Synthesis and Optimization of Anodic Aluminium Oxide Thin Film Electrode for DNA Sensing

by

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LIST OF ABBREVIATIONS

AAO	Anodic aluminum oxide
AC	Alternating current
Ag/AgCl	Silver/silver chloride
Al	Aluminum
ANOM	Analysis of means
ANOVA	Analysis of variance
APTES	3-aminopropyltrimethoxysilane
ASTM	American Society for Testing and Materials
CNTs	Carbon nanotubes
CPE	Constant phase element
CV	Cyclic voltammetry
DENV2	Dengue-virus particles serotype 2
DI	Deionized water
DNA	Deoxyribonucleic acid
DOE	Design of experiment
DPV	Differential pulse voltammetry
dsDNA	Double stranded DNA
EIS	Electrochemical impedance spectroscopy
FESEM	Field emission scanning electron microscope
FFT	Fast Fourier Transform
FTIR	Fourier-transform infrared spectroscopy
GA	Glutaraldehyde
GPMS	3-glycidoxypropyl) trimethoxysilane
HA	Hard anodizing
IPA	Isopropyl alcohol
KCL	Potassium chloride
L9	Taguchi orthogonal array with 9 Lines of experimental arrays
LED	Light-emitting diode
LOD	Limit of detection
LOD-AAO	LOD optimized AAO
LTB	Larger the better
M	Molarity

MA	Mild anodizing
PBR	Pilling-Bedworth ratio
PL	Photoluminescence
PVD	Physical vapour deposition
R_{ct}	Resistance charge transfer
R_s	Solution resistance
S-AAO	Sensitivity optimized AAO
SAM	Self-assembled monolayer
SEM	Scanning electron microscopy
SNR	Signal to noise ratio
ssDNA	Single-stranded DNA
STB	Smaller the better
T	Temperature
TEM	Transmission electron microscopy
V	Voltage
W	Warburg
XRD	X-ray diffraction
XRF	X-ray fluorescence

LIST OF SYMBOLS

Al_2O_3	Aluminium oxide
Al^{3+}	Aluminum ions
Au	Aurum
°C	Celcius
cm	Centimeter
cm^2	Centimeter square
ρ	Density
e-	Electrons
$Fe(CN)_6^{3-}$	Ferrycyanide ions
Hz	Hertz
h	Hour
H_2	Hydrogen
H^+	Hydrogen ions
H_2O_2	Hydrogen peroxide
H_3O^+	Hydronium ions
-OH	Hydroxyl
L	Liter
Mn	Manganese
μ	Micro
μL	Microliter
μM	Micromolar
mA	Miliampere
mL	Mililiter
mV	Milivolts
ng	Nanogram
nm	Nanometer
nM	Nanomolar
Ω	Ohm
COO-	Oxalate
$H_2C_2O_4$	Oxalic acid
O^{2-}	Oxygen ions

O_2	Oxygen ions
O-	Oxylate
%	Percentage
H_3PO_4	Phosphoric acid
π	Pi
pM	Picomolar
Pt	Platinum
ρ_p	Pore density
pH	Scale used for acidity or basic
σ	Sigma
SiO_2	Silicone oxide
H_2SO_4	Sulphuric acid
H_2O	Water

Sintesis Dan Pengoptimuman Elektrod Saput Nipis Aluminium Oksida Teranod Untuk Penderiaan DNA

