*Int. J. Electrochem. Sci.*, 11 **(2016)** 872 - 881

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

# Development of Impedimetric Immunosensor for The Detection of Amyloid Beta (1- 40) on maβa/*ortho*-Polyphenylenediamine Modified Platinum Micro Disk Electrode

*Z. Norazreen and M.Z. Zainiharyati*[*]

Faculty of Applied Sciences, Universiti Teknologi MARA 40450 Shah Alam Selangor, MALAYSIA
[*]E-mail: zainihar@salam.uitm.edu.my

*Received:* 17 April 2015 / *Accepted:* 10 September 2015 / *Published:* 1 January 2016

The development of an electrochemical detection of amyloid beta (Aβ) (1-40) peptides as a bio marker of Alzheimer's disease (AD) was carried out on a Pt disk microelectrode. The electrode was modified with poly-ortho- Phenylenediamine (PPD) and mouse monoclonal beta amyloid antibody (mAβA) was immobilized to form Pt/PPD/mAβA immunosensor. The measurements were done in Phosphate Buffer Solution (PBS) by applying Electrochemical Impedance Spectroscopy (EIS) technique. The detection is evaluated by the changes in the Nyquist spectra and values of charge transfer resistance (Rct) that were extracted from a modified Randles equivalent circuit. A semi-circle Nyquist plot was observed in the presence of Aβ (1-40) whereas a linear spectra were exhibited in the absence of Aβ (1-40). In addition, as the concentrations of Aβ (1-40) solution increased, the diameter of Nyquist plots was also increased. Surface morphology of Aβ (1-40) adsorbed on microelectrode was observed using Field-emission Scanning Electron Microscope (FESEM) and Optical Microscopic. Based on these findings, a promising in vivo immunosensor for Aβ (1-40) detection that can be used as an alternative in monitoring inhibitors of Aβ (1-40) aggregation research.

**Keywords:** Electrochemical Impedance Spectroscopy, Amyloid Beta, Alzheimer's disease, Platinum, Biosensor.

## 1. INTRODUCTION

Fibrillogenisis is the main pathological effect observed in AD. AD is triggered due to the shrinkage of neurons, followed by several steps of amyloid precursor protein (APP) cleavage process. This cleavage produces toxic proteins which resulted in the accumulation of the amyloid plaques in the brain and it is also known as amyloid beta protein [1]. However, studies on this protein become critical since the information on the identification of the toxic species, the mechanism of its formation and

how it polymerized into fibrils are still not well understood [2]. Previously, there are some reported techniques in studying the fibril formation includes circular dichroism [3], fluorescence spectroscopy [4], electron microscopy [5], dynamic light scattering [6], centrifuging and mass-based analyses, black lipid membranes, microscopy technique and Nuclear Magnetic Resonance (NMR) have been well reviewed [7]. In addition, the most common conventional laboratory technique for Aβ (1-40) determination is ELISA-type assays [8]. However, results obtained from the above might require expensive instrument and tedious sample preparation step.

On the contrary, immunosensor technology would offer a wider platform to study the degeneration of proteins in brain [9]. Generally, immunosensor is prepared by immobilizing a bio recognition molecular on a substrate. Then, this bio recognition (here we used mAβA) will recognize an antigen (Aβ (1-40)) according to its specificity [10]. Most of immunosensors were built based on indirect method. One of the disadvantages of working on indirect method is the requirement of electron transfer agents layered on an electrode surface to enhance the sensitivity which leads towards a more complex electrode surface preparation and time consuming [10]. Meanwhile, a direct method also known as label free assay is one of the alternatives to be used due to rapid, simple and lower cost of field detection [11-12]. Therefore, one from the electrochemical technique that can be applied for this immunosensor is EIS. EIS technique is one of a direct method that could offer an alternate approach for the detection of antigen-antibody reaction which permits real-time detection and capable to be used for the detection of a wide variety of biomolecules [13]. Besides that, EIS provides important informations on the electrical properties of many biological systems. EIS function is to measure the changes of interfacial properties of the electrode surface and the resistance or capacitance changes which referring to the antibody-antigen reaction [13]. Hence, this feature is applicable to monitor the morphology, viability and environmental changes of the system [14].

