DEVELOPMENT OF ELECTROCHEMICAL BIOSENSOR BASED ON NYLON-6 MEMBRANE

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DEVELOPMENT OF ELECTROCHEMICAL BIOSENSOR BASED ON NYLON-6 MEMBRANE

by

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LIST OF SYMBOLS

E_{we}	Applied potential (V)
ca.	Approximately
R_{ct}	Charge transfer resistance (Ohm)
NYL	Commercial nylon-6 membrane
C	Concentration of AA (mM)
I	Current response (mA)
Z_{im}	Imaginary part of Nyquist plots on the *y*-axis
R^2	Linear regression correlation coefficients
I_{max}	Maximum current
V_A	Membrane‟s apparent volume (mm^3)
V_E	Membrane‟s existent volume (mm^3)
K_m	Michaelis–Menten constant (mM)
N-16	Nylon-6 polymer with 16 wt. %
N-18	Nylon-6 polymer with 18 wt. %
N-23	Nylon-6 polymer with 23 wt. %
N-25	Nylon-6 polymer with 25 wt. %
N-28	Nylon-6 polymer with 28 wt. %
N-16A	Nylon-6 membrane (polymer 16 wt. %) with water
N-16B	Nylon-6 membrane (polymer 16 wt. %) with methanol
ε	Porosity of the nylon-6 membranes (%)
Z_{re}	Real part of Nyquist plots on the *x*-axis
I_{ss}	Steady-state current
wt. %	Weight percentage

LIST OF ABBREVATIONS

AA	Ascorbic acid
Ag/AgCl	Silver/silver chloride
AM	Aniline Monomer
APS	Ammonium peroxodisulfate
ANOVA	Analysis of variance
BCA	Bicinchoninic acid assay
b-IgG	Biotinylated goat anti-mouse IgG
b-NHS	N-hydroxysuccinimidobiotin
BSA	Bovine serum albumin
CA	Citric acid
CP	Conducting polymers
CV	Cyclic voltammetry
DHA	Dehydroascorbic acid
DMF	*N, N*-dimethylformamide
DoE	Design of Experiment
DPV	Differential pulse voltammograms
EB	Emeraldine base
EIS	Electrochemical impedance spectroscopy
FESEM	Field emission scanning electron microscope
FTIR	Fourier transform infrared spectroscopy
GA	Glutaraldehyde
GCE	Glassy carbon electrode
HCl	Hydrochloric acid
Fe_2O_3	Iron oxide

LB	Leucoemeraldine base
LOD	Limit of detection
PANI	Polyaniline
PANI/Fe_2O_3	Polyaniline-iron oxide
PB	Pernigraniline base
Pt	Platinum
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
PVDF	Polyvinylidene fluoride
TGA	Thermogravimetric analysis
TEM	Transmission electron microscopy

PEMBANGUNAN BIOPENDERIA ELEKTROKIMIA BERASASKAN MEMBRAN NILON-6

ABSTRAK

Jutaan manusia mati disebabkan ketiadaan dan ketidakbolehcapaian kemudahan diagnostik terutamanya dalam mengawal penyakit-penyakit penting, yang mana membawa kepada pembangunan biopenderia yang progresif. Malangnya, pengoksidaan secara langsung analit sasaran pada elektrod terdedah bagi biopenderia adalah proses tidak boleh balik dan memerlukan upaya lebih yang tinggi, menyebabkan kotoran elektrod dan kepekaan yang rendah. Elektrod yang terubahsuai membran nilon-6 telah dicadangkan untuk mengatasi masalah ini. Sebagai satu immunocerakin, tindakbalas biokimia antara analit sasaran dan tawanan berlaku pada permukaan membran nilon-6, kemudian diterjemahkan kepada isyarat rintangan yang boleh diukur. Kajian ini menjelaskan ciri morfologi bagi nilon-6 sebagai membran penjerapan protin dan pengaruh mereka dalam antaramuka pengecaman biologi. Membran nilon-6 disintesis melalui penyongsangan fasa kering dengan kepekataan berbeza bagi polimer nilon-6 dalam julat 16 wt. % hingga 28 wt. % dan pelbagai jenis bahan tambah (air dan metanol) dalam larutan '*dope*'. Membran nilon-6 yang dibangunkan, N-16B, dengan 16 wt. % nilon-6 polimer dan metanol sebagai bukan pelarut telah memperlihatkan kelajuan sisi penyumbuan yang terpantas (1.07 mm/saat) dan kapasiti penjerapan protin yang sangat baik, (1,650.00 $\pm$ 85.84 µg/cm^3). Kajian semasa mendedahkan kepentingan morfologi membran yang mempengaruhi kepekaan dan keberkesanan peranti pengesan imun. Isu kestabilan dalam penjerapan biomolekul telah diatasi dengan mengintegrasi glutaradehid (GA) ke atas membran nilon-6 sebelum penjerapan