ABSTRAK

Dalam beberapa tahun kebelakangan ini, aluminium oksida teranod (AAO) telah dieksplorasi secara meluas sebagai peranti biopenderiaan DNA yang murah, mudah alih dan sensitif. Selain dari kemampuan mereka melakukan pertumbuhan sendiri liang nano yang tersusun, keliangan yang tinggi dan keluasan permukaan yang besar, AAO mempunyai satu ciri yang sangat istimewa dimana sifat-sifat dimensinya boleh diubahsuai dan direka bentuk dengan hanya mengawal parameter utama penganodan iaitu voltan, suhu, masa dan kepekatan elektrolit. Oleh kerana kepekaan dan had pengesanan biopenderia AAO-DNA bergantung pada ukuran dimensi liang nano itu sendiri, maka ramai penyelidik telah berusaha untuk cuba memahami kesan bagi setiap parameter anodisasi terhadap dimensi liang nano disamping mencuba untuk mengoptimumkan dan meningkatkan kepekaan AAO dalam mengesan hibridisasi DNA. Walau bagaimanapun, kajian-kajian tersebut hanya tertumpu pada trend pemboleh ubah tunggal pada satu-satu masa dengan kaedah *one-factor-at-a-time* (OFAT), berbanding dari menyiasat semua interaksi yang mungkin di antara parameter anodisasi secara serentak. Oleh itu, mereka hanya melaporkan peningkatan terhadap kepekaan biopenderia AAO-DNA yang telah mereka peroleh pada julat parameter tertentu yang telah mereka uji tanpa memberikan tahap kombinasi optimum dari semua parameter anodisasi sementara parameter anodisasi yang paling kritikal dan berpengaruh masih tetap tidak diketahui. Sebagai sumbangan untuk menyelesaikan masalah ini, kaedah Taguchi telah diusulkan dalam penyelidikan ini sebagai alat pengoptimuman untuk mengkaji interaksi yang ada di antara semua parameter dan pada masa yang sama memberikan kombinasi terbaik dari semua tahap parameter untuk meningkatkan kepekaan biopenderia AAO-DNA pada kadar optimumnya. Sementara itu, analisis ANOVA telah dicadangkan untuk mendapatkan parameter anodisasi yang paling berpengaruh terhadap prestasi kepekaan biopenderia AAO-DNA. Sebelum pengoptimuman dilakukan, kajian korelasi antara dimensi liang pori dan pemindahan caj rintangan (R_{ct}) yang mempengaruhi kepekaan biopenderia AAO-DNA telah dilakukan. Untuk penyelidikan ini, elektrod biopenderia DNA saput nipis AAO akan disintesis menggunakan kaedah anodisasi langkah tunggal di dalam asid oksalik. FESEM digunakan untuk mengamati permukaan AAO, sementara EIS digunakan untuk mempelajari sistem elektrokimia bagi pengesanan hibridisasi DNA. Dari penemuan pertama, kepekaan biopenderia AAO-DNA didapati dipengaruhi oleh nisbah ketebalan AAO kepada ukuran liang pori. R_{ct} kekal di bawah 100 kΩ selagi nisbah ketebalan AAO kepada ukuran liang pori dikekalkan dalam julat antara 1:11 kepada 1:16. Melebihi nisbah lebih dari 1:25 akan mengakibatkan kenaikan R_{ct} secara mendadak dan mempengaruhi penurunan pada kepekaan. Dari penemuan kedua, pengoptimuman melalui kaedah Taguchi merupakan suatu pembaharuan di dalam kajian ini. Kombinasi parameter anodisasi optimum didapati pada suhu 40 V, 17°C, 0.3 M asid oksalik pada 1 jam. Pengulangan eksperimen telah dilakukan untuk mengesahkan kecekapan kaedah Taguchi di mana kepekaan electrod biopenderia AAO-DNA yang telah dioptimumkan berjaya ditingkatkan kepada 62.57% pada 0.278 kΩ/M (LOD pada 6.497×10^{-15}M)

berbanding dengan nominal. Dari penemuan ketiga melalui analisis ANOVA, urutan parameter anodisasi yang paling berpengaruh terhadap kepekaan AAO-DNA biopenderia adalah mengikut susunan: voltan anodisasi (34.13%) > masa anodisasi (29.85%) > suhu (20.27%) > kepekatan elektrolit (15.74%). Pada akhir penyelidikan ini, pengoptimuman sintesis elektrod saput nipis AAO dengan kaedah Taguchi untuk penginderaan DNA telah berjaya dicapai. Oleh kerana penyelidikan ini telah menggunakan jenis DNA sasaran yang tidak spesifik bagi tujuan pembangunan prototaip, oleh itu, disasarkan bahawa elektrod saput nipis AAO yang telah dioptimumkan sensitifnya ini akan mempunyai peluang yang lebih besar untuk digunakan secara meluas di masa depan bagi mengesan pelbagai jenis DNA seperti denggi, *E-Coli* atau *salmonella.* Diharapkan agar hasil kajian ini dapat membantu para penyelidik lain untuk mensintesis saput nipis AAO pada tahap prestasi anodisasi optimumnya agar kepekaan biopenderia AAO-DNA dapat ditingkatkan lagi.