The aim of this work is to develop an immunosensor for the detection of Aβ (1-40) on PPD modified Pt microelectrode. Pt surface modification with PPD and the PPD performance as reliable conductive polymer towards the application in immunosensor have been demonstrated in previous work [15]. Here, we outlined the findings which offer a simpler preparation step producing a labeless immunosensor for specific target towards Aβ (1-40). To the best of our knowledge, the detection of Aβ (1-40) based on modified micro platinum electrode using EIS has not yet been reported. Here, the effect of potential applied, mAβA-Aß (1-40) interaction, the effect of the concentration of Aβ (1-40) as well as analyzing the EIS equivalent circuit are reported.

## 2. EXPERIMENTAL

### *2.1 Reagents and Chemicals*

Ortho-phenylenediamine (o-PD), Sodium Chloride (NaCl), Sodium Phosphate monobasic (NaH2PO4), Glutaraldehyde (GA), Glutamate (Glu), Bolvine Serum Albumin (BSA), Nafion (Naf) and horseradish peroxide (HRP) were purchased from Sigma Aldrich (Germany). Sodium Hydroxide (NaOH) was purchased from Merck (Germany). All chemicals were used without further purification.

Beta amyloid fragment (1-40) (1.0 mg) and mouse monoclonal beta amyloid antibody (mAβA) (1.5 mg/mL) were purchased from Sigma Aldrich.

### *2.2 Preparation of standard Aβ (1-40) solution*

1 mg/mL of standard Aβ (1-40) solution was prepared by diluting 1 mg of Aβ (1-40) peptide with 1.0 L of ultra-pure water. Then, approximately 0.5 mL of prepared standard Aβ (1-40) peptide was mixed with 0.5 mL of PBS solution. This standard was used during this work. The Aβ (1-40) standard solution was kept at -0.2 $^{o}$C when not in used.

### *2.3 Preparation of 100 μM antibody from mAβA.*

A 1.5 mg/mL mAβA produced in mouse was purchased from Sigma Aldrich (Germany). An optimum studied on the optimum concentration of mAβA immobilized on Pt/PPD's surface was 1.0 mm. This standard monoclonal mAβA was diluted with PBS solution at pH 7.4 to make a 100 μM of new mAβA concentration. Then, this mAβA was incubated for 5 minutes for stabilization before been used.

### *2.4 Electrochemical Analysis*

EIS and Cyclic Voltammetry (CV) measurements were performed using Autolab PG STAT302 potentiostat/galvanostat (Eco Chemie, Netherlands) controlled by Nova 1.10 electrochemical software. The electrochemical cell was set up using three electrodes. 99.9% Teflon coated Pt micro with internal diameter of 50 μm (Advent Material, UK) acted as working electrode as described elsewhere [16]. Approximately 4 cm length of the bare electrode was cut using a scalpel. Next, about 0.5 cm of Teflon coated at the end of the Pt electrode was removed in order to expose the bare Pt inside it. Then, this end was soldered in the gold connector. Other requirements to set up were Pt rod as the counter electrode and Ag/AgCl as the reference electrode.

Electropolymerization on Pt electrode was done in o-PD solution (10 mM) at constant potential +0.7 V by Chronoamperometry as described from previous paper [15, 17]. CV scan was varied from -1.0 V to +1.0 V with a scan rate at 0.01 V/s. While Impedance measurements were performed at voltage perturbation of 5 mV over a wide frequency range from 0.1 Hz to 100 kHz at 50 numbers of frequencies. The resulting impedance data are displayed as a Nyquist plot as a function of real component of impedance (Z') and imaginary component of impedance (Z"). The experiment data was interpreted by lying out or fitted with the equivalent circuit which is a useful tool in interpreting EIS data in terms of electrical components that matches with the system's behavior.

All the experiments were performed in Phosphate Buffer Solution (PBS). Meanwhile, the surface morphology of Aβ (1-40) on the sensor was observed using FESEM, Carl Zeiss SMT Supra 40VP and Optical microscopic.