protin. Kajian awal telah dijalankan untuk mengkaji kesan masa inkubasi, pH dan kepekatan GA pada pengikatan protin ke atas polimer nilon-6. Keadaan optimum bagi integrasi GA ditemui pada 40 minit masa inkubasi, pH 7.5 dan 1 wt. % kepekatan GA. Analisis statistik dengan program rekabentuk silang dilakukan dan keadaan integrasi GA optimum dari analisis statistik didapati pada 25 wt. % bagi polimer nilon-6, 75 wt.% campuran pelarut + tak larut, pH 9.0 dan 70 minit masa inkubasi. Keputusan eksperimen menunjukkan bahawa GA sebagai penyambung lintang pada keadaan optimum mampu mencapai sangkutan GA yang lebih baik untuk penjerapan protin yang terakhir. Kajian lanjut telah meneroka penyediaan polianilina-ferum (III) oksida (PANI/Fe_2O_3) yang konduktif yang berfungsi sebagai pemindaharuh isyarat elektrik, untuk menukar interaksi elektrokimia kepada satu isyarat ketahanan yang dapat diukur. PANI disintesis melalui pempolimeran beroksida bagi monomer anilina (AM) dengan kehadiran ammonium persulfat (APS). 0.2 M kepekatan AM and 1:3 nisbah sukatan AM:APS didapati menghasilkan PANI dengan tindakbalas konduktiviti ionik yang tertinggi pada 7.565 $\pm$ 0.262 mS/cm. Kajian telah mengkaji semula aspek menarik bagi keadaan yang berlainan oleh PANI sebagai satu bahan konduktif yang penting untuk peranti elektronik/elektrik. Seterusnya, penderia amperometri berasaskan-membran telah dipasang dan aktiviti elektrokimia antara asid askorbat (AA, analit sasaran) dan askorbat oksidase (analit tawanan) telah dinilai. Had pengesanan bagi penderia didapati pada 5.77 mM dan pemalar *Michaelis-Menten* (K_m) dikira sebanyak 26.76 mM. Tindakbalas bagi spektroskopi galangan elektrokimia (EIS), voltammetry kitaran (CV) dan voltammograms denyutan kebezaan (DPV) dijalankan untuk menganalis dua lapis elektrokimia ke atas elektrod kerja. Membran nilon-6 yang dibangunkan telah menyediakan satu pelantar pengesanan yang menjanjikan untuk

pembinaan penderia dan sesuai untuk aplikasi praktikal dalam analisis farmaseutikal atau klinikal dan tanaman pertanian.