Synthesis and Optimization of Anodic Aluminium Oxide Thin Film Electrode for DNA Sensing

ABSTRACT

Recent years, anodic aluminum oxide (AAO) has been extensively explored as an inexpensive, portable and sensitive DNA biosensing device. Apart from their ability to self-grow into well-ordered nanoporous with high porosity and huge surface area, AAO has one special feature wherein their nanopores' dimension are capable to be altered and engineered by controlling the main anodizing parameters namely voltage, temperature, time and electrolyte concentration. Since sensitivity and limit of detection of AAO-DNA biosensor depends on the nanopores' dimension itself, therefore, many researchers have tried to understand the effect of each anodizing parameters to the nanopores' dimension while tried to optimize and improve the sensitivity of AAO in detecting DNA. However, those studies were only focused on the trend of single variable parameter at one time by one-factor-at-a-time method (OFAT), rather than investigating all the interactions between the anodizing parameters simultaneously. Hence, they only reported the improvement made to the sensitivity of their AAO-DNA biosensor at the particular parameter range that they have tested without providing the best of optimum combination levels of all anodizing parameters while the most critical and influential anodizing parameter is still remained unknown. As a contribution to solve these problems, Taguchi method has been proposed in this research as an optimization tool to study the existing interaction between the parameters while at the same time providing the best combination of all parameters levels to improve the AAO-DNA biosensor sensitivity at its optimum performance. Meantime, ANOVA analysis has been proposed to obtain the most influential anodizing parameter to the sensitivity of AAO-DNA biosensor. Prior to optimization, correlation study between the pores' dimension and resistance charge transfer (R_{ct}) that affects the sensitivity of AAO-DNA biosensor has been conducted. For this research, AAO thin film-DNA biosensor electrode was synthesized using a single step anodization method in oxalic acid. FESEM was used to observe the AAO surface, while EIS was utilised to study the electrochemical system for DNA hybridization detection. From the first finding, sensitivity of AAO-DNA biosensor was found to be influenced by the ratio of AAO thickness to the pore size. R_{ct} remained under 100 kΩ as long as the ratio of AAO thickness to the pore size was maintained in the range between 1:11 to 1:16. Exceeding the ratio of more than 1:25 will result to the sudden increased in R_{ct} and hence affecting the sensitivity to be reduced. From the second finding, optimization through Taguchi method is the main novelty of this research. The optimum combinations of anodizing parameters were found at 40 V, 17 °C, 0.3 M of oxalic acid at 1 hour. A repetition in experiment was conducted to confirm the efficiency of Taguchi where the sensitivity of the optimized AAO-DNA biosensor electrode was successfully improved to 62.57 % at 0.278 kΩ/M (LOD at 6.497 x 10^{-15} M) compared to the nominal. From the third finding through ANOVA analysis, the sequences of the most influential anodizing parameter to the AAO-DNA biosensor sensitivity are following the order: anodizing voltage (34.13%) > anodizing time (29.85%) > temperature (20.27%) > electrolyte concentration (15.74%). At the end of this research, optimization of the synthesis of AAO thin film electrode by Taguchi method for DNA sensing was successfully achieved. Since this

research had used nonspecific type of DNA target analytes for prototyping development purposed, therefore, it is targeted that this optimized and sensitivity improved AAO thin film electrode will have greater chance to be used widely in the future for detecting various types of DNA, such as dengue, E-coli, or salmonella. Besides, it is of great hope that the outcome from this research may help other researchers to synthesize AAO thin film at its optimum anodizing condition as to improve the sensitivity of the AAO-DNA biosensor.

CHAPTER 1 : INTRODUCTION

1.1 Background

Biosensors have emerged since a few decades ago and its application has become popular primarily in the field of food analysis, control of environmental pollution as well as in the area of human healthcare and diagnostic (Aw, et. al., 2015; Gotoh, et. al., 2014; Rothberg, et. al., 2011; Santos, Kumeria, & Losic, 2013). Since 1996, statistic shows that the world market for biosensor has been continuously increasing up to this date. Unlike conventional methods such as bio-labelled fluorescents technique that requires an extensive preparation as well as expensive label, biosensor on the other hands offers a rapid, label-free and significantly low cost measurement technique. Thus, biosensors help to improve the measurement and detection methods employed by the traditional techniques used previously.