## 3. RESULTS AND DISCUSSION

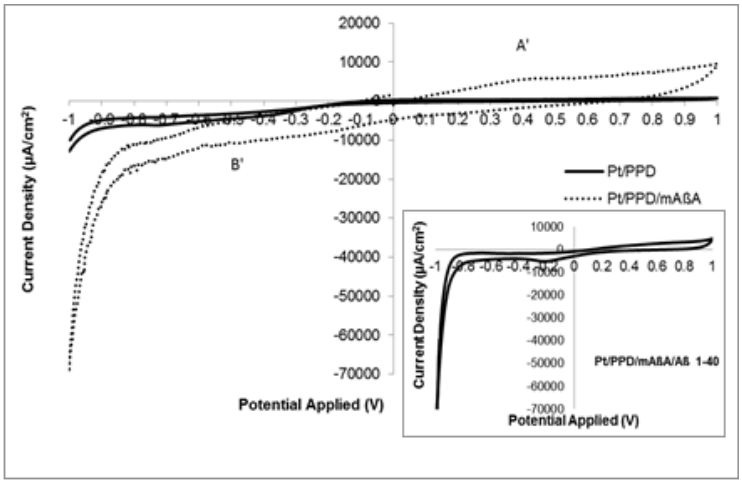
### *3.1 Determination of Potential Applied For EIS System*

The CV technique is used in order to determine the potential applied in EIS. This work is studied on how a changes in the voltametric behavior of an antibody immobilized on PPD polymer was investigated in a PBS (pH 7.2) solution. Figure 1(a) shows the increasing of current density after immobilization of mAßA on Pt/PPD. The figure is indicates a small peak (A') located at + 0.35 to + 0.40 V when the potential was applied between -1.0 to +1.0 V. Meanwhile the B' was proposed to be between -0.2 to -0.5 V. Then, the immunosensor was conducted in the PBS solution containing Aβ (1-40) solution. Upon exposure to PBS electrolyte containing Aβ (1-40) solution, the appearance of small peak at potential -0.2 V appeared as shown in Figure 1(a) insert. Therefore, this voltametric behavior can be applied to determine the optimum potential applied in EIS study.

Based on the cyclic voltammogram in Figure 1 (a), in the particular potentials of +0.4 V, + 0.35 V, + 0.2 V, -0.3 V and -0.2 V were selected for EIS experiment. Figure 1(b) shows the trend of the Nyquist plot which is represented of capacitive behavior (bode phase (0) > 900) at negative potential and a diffusion behavior at positive potential at the lower of frequency.

Nyquist plots appeared as a straight line (at lower frequency < 10Hz) can be categorized as a diffusion behavior. At potential -0.2 V, a large semi-circle obtained due to the charge transfer occurred as the Aβ (1-40) was present in the solution. The occurrence of charge transfer resistance at -0.2 V explained that the mAβA was interacting with the Aβ (1-40) monomer at the electrode surface and maintaining the charged of interaction. Nevertheless, a non-perfect semi-circle appeared at -0.3 V was due to the fast rate of charge transfer and a CV result from Fig. 1(a) insert proved that after -0.2 V, the rate of electron transfer start to become stabilized.

Combination of EIS and CV are due to the fact that impedance spectra are recorded under transient conditions of CV and kinetic parameters based on electrode-equivalent circuit models are obtained as functions of CV scans [18]. Therefore, the optimum potential applied throughout this experiment was determined at -0.2 V.



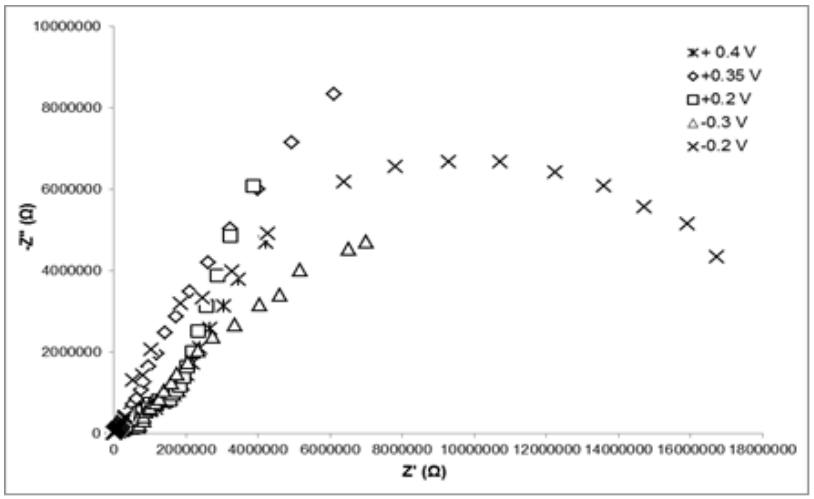



**Figure 1.** Results corresponding to (a) Cylic voltammogram between Pt/PPD electrode and Pt/PPD/mAßA; insert: Pt/PPD/mAßA after introducing an Aß (1-40) solution in PBS solution; and (b) Nyquist spectra obtained at the different of potentials applied.