DEVELOPMENT OF ELECTROCHEMICAL BIOSENSOR BASED ON NYLON-6 MEMBRANE

ABSTRACT

Millions of people die due to the unavailability and inaccessible of diagnostics facilities especially in controlling crucial diseases, which led to the progressive development of biosensor. Unfortunately, direct oxidation of target analyte at the bare electrode of a biosensor is an irreversible process and requires a high overpotential, resulted in electrode fouling and low sensitivity. Nylon-6 membrane modified electrodes have been proposed to overcome this problem. As an immunoassay, the biochemical reaction between target and capture analyte takes place on the surface of the nylon-6 membrane, then translated to measurable resistance signal. The present study elucidates the morphology characteristic of nylon-6 as protein immobilization membrane and their influences in biological recognition interface. The nylon-6 membranes were synthesized via dry phase inversion with different concentration of nylon-6 polymer in a range of 16 wt. % to 28 wt.% and different types of additives (water and methanol) in dope solution. The developed nylon-6 membrane, N-16B, with 16 wt. % nylon-6 polymer and methanol as non-solvent had demonstrated the fastest lateral wicking speed (1.07 mm/sec) and excellent protein immobilization capacity (1,650.00 $\pm$ 85.84 μg/cm^3). The current study revealed the importance of membrane morphology that affects the sensitivity and effectiveness of an immuno-sensing device. The stability issue in biomolecule immobilization has been overcomed by integrating glutaraldehyde (GA) onto nylon-6 membrane prior to protein immobilization. The preliminary study was carried out to study the effect of incubation time, pH and concentration of GA on protein

binding of the nylon-6 polymer. The optimum conditions of GA integration were found at 40 minutes of incubation time, pH 7.5 and 1 wt. % of GA concentration. Statistical analysis using crossed design programme was performed and the optimum GA integration conditions from the statistical analysis were found at 25 wt. % of the nylon-6 polymer, 75 wt. % of mixture solvent + nonsolvent, pH 9.0 and 70 minutes of incubation time. The experimental results showed that the GA as a cross-linker reagent at optimum conditions was able to achieve better GA attachment for latter protein immobilization. Further study has been explored on the preparation of conductive polyaniline-iron oxide (PANI/Fe_2O_3) that served as the electrical signal transducer, to convert the electrochemical interactions to a measurable resistance signal. PANI was synthesized via oxidative polymerization of aniline monomer (AM) in the presence of ammonium persulfate (APS). 0.2 M concentration of AM and 1:3 volume ratio of AM:APS were found to produce PANI logged with the highest ionic conductivity response at 7.565 $\pm$ 0.262 mS/cm. The study had reviewed the interesting aspect of different state of PANI as one of the important conducting material for electronic/electrical devices. Subsequently, a membrane-based amperometric sensor was assembled and electrochemical activities ascorbic acid (AA, target analyte) and ascorbate oxidase (capture analyte) were evaluated. The detection limit of the sensor was found at 5.77 mM and the Michaelis–Menten constant (K_m) was calculated as 26.76 mM. Electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and differential pulse voltammograms (DPV) responses were carried out to analyze the electrochemical double layer on the working electrode. The developed nylon-6 membrane has provided a promising detection platform for sensor construction and suitable for practical application in pharmaceutical or clinical analysis and agricultural crops.

CHAPTER 1

INTRODUCTION

1.1 Membrane as biological recognition interface for immunosensor

Modern technology has achieved better enhancement and sophistication in providing treatment, monitoring and controlling the spread of crucial diseases, especially those related to waterborne and foodborne outbreaks (Ivnitski *et al.*, 2000, Low *et al.*, 2012). Due to the unavailability or inaccessibility of diagnostic facilities, a rapid construction, on-site examination, and user-friendly detection system is progressively developed to overcome such problem. Under such a scenario, immunosensor has emerged as a potential detection system for an effective monitoring of pathogen (Donmez *et al.*, 2015, Hosseini *et al.*, 2014, Kolosovas-Machuca *et al.*, 2015, Nandakumar *et al.*, 2008, Pohanka *et al.*, 2007, Waiyapoka *et al.*, 2015), contaminant and toxic material in the biotechnology applications (Grover *et al.*, 2015, Hsiue *et al.*, 2004, Khaksarinejad *et al.*, 2015, Srivastava *et al.*, 2014, Zahedi *et al.*, 2016). The biomolecules immobilization is the key factor that determined the sophisticated development of an immunosensors (Dong *et al.*, 2013, García *et al.*, 2012).

The effectiveness of biomolecule immobilization strongly depends on the membrane material (the detection platform) (Betty, 2016, Sadeghi *et al.*, 2015) that provides excellent stability, fast lateral wicking speed and reduces biomolecule desorption (Yong *et al.*, 2010). As an immunoassay, the biochemical reaction between antigen and antibody takes place on the surface of the porous membrane, which is greatly influenced by the membrane morphology and its intrinsic chemical composition. The membrane material should be insoluble in water and has a high

binding capacity to the specifically targeted biomolecules (Pundir *et al.*, 2008). By manipulating the structure of the membrane support, a sensor's bio-catalytic efficiency can be easily achieved to produce an effective and accurate immunological analysis (Ahmad *et al.*, 2016). Recently, polymeric porous membranes offer the most available support material for recognition interface in biosensing applications. The drive to improve the response signal, increase sensitivity, lower detection limit and better reproducibility are the reason of the growing need of the polymeric porous membrane (Luo *et al.*, 2017, Santhy *et al.*, 2018, Tang *et al.*, 2019). Polyvinyl alcohol (PVA) (Braiek *et al.*, 2018, Luo *et al.*, 2017), polyvinyl chloride (PVC) (Afkhami *et al.*, 2014, Ezzeldin *et al.*, 2012, K Gupta *et al.*, 2011, Santhy *et al.*, 2018), polysulfone (Zhao *et al.*, 2016), nylon-6 (Tang *et al.*, 2019, Yaqoob *et al.*, 2016) and polyvinylidene fluoride (PVDF) (Chen *et al.*, 2015, Zhao *et al.*, 2015) are the most potential polymeric porous membrane in development of biosensing application.

Among these polymeric membranes, nylon-6 is the most desirable material and is proven to be a suitable immobilization platform (Jackeray *et al.*, 2010). This polymer comprises of high mechanical strength (Salapare *et al.*, 2015, Shakaib *et al.*, 2013, Zhou *et al.*, 2013), good stability and high resistance towards microbial attack (Pant *et al.*, 2013b). Nylon-6 membrane is commonly produced by phase inversion technique through immersion precipitation (Chang *et al.*, 2013, Lin *et al.*, 2002, Shakaib *et al.*, 2013, Shih *et al.*, 2012, Sobhanipour and Karimi, 2013) or dry phase inversion (Fatarella *et al.*, 2014, Leo *et al.*, 2011, Pant *et al.*, 2013a, Pant *et al.*, 2013b, Yan *et al.*, 2007). Liu *et al.* (2013), reported the use of nylon-6 membrane as a porous matrix to immobilize antibody in the detection of two main serum biomarkers for down syndrome, which were β-human chorionic gonadotrophin (β-

HCG) and α-fetal protein (AFP). The nylon-6 membrane was chosen as the sensor's detection platform because of its large total surface area, high mechanical strength, and good biocompatibility. Antibodies were first immobilized onto the nylon-6 membrane surface, so as to capture the target antigens to form the immunocomplexes (antibodies–antigens) prior to react with the labelled antibodies to form the triplex "sandwich" structures. Then the target antigen will be detected through photoluminescence. A low detection limit of the biosensor at 1×10^{-6} IU/L for β-HCG and 1 ng/mL for AFP were detected within the short assay time (Liu *et al.*, 2013).

Biosensor is the most effective method in monitoring and detection of target interest. In biosensing application, biomolecules immobilization is the key factor to determine the effectiveness of the developed biosensor. Biomolecule immobilization strongly depends on the membrane material (the detection platform). Polymeric membrane is the most desirable material for biomolecules immobilization. Among of the polymeric membrane, nylon-6 has proved to be an effective detection platform. However, due to the lower hydrophilicity of the membrane, had limit the usage of the membrane in biosensing application.

1.2 Challenges of protein immobilization

Immobilization process is defined as the attachment of biomolecules to a substrate surface resulting in a reduction or loss of mobility (Rusmini *et al.*, 2007). The attachment of the protein onto a surface should not affect the protein conformation and its function for fully retain biological activity. There are many immobilization techniques, includes physical adsorption, covalent and bioaffinity