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HYDROGEN PEROXIDE IMPEDIMETRIC DETECTION ON POLY-ORTHO-PHENYLENEDIAMINE MODIFIED PLATINUM DISK MICROELECTRODE

(Pengesanan Hidrogen Peroksida Secara Impedimetrik Di Atas Elektrod Mikro Cakera Platinum Yang Di Ubah Suai Dengan Poli-Orto-Fenilinadiamina)

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Abstract

This work describes the development of hydrogen peroxide detection based on Poly-ortho-phenylenediamine modified Platinum disk microelectrode (50 µm in diameter). The electrochemical performances of H_2O_2 detection were studied using Chronoamperometry, Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) techniques in Phosphate Buffer Solution (PBS) pH 7.4. Effect of potential, electrode size, and various concentrations of H_2O_2, among others, were investigated by tracking the impedance changes at a specific perturbation frequency. To obtain the Charge transfer resistance (R_{ct}) values, a modified Randles Equivalent Circuit was modelled and fitted to Nyquist Plot. Then, this sensor was further applied in the detection of H_2O_2 in antiseptic mouthwash with percent recovery of 97% ± 0.14 (x10^3 kΩ).

Keywords: Electrochemical Impedance Spectroscopy, Platinum microelectrode, hydrogen peroxide, poly-ortho-phenylendiamine

Abstrak

Kajian ini menerangkan pembangunan pengesanan Hidrogen Peroksida berasaskan modifikasi poli ortho- fenilinadiamina di atas mikro elektrod cakera Platinum pada diameter 50 µm. Prestasi elektrokimia bagi pengesanan Hidrogen Peroksida di kaji menggunakan teknik Kronoamperometri, Voltammetri berkitar, Spektoskopi Rintangan Elektrokimia di dalam larutan penimbal fosfat, pH 7.4. Kesan potensial, saiz elektrod dan kepekatan Hidrogen Peroksida di kaji dengan mengenal pasti perubahan rintangan pada frekuensi tetap. Bagi mendapatkan nilai pemindahan cas rintangan, litar Randles terubah suai di padankan bersama plot Nyquist. Kemudian, sensor ini diaplikasikan untuk mengesan Hidrogen Peroksida di dalam ubat kumur antiseptik dengan peratus pemulihan sebanyak 97% ± 0.14 (x10^3 kΩ).

Kata kunci: : Spektroskopi Rintangan Elektrokimia, mikroelektrod cakera platinum, hidrogen peroksida, poliorthofenilindiamina

Introduction

An impedimetric analysis of hydrogen peroxide (H_2O_2) has been intensively investigated due to the fact that H_2O_2 has wide significance in medicine, food investigation, biological pathway, and industries such as disinfectant, antiseptic, oxidizer and often used in personal usage such as hair dye, conditioner and cosmetics [1]. Detection of H_2O_2 plays crucial role as it is an essential mediator in pharmaceutical, clinical, and environmental research. In addition, it is also a byproduct of highly selective oxidase and an important contaminant in several industrial products and wastes [2]. Many other analytical methods such as UV-Vis [3-4] titrimetry [5] as well as electrochemical techniques have been applied for this purpose [6]. However, electrochemical techniques provide simpler, speed, high sensitivity and stability of the sensing element. Electrochemical impedance spectroscopy (EIS) technique is an interesting alternative because it provides more information about the electrochemical system, for example, envision the true condition that passes from the bulk solution towards the surface of electrode [7].

Nyquist plot is resulted from graphing the real (Z') versus the imaginary (-Z") impedance values. Some information can be well-read about the electrochemical system from the Nyquist Plot [8] as such the spectrum is usually present in a semicircle and a straight line. The semicircle reflects the charge transfer resistance (R_{ct}) which describes the properties of interface electrode at higher frequency. While the straight line describes the diffusion process on the electrode at lower frequency [9]. Impedance data on H_2O_2 detection can be analyzed and determined using the components of the Randles circuit. From this circuit, the a value of charge transfer resistance can be exploited, hence, a calibration graph of $1/R_{ct}$ versus concentration is obtained. A similar study by Zhang *et al.* [10] reported on modified glassy carbon by poly dopamine-hyaluronic acid (PDA-HA) was able to detect H_2O_2. They interpreted that detection of H_2O_2 was observed by degradation of HA on active oxygen material which is observed by the decrement in the semicircle diameter.

Previously, studies on permselective membrane such as Poly-ortho-phenylenediamine (PPD) on sensor application have been investigated [11-14]. Therefore the efficiency of PPD as a coating polymer on this sensor was undoubted to be useful in this work, not only towards H_2O_2 detection, but also 'blocking' other interferences such as ascorbic acid (AA), uric acid and acetaminophen [13-15]. The objective of this study is to examine the characteristics of H_2O_2 detection on Platinum microelectrode deposited with PPD using EIS technique. In addition, in order to evaluate the efficiency of the sensor towards the presence of H_2O_2, this sensor was also practically applied in antiseptic mouthwash which contain of H_2O_2 as one of the ingredients.

Materials and Methods

Reagents and chemicals- Ortho-phenylenediamine (o-PD), Hydrochloric acid (HCl) 37%, and Sodium Phosphate monobasic (NaH_2PO_4) were purchased from Sigma Aldrich (Germany). Sodium Hydroxide (NaOH) was purchased from Merck (Germany). All chemicals were used without further purification. Antiseptic mouthwash brand PolyLab was purchased from a pharmacy outlet. Autolab PG STAT302 potentiostat/galvanostat (Eco Chemie, Netherlands) controlled by Nova 1.7 electrochemical software was employed in this study. The three electrode cell comprised of Ag/AgCl (0.3 KCl) as reference electrode, PPD modified 99.9% Teflon-coated Pt micro (i.d. 25 µm and 50 µm, purchased from Advent Material, UK) electrode as working electrode as described elsewhere [16] and Pt rod as the counter electrode.

Electropolymerization of Pt was done in o-PD solution (10 mM) at constant potential + 0.7 V . The oxidation potential of H_2O_2 was investigated at a constant scan rate 10 mVs^{-1} with potential ranges between -1.0 to +1.0 V using CV. Impedance measurements were performed at voltage perturbation of 10 mV over a wide frequency range from 0.1 Hz to 100 kHz at 30 to 50 numbers of frequencies.

Results and Discussion

Impedimetric characterization of PoPD electropolymerized on Pt surface

Figure 1 showed Nyquist spectra in comparison of bare Pt and modified Pt/PPD in pH 7.4 PBS solution. Pt bare showed a straight line which is further supported by Miao et al [17]. This is due to the diffusion limiting step of the electrochemical process. Meanwhile, modified Pt/PPD showed a small semicircle indicated that the appearance of impedance caused by PPD polymerization on the surface of the electrode. Moreover, the large difference in charge transfer resistance (R_{ct}) value between the presence of PPD on electrode (92.2 kΩ) compared to bare electrode (9.51 kΩ) confirmed that the PPD was successfully electropolymerized on Pt surface.

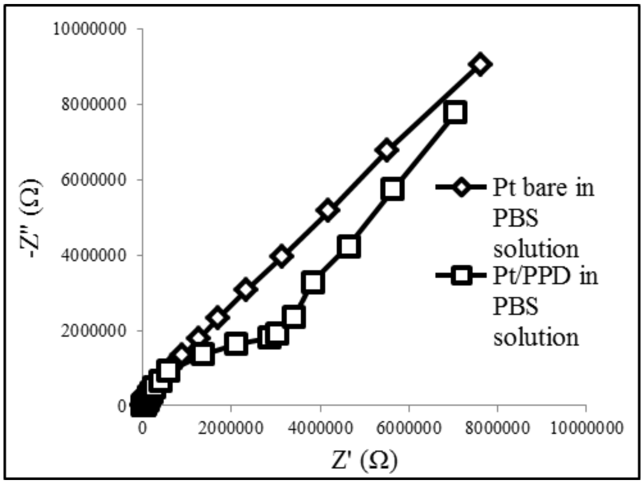

Figure 1: Comparison between Pt bare and Pt/PPD in PBS solution. The appearance of semicircle indicates the formed layer of PPD polymer on Pt surface.

H_2O_2 and AA Amperometric responses

Current response from chronoamperometric oxidation of H_2O_2 at constant +0.7 V potential is shown in Figure 2. This was to determine the efficiency of sensor's behavior towards H_2O_2. From the calibration curve, it produced a linear H_2O_2 calibration in the range from 5 to 100 µM in PBS (pH 7.4) with R^2= 0.9919. The response time ($t_{90\%}$) of the increment H_2O_2 spiked was in 0.3 s which is lower than previously reported work [12, 18] and the lowest detection limit (S/N x 3) was about 3 µM. Meanwhile, Equation (1) was used to define the percentage permeability of sensor (100% ± 1.1 n=4), which was close to the ideal values of 100% [13]. $\%P_{H2O2}$ is other important parameters in sensor fabrication to ensure acceptable H_2O_2 oxidation on the electrode. While, the sensitivity of electrode towards H_2O_2 was represented by the slope of linear calibration graph of H_2O_2 which is 188.1 μAcm^{-2} ± 8.5, n= 4 [15].

$$\%\ P_{H_2O_2} = \frac{\text{Slope Pt/PoPD}}{\text{Slope Pt Bare}} \times 100\% \qquad (1)$$

In order to exclude the nonspecific species adsorption on surface electrode, the amperometric measurement was carried out with AA [19-20] and resulted in no response of AA spiked into a PBS solution for up to 30 minutes. The percentage permeability of sensor towards AA was 0.08% ± 7.4, n=5, further indicated that this sensor is acceptable at blocking the interference species.

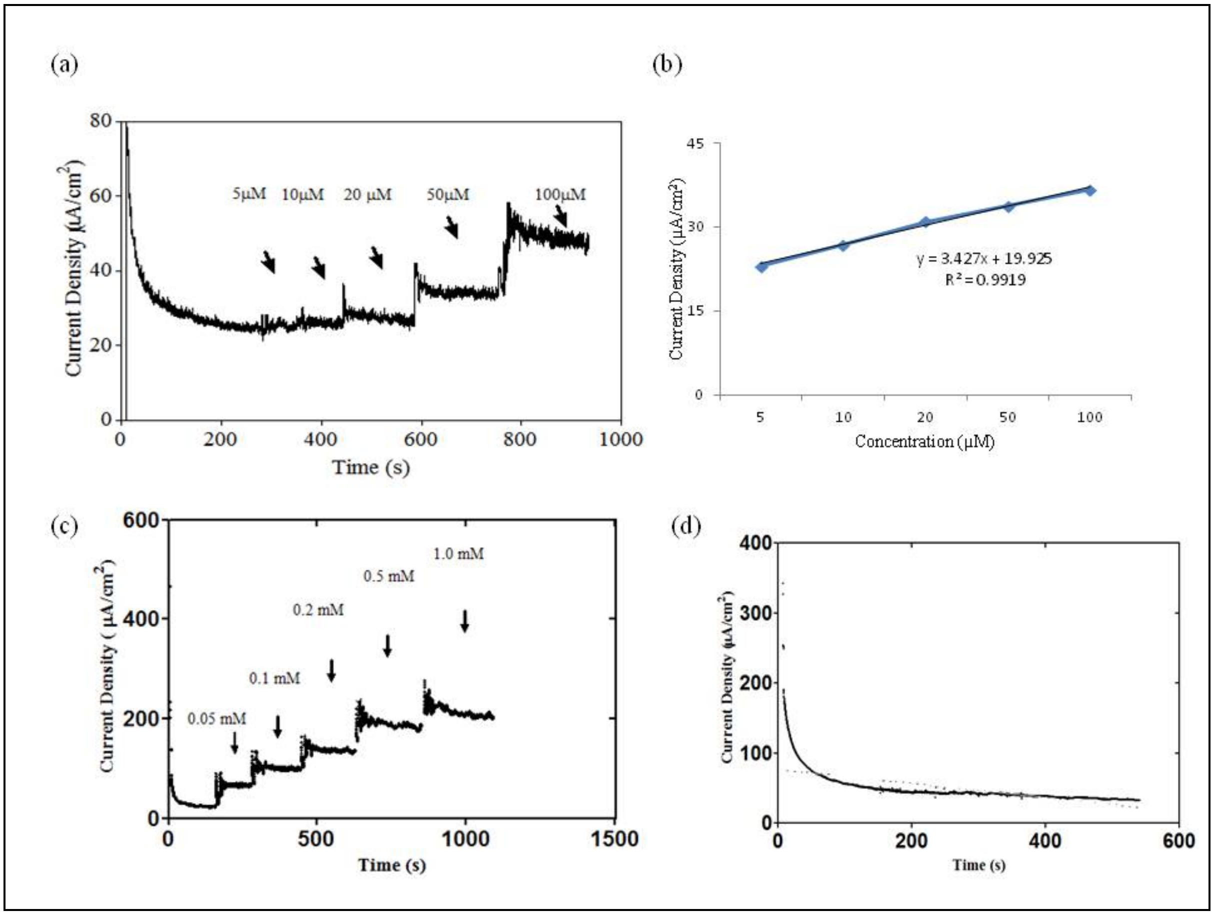

Figure 2: Chronoamperometry of H_2O_2 and AA were added in PBS solution with different range of concentrations.

(a) increasing the current density with increasing the H_2O_2 concentrations (5-100 μM),
(b) linear calibration of Pt/PPD with concentrations of H_2O_2,
(c) different AA concentrations were spiked on bare Pt, and
(d) graph showed there is no signal being detected when AA was spiked in solution after the Pt microelectrode was insulated with PPD.

Effect of potential

The dependent of H_2O_2 oxidation towards applied potential was at +0.4V as shown in Figure 3 and was supported by the others [21- 22]. The trend was also exhibited by EIS result. Hence, the potential of oxidation peak, which is +0.4V was further applied in performing EIS, which is in line with other literature [23].

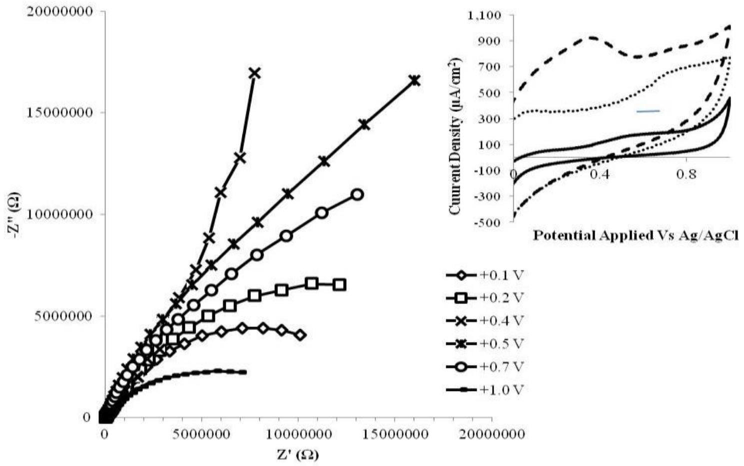

Figure 3: Nyquist plots resulted from the effect of applied potential on EIS measurement by using Pt_{50}/PPD in 5 µM H_2O_2. Inset: CV voltamogram of Pt bare in PBS solution (-),Pt/PoPD in PBS solution(…) and Pt/PPD (- -) spiked in 5 µM H_2O_2 showed the oxidation peak at +0.4 V vs. Ag/AgCl.

Effect of electrode size

Two different electrode sizes were studied in this work. The efficiency of Pt/PPD with internal diameter of 50 µm (Pt_{50}/PPD) and 25 µm (Pt_{25}/PPD) were calibrated with the standard addition of H_2O_2 in PBS solution (pH 7.4) at 30 number of frequency measurement. Figure 4 showed Pt_{25}/PPD gave a straight line in the Nyquist spectrum while Pt_{50}/PPD showed the appearance of a small semi circle which indicates the charge transfer resistance occurred after 5µL of standard H_2O_2 was spiked in solution. This observation is in line to Daniels and Pourrmand [8] which discussed on the reduction of electrode area will increase the capacitor behavior at higher frequency and further resulted in lack of analyte oxidation. Thus, on Pt_{25} /PPD it is difficult to measure H_2O_2 due to higher resistance of the solution (R_s) value (1.1 x 10^8 Ω) as compared to Pt_{50} /PPD (2.4 x 10^7 Ω) and supported by Kartika et al [24] whereby the oxidation of H_2O_2 on Pt_{25}/PPD surface could not be detected. This can be proven by referring to the equation (2),

$$Z_{re} = R_s + \frac{R_{ct} d^2}{d^2 + \omega^2 R_{ct} 2 \, (\varepsilon_r \varepsilon_o A)^2} \tag{2}$$

whereby, Z_{re} is the real impedance, R_s is the resistance of solution and A is the active surface area that is in contact with the electrolyte. From Figure 4, the impedance decreases as the effective area is in the electrolyte increases. Thereby, the active surface area does not play an appreciable role and electrolyte resistance (R_s) is only responsible for impedance response. Hence, Pt_{50} was used as a working electrode throughout this work [25].

Effect of concentration

A range of concentrations from 5 mM to 30 mM of standard H_2O_2 were prepared. The Nyquist plot in Figure 5 was observed, which gave lower detection of standard H_2O_2 [12]. At higher frequency, 10 0000 kHz this electrode behaved as capacitor due to the phase angle from the bode phase was more than 55^o. However, at frequencies less than 10 kHz on bode phase the diffusion process was controlled on electrode surface since the angle of the bode phase was at 40^o. In order to interpret the charge transfer resistance, R_{ct} the impedance spectrum was fitted into a modified equivalent circuit (Figure 6). From the best fitted model we have calculated the charge transfer resistance ($R_{ct)}$ values. Equation (3), gives the relationship between R_{ct} and the concentration of bulk species as below [7]

$$R_{ct} = \frac{\mathrm{RT}}{(\mathrm{n^2 F^2 A K_{ct} [S]})} \tag{3}$$

where, K_{ct} is the potential dependent charge transfer rate constant, [S] is the concentration of the redox species and the other symbols have their usual meanings. We found that as the concentration of H_2O_2 increased, the value of R_{ct} was decreased (583 to 126 kΩ).