Biosensors can be classified and named based on several criteria such as the transduction principle used (e.g. electrochemical, mass dependent or optical), the types of bio elements used as the receptor (e.g. enzyme, nucleic acid, proteins or oligonucleotides) or the types of analytes that can be detected (e.g. DNA, glucose, toxin or drugs) (Bhalla, Jolly, Formisano, & Estrela, 2016). The most basic biosensor system is usually contains of three elements, namely receptor as the sensing element, transducer as well as processor (Minaei, Saadati, Najafi, & Honari, 2015). A complete illustration on the basic of biosensor system is presented in Figure 1.1.

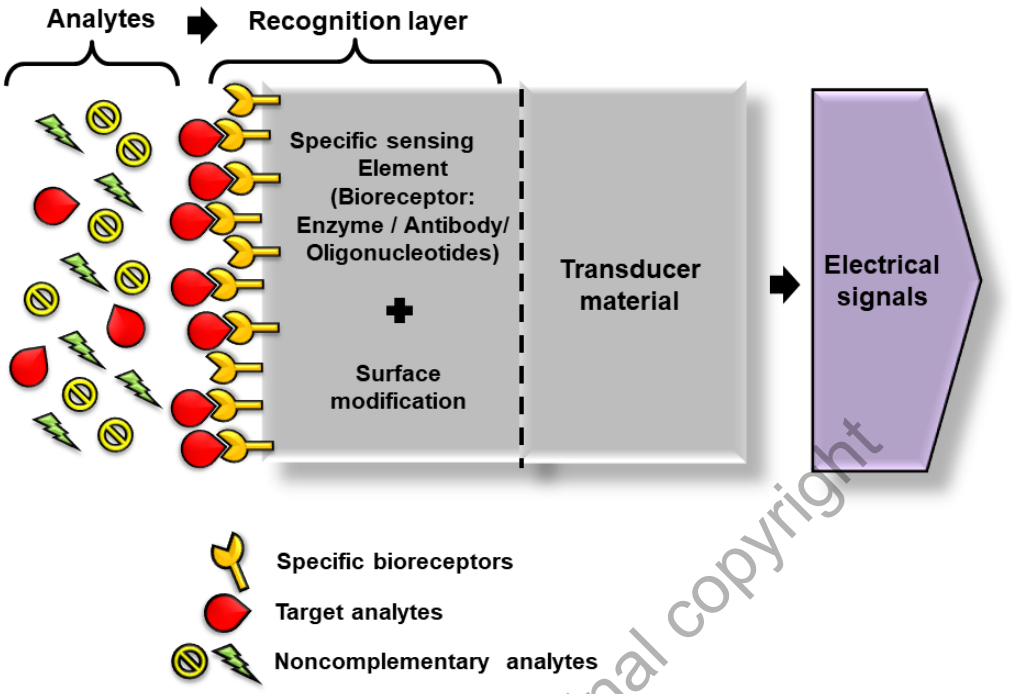

Figure 1.1 Illustration of the basic biosensor system.

Receptor is attached onto the transducer surfaces to form a recognition layer. For example, DNA biosensors use single-stranded DNA (ssDNA) probe containing 20 to 40 base pairs to be functioning as the receptor. Selected sequence of ssDNA probe is then immobilized onto the electrode surfaces by using the surface modification method. Methods that used for immobilization and surface modification are depending on the functionality properties of the electrode surface. During hybridization, receptor will selectively combine with the specific target analytes to release hydrogen ions. At the same time, the recognition layer will thickens and thus increases the resistance of the charge transfer at the transducer surfaces. Depending on the detection method used, hybridization of DNA can be detected by measuring analytical signals from the amounts of H^+ released or the changes in the resistance of the charge transfer (R_{ct}) at the transducer surfaces. These analytical signals will then receive by the transducer and converted into electrical signals to be measured. Finally, electrical signals will be