reactance percentage sensitivity for DEHP in DI water	111
Figure 5.12 Test bench setup for DEHP-spiked energy drinks	112
Figure 5.13 $Z_{\text{real}}$ and $Z_{\text{imag}}$ for DEHP dip-test of spiked energy drink	112
Figure 5.14 Bode plot showing measured spectra for the spiked drink	113
Figure 5.15 Nyquist plot for DINP concentration test in EtOH	114
Figure 5.16 Real part of impedance for DINP-in-EtOH	115
Figure 5.17 Imaginary Part of Impedance for DINP-in-EtOH	115
Figure 5.18 Real part of measured impedance for DINP in orange juice	116
Figure 5.19 Reactance plot for DINP-in-Orange juice	117
Figure 6.1 Synthesis process of Molecular Imprinted Polymer	120
Figure 6.2 Extraction of template molecule forming molecular recognition site in MIP	120
Figure 6.3 Polymerisation reaction	122
Figure 6.4 Polymerized MIP	122
Figure 6.5 Soxhlet Extraction	123
Figure 6.6 Filter caplet 0.22 $\mu\text{m}$	123
Figure 6.7 DEHP extracted MIP	124
Figure 6.8 MIP coated sensor using SAM of APTES	124
Figure 6.9 Confocal micrograph image of MIP coated sensor	124
Figure 6.10 Dip Coating process	125
Figure 6.11 Drying in nitrogen flow	125
Figure 6.12 Laboratory test bench setup for MIP coated sensor	126
Figure 6.13 Isotherm for the static adsorption studies of DEHP to MIP	128
Figure 6.14 Isotherm for adsorption uptake kinetics of DEHP to MIP	129
Figure 6.15 Nyquist plot for EIS testing of MIP coated Sensor	130
Figure 6.16 Equivalent circuit estimated by CNLS analysis	131
Figure 6.17 DIONEX-Ultimate 3000 HPLC apparatus	132

Figure 6.18 Absorption spectrum of DEHP	133
Figure 6.19 MIP eluent tested with HPLC for EIS results validation	133
Figure 6.20 DEHP eluent extracted from MIP immobilized on ID sensor	134
Figure 6.21 Nyquist plot for DEHP spiked drink by MIP coated sensor	135
Figure 6.22 HPLC chromatogram showing the DEHP peaks in the eluent extracted out of the functionalized MIP	136
Figure 7.1 New sensor design to overcome heat and stray capacitance problems	145

## List of Tables

Table 1.1 Endocrine system's glands, hormones and their function	2
Table 1.2 Advantages and disadvantages of contemporary techniques used for biochemical analytes	18
Table 2.1 Hioki Hi Precision LCR 3522-50 and 3532-50 Specifications	37
Table 2.2 Hioki Hi Precision LCR test terminals description	38
Table 2.3 LCR testing parameters and calculation equations	39
Table 3.1 Geometric design parameters for all four types of modelled ID sensors	46
Table 3.2 Calculated values of capacitance and total electrical energy stored for all four sensors using COMSOL Multiphysics®	52
Table 3.3 Component parameters for the best fit circuit	70
Table 3.4 Table of variance ECI (30°C – 90°C) anneal	73
Table 3.5 Table of variance EC2 (91°C – 150°C) anneal	75
Table 3.6 Table of variance EC3 (151°C – 210°C) anneal	77
Table 4.1 Equivalent circuit components' parameters deduced by CNLS curve fitting technique for electrochemical spectrum analysis	90
Table 4.2 Progesterone in deionized water- Samples' nomenclature and concentration	95
Table 5.1 List of the most ubiquitous EDCs	101
Table 5.2 Risk assessment of phthalates by world agencies [130].	103
Table 5.3 Equivalent circuit parameters evaluated by CNLS algorithm	110
Table 6.1 CNLS curve fitted equivalent circuit parameters	131
Table 6.2 Equivalent circuit parameters evaluated by CNLS fitting for DEHP detection in 'Lift Plus' drink by MIP functionalized sensor	135

## Abbreviated Terms

<b>AC</b>	Alternating Current
<b>AFM</b>	Atomic Force Microscope
<b>Alumina-Al<sub>2</sub>O<sub>3</sub></b>	Aluminium Oxide
<b>APTES</b>	3-aminopropyltriethoxysilane
<b>Au</b>	Gold
<b>BBP</b>	Butylbenyl phthalate
<b>Bode</b>	Data presentation as a function of frequency
<b>BSA</b>	Bovine serum albumin
<b>C</b>	Capacitance
<b>C<sub>ad</sub></b>	Adsorption capacitance
<b>C<sub>dl</sub></b>	Double-layer capacitance
<b>CNLS</b>	Complex Non-linear Least Square
<b>CPE</b>	Constant Phase-Element
<b>Cr</b>	Chromium
<b>D</b>	The electric displacement
<b>DBP</b>	Di- <i>n</i> -butyl phthalate
<b>DEHP</b>	Di-(2-ethylhexyl) phthalate
<b>DEP</b>	Di-ethyl phthalate
<b>dH<sub>2</sub>O</b>	Distilled water
<b>DINP</b>	Di-isononyl phthalate
<b>DMF</b>	dimethyl formamide
<b>DMP</b>	Di-methyl phthalate
<b>DNA</b>	Deoxyribonucleic Acid
<b>DOP</b>	Di- <i>n</i> -octyl phthalate
<b>DUT</b>	Device Under Test
<b>E</b>	The electric field intensity
<b>EC</b>	Electrolyte Conductivity
<b>EDCs</b>	Endocrine Disrupting Compounds
<b>EDs</b>	Endocrine Disruptors
<b>EIS</b>	Electrochemical Impedance Spectroscopy
<b>ELISA</b>	Enzyme Linked Immunosorbent Assays
<b>EtOH</b>	Ethanol
<b>EU</b>	Endotoxin Unit
<b><i>f</i></b>	Frequency
<b>FDA</b>	United States Food and Drug Administration
<b>FR4</b>	Fiberglass reinforced epoxy laminates for PCB fabrication
<b>FTIR</b>	Fourier Transform Infrared Spectroscopy

<b>GND</b>	Electrical ground
<b><math>H^+</math></b>	Hydrogen ions
<b><math>H_{CUR}</math></b>	Carries the signal current source.
<b>HPLC</b>	High performance liquid Chromatography
<b>HPLC</b>	High Performance Liquid Chromatography
<b><math>H_{POT}</math></b>	Monitors the excitation potential, V.
<b>I</b>	Current
<b>IDAM</b>	Interdigitated Array Microelectrode
<b>IDES</b>	Interdigitated Electrode Structures
<b>IDTs</b>	Interdigital Transducers
<b>IS</b>	Impedance Spectroscopy
<b>ISE</b>	Ion-Selective Electrodes
<b>ISFET</b>	Ion-Sensitive Field Effect Transistors
<b>LCR3522-50</b>	Measuring instrument for impedance (inductance, capacitance and resistance) (1mHz to 100kHz)
<b>LCR3532-50</b>	Measuring instrument for impedance (inductance, capacitance and resistance) (42Hz to 5MHz)
<b><math>L_{CUR}</math></b>	Accepts the signal current return.
<b>LOD</b>	Limit of Detection
<b>LOD</b>	Limit of Detection
<b><math>L_{POT}</math></b>	Connected to the sensor's sensing electrodes
<b>MEMS</b>	Micro-electromechanical Systems
<b>MilliQ</b>	Ultra-pure water which undergo the proses filtration and deionisation that has been characterised in terms of resistivity (typically 18.2 $M\Omega \cdot cm$ )
<b>MRL</b>	Minimal Risk Level
<b>MUT</b>	Material Under Test
<b>NaCl</b>	Sodium Chloride
<b>NMR</b>	Nuclear Magnetic Resonance
<b>Nyquist</b>	Data presentation in complex impedance plot
<b><math>OH^-</math></b>	Hydroxide ions
<b>PC</b>	Principal Component
<b>PCA</b>	Principal Component Analysis
<b>PCB</b>	Printed Circuit Board
<b>PCR</b>	Polymerase Chain Reaction
<b>PDEs</b>	Partial differential equations

<b>PECVD</b>	Plasma enhanced chemical vapour deposition
<b>PETE</b>	Polyethylene Terephthalate
<b>pH</b>	Potential of Hydrogen
<b>PPB</b>	Parts per billion
<b>PPM</b>	Parts per million
<b>PR</b>	Photo Resist
<b>PVC</b>	Polyvinyl chloride
<b>Q</b>	Charge
<b><math>\theta</math></b>	Phase Angle, theta
<b>QCM</b>	Quartz Crystal Microbalance
<b>R</b>	Resistance
<b><math>r^2</math></b>	Residual mean squares
<b><math>R_{ct}</math></b>	Charge/electron transfer resistance
<b>RIE</b>	Reactive ion etching
<b><math>R_s</math></b>	Solution resistance
<b>RS232</b>	Serial communication port
<b>SAW</b>	Surface Acoustic Wave
<b>SEAT</b>	School of Engineering and Advanced Technology
<b>SEM</b>	Scanning Electron Microscope
<b>Si</b>	Silicon
<b><math>Si_3N_4</math></b>	Silicon Nitride
<b><math>SiO_2</math></b>	Silicon Dioxide
<b>SNR</b>	Signal to Noise Ratio
<b>SPR</b>	Surface Plasmon Resonance
<b>SPSS</b>	IBM Statistical Analysis software
<b><math>\sigma_w</math></b>	Warburg coefficient
<b>TDI</b>	Tolerable Daily Intake
<b>TEOS</b>	Tetraethylorthosilicate
<b>TMAH</b>	Tetra-Methyl Ammonium Hydroxide
<b>UV</b>	Ultra-violet
<b>V</b>	Voltage
<b><math>W_e</math></b>	The electrostatic energy density
<b>Z</b>	Impedance
<b><math>Z_w</math></b>	Warburg impedance
<b><math>\omega</math></b>	The angular frequency
<b><math>\epsilon_0</math></b>	The permittivity of vacuum which sets to be $8.854 \times 10^{-2}$ F/m
<b><math>\epsilon_r</math></b>	The relative permittivity
<b><math>\epsilon</math></b>	The permittivity