*3.2 Impedimetric Immunosensor Performance*

Pt/PPD/mAßA as a probe for the detection of Aß (1-40) were successfully prepared as a sensitive immunosensor. The experiment was controlled by using EIS after preparation of the Pt modified surface. The measurements were done in PBS solution.

Figure 2 shows the impedance responses of the (1) Pt/PPD electrode, (2) Pt/PPD/ immobilized mAßA and (3) Pt/PPD/ mAßA/Aß 1-40 that have been spiked in the PBS solution. The Pt/PPD electrode exhibit almost as straight line of the impedance spectra which indicating the characteristic for a limiting step of the electrochemical process (Figure 2, curve 1). On the other hand, after immobilization of mAßA a small semi-circle appeared at the higher frequency which indicated a lower charge transfer resistance at the electrode interface (Figure 2, curve 2). However, with the present of Aß (1-40) peptide in PBS solution the impedance turns to a large depressed of semi-circle as increasing material on the surface provided a larger charge transfer resistance. This increasing of diameter of semi-circle indicated a greater blocking effect between the redox probe and the immunosensor surface [19].

The impedance spectra were interpreted by the equivalent circuit that has been extracted by using FRA software. The circuit as displayed in Figure 3 indicates a good agreement between the circuit model and the measurement system [19]. This is due to the lowest chi square ($\chi 2$) obtained, 0.06. The circuit element consists of ohmic resistance representing the electrolyte solution, Rs; the constant phase element, CPE which indicating the depressed semi-circle obtained and the charge transfer resistance, Rct. In this system, the Rs is represented the properties of electrolyte solution and the diffusion of the PBS redox probe. This element is not affected by the modification occurring on the surface of electrode [20].

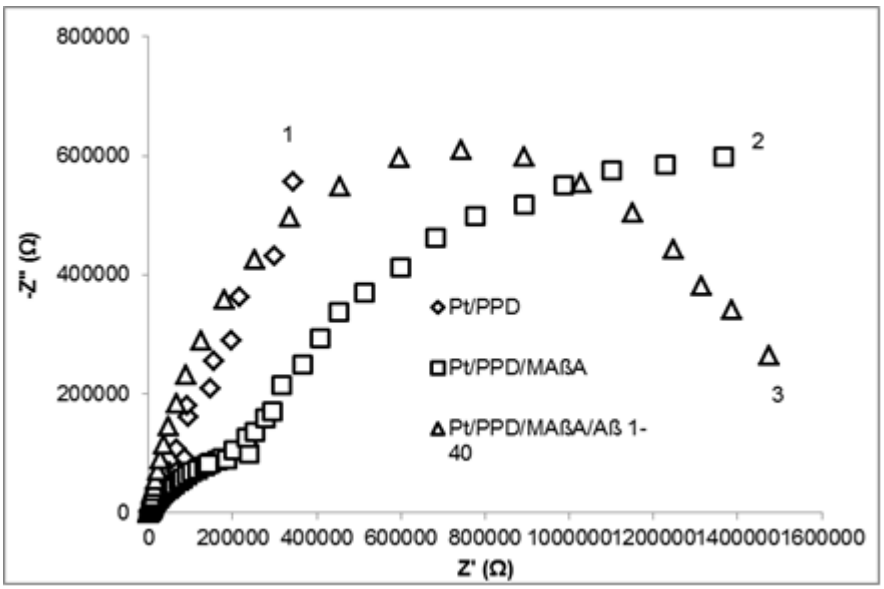



**Figure 2.** Nyquist diagram corresponding to: (1) Pt/PPD electrode, (2) Pt/PPD/mAßA and (3) Pt/PPD/mAßA/Aß (1-40).

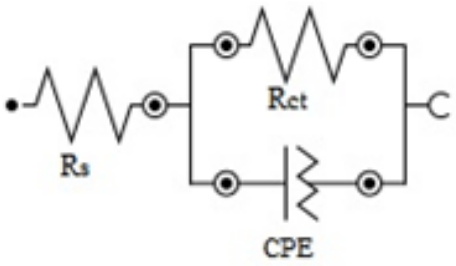



**Figure 3.** An equivalent circuit fitted with the impedance measurement in Figure 2 above.

*3.3 Effect Concentrations of Aß (1-40) towards on Pt/PPD/mAßA*

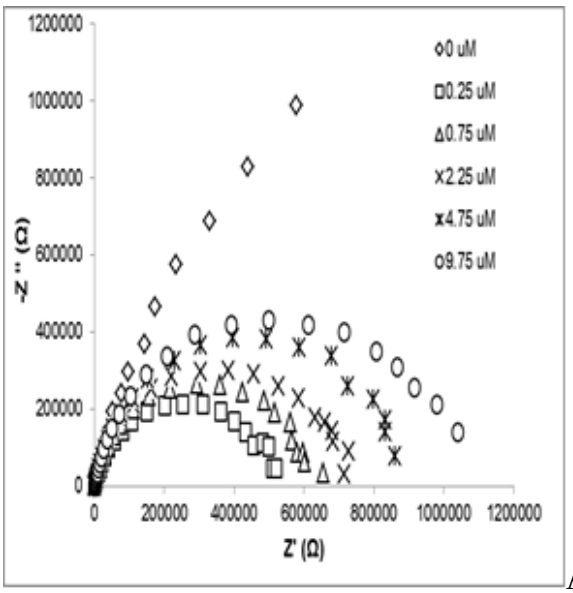
Impedimetric data were collected for the Pt/PPD/mAβA/ Aβ (1-40) immunosensor between 10 kHz -10 Hz in PBS, pH 7.4, in a buffer containing no antigen, as a baseline trace. This experiment was carried out in triplicate for reproducibility. Figure 4 shows the impedimetric data presented in the form of a Nyquist plot depicting the real (Z') and imaginary (Z'') components of the AC impedance analysis when the Pt/PPD/mAβA/Aβ (1-40) exposed to various concentrations of Aβ (1-40) standard solution (0.25 – 9.75µM).

Generally, the changes in the semicircle diameter resulted in the change of the Rct. So, it can also be deduced that a charge-transfer mechanism between the interaction of mAβA-Aβ(1-40) has occurred. As seen from Fig. 4, it is apparent that the real Z' component and the imaginary -Z'' component of impedance both increase with decreasing frequency from the baseline trace [24]. The cause of this increasing order is because of the saturation effect of mAβA binding site to adsorb more Aβ (1-40) [21, 22].

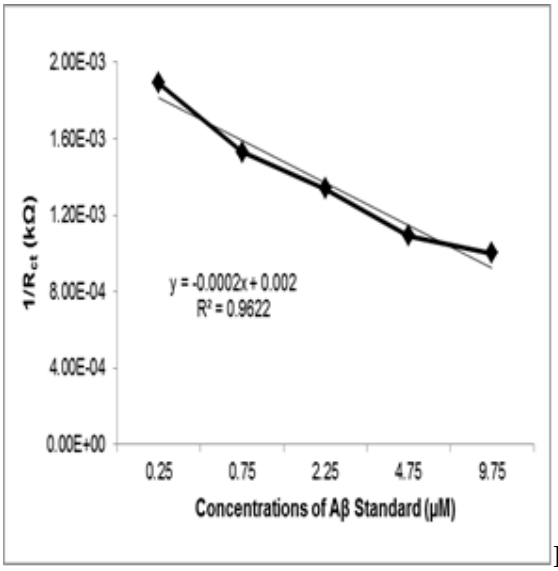
The Rct values were extracted from the equivalent circuit as shown if Figure 3 and a calibration graph was plotted corresponding of 1/Rct (kΩ) Vs. concentration of Aβ (1-40) standard (μM). As witnessed in the Figure 4(b), the 1/Rct was linearly increased with increasing the concentration of Aβ 1-40 standard. Moreover, a perfect linear plot obtained, conclude that the standard solution was suitable used for this immunosensor which is in agreement with Equation [1]:

$$R_{ct} = \frac{RT}{(n^2F^2AK_{ct}[S])} \quad (1)$$

Where, $K_{ct}$ is the potential dependent charge transfer rate constant, [S] is the concentration of the analyte and the other symbols have their usual meanings. In addition, the lowest of detection limit recorded by using the impedance technique was 0.25 μM [23].




A




B

**Figure 4.** (a) The Impedance spectra of Real Impedance (Z') vs Imaginary Impedance (-Z") of different Aβ (1-40) concentrations solution in PBS, pH 7.4. (b) A calibration plot corresponding to the concentration of (Aβ 1-40) solution vs 1/Rct (MΩ) and the insert was a Randles circuit applied to get the Rct values that matched with the Nyquist plots above.

Remarkably, Pt/PPD/ mAβA microelectrode was also tested on difference interferences such as glutamate (Glu), Bolvine Serum Albumin (BSA), Aβ (1-42) standard and Glu. We found that the EIS respond of these interferences did not exhibited the similar Nyquist spectra as Aβ (1-40). Most of them show a lower values of capacitance. Figure 5 shows the selectivity of this immunosensor towards the variable of proteins detection. The result concluded that this immunosensor is highly selective upon Aß (1-40) detection.

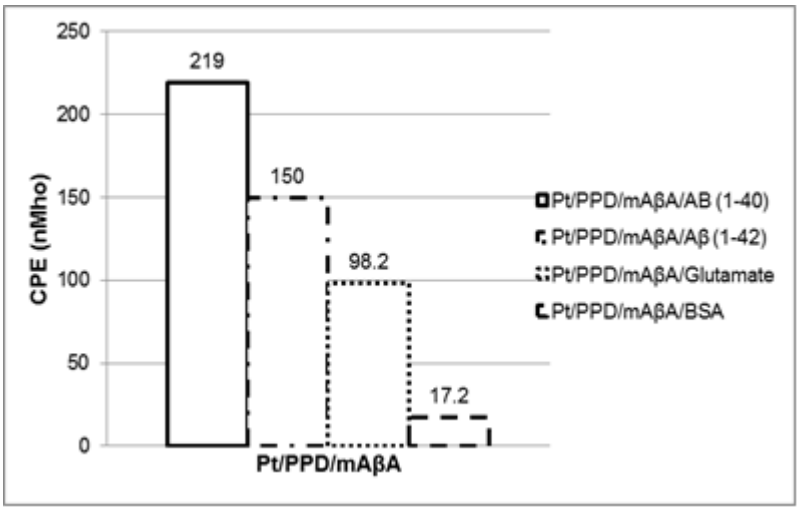
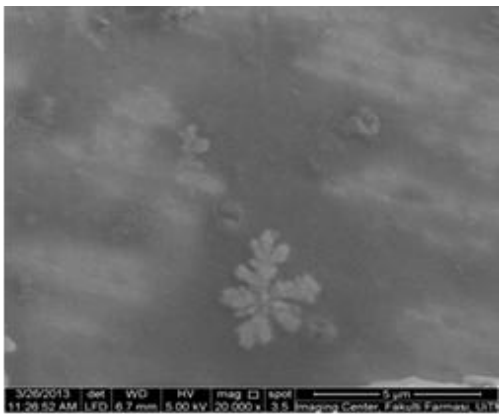



**Figure 5.** A chart of immobilized the MAβA on Pt/PPD Vs CPE (nMho) values in different types of proteins.

*3.4 Morphology of Aβ monomer*

The morphology of Aβ monomer that had adsorbed on the sensor surface was captured using FESEM and an optical microscopic. The immunosensor was incubated in the PBS solution containing Aβ (1-40) standard solution for a day. Then, the sensor surface was exposed under FESEM to observe the adsorbed Aβ (1-40) on Pt/PPD/ mAβA microelectrode surface.

A 

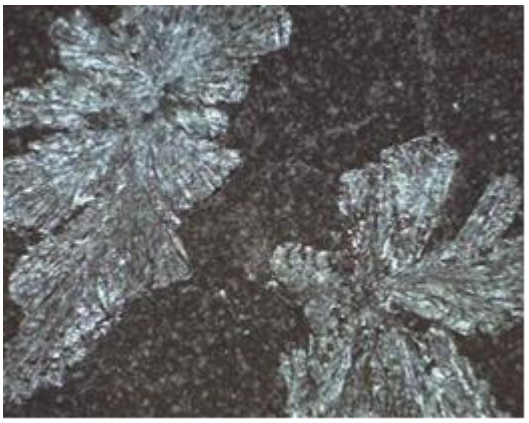
B 

**Figure 6.** The morphology of Aβ monomer has been observed using (A) FESEM, at 20 K magnification and (B) Optical microscopic at 50 x magnification.

## 4. CONCLUSION

In this work, a simple, sensitive and fast electrochemical immunosensor for Alzheimer's disease biomarkers is presented. The changes signal from impedance generated due to the detection of Aβ 1-40 peptide was demonstrated to be practicable in the development of a direct, one-step amyloid beta biosensor. The reagentless detection process based on the inherent adsorption of Aβ (1-40) peptide was simplified for the sensor utilization.

ACKNOWLEDGMENT
Authors would like to thank MyBrain15 scholarship awarded to Z. Norazreen from Ministry Of Education, Malaysia for her financial support.

## References

1. D. Brambilla, B. L. Droumaguet, J. Nicolas, S. H. Hashemi, L. P. Wu, S. M. Moghimi, P. Couvreur and K. Andrieux, *Nanomedicine; NBM,* 7 (2011) 521.
2. M. Fändrich, M. Schmidt, and N. Grigorieff, *Trends Biochem. Sci.*, 36 (2011) 338.
3. A. R. Salomon, K. J. Marcinowski, R. P. Friedland and M. G. Zagorski, *Biochemistry*, 35 (1996) 13568.
4. L. O. Tjernberg, D. J. E. Callaway, A. Tjernberg, S. Hahne, C. Lilliehöök, L. Terenius, J. Thyberg and C. Nordstedt, *J. Biol. Chem*., 274 (1999) 12619.
5. M. Bartolini, M. Naldi, J. Fiori, F. Valle, F. Biscarini, D. V. Nicolau and V. Andrisano, *Anal. Biochem.*, 414 (2011) 215.
6. A. M. Streets, Y. Sourigues, R. R. Kopito, R. Melki and S. R. Quake, *PloS One*, 8 (2013) e54541.
7. R. Jelinek and T. Sheynis, *Curr. Protein Pept. Sci.*, 11 (2010) 1.
8. S. Prabhulkar, R. Piatyszek, J. R. Cirrito, Z. Z Wu and C. Z. Li, *J. Neurochem.*, 122(2012) 374.
9. M. A. Cooper, *Label-Free Biosensors Techniques and Applications*, Cambridge University Press, New York (2009).
10. F. Darain, D. S. Park, J. S. Park and Y. B. Shim, *Biosens. Bioelectron.*, 19 (2004) 1245.

11. M. Vestergaard, K. Kerman and E. Tamiya, *Sens.*, 7 (2007) 3442.
12. D. W. Kimmel, G. LeBlanc, M. E. Meschievitz, and D. E. Cliffel, *Anal. Chem.*, 84 (2012) 685.
13. E. Katz and I. Willner. , *Electroanal.*, 15 (2003) 913.
14. X. Jiang, L. Tan, B. Zhang, Y. Zhang, H. Tang, Q. Xie and S. Yao, *Sens. Actuators, B*, 149 (2010) 87.
15. Z. M. Zain and N. Zakaria, *MJAS*, 18 (2014) 107.
16. J. H. Han, H. Boo, S. Park and T. D. Chung, *Electrochim. Acta*, 52 (2006) 1788.
17. A. A. Ariffin, R. D. O. Neill, M. Z. A. Yahya and Z. M. Zain, *Int. J. Electrochem. Sci.*, 7 (2012) 1015.
18. C. M. Pettit, P. C. Goonetilleke, C. M. Sulyma and D. Roy, *Anal. Chem.*, 78 (2006) 3723.
19. J. V. Rushworth, A. Ahmed, H. H. Griffiths, N. M. Pollock, N. M. Hooper and P. A. Millner, *Biosens. Bioelectron.*, 56 (2014) 83.
20. M. G. Silva, S. Helali, C. Esseghaier, C. E. Suarez, A. Oliva, and A. Abdelghani, *Sens. Actuators, B*, 135 (2008) 206.
21. J. T. L. Belle, K. Bhavsar, A. Fairchild, A. Das, J. Sweeney, T. L. Alford, J. Wang, V. P. Bhavanandan and L. Joshi, *Biosens. Bioelectron.*, 23 (2007) 428.
22. R. Ohno, H. Ohnuki, H. Wang, T. Yokoyama, H. Endo, D. Tsuya and M. Izumi, *Biosens. Bioelectron.*, 40 (2013) 422.
23. G. Chornokur, S. K. Arya, C. Phelan, R. Tanner and S. Bhansali, *J. Sens.*, 2011 (2011) 1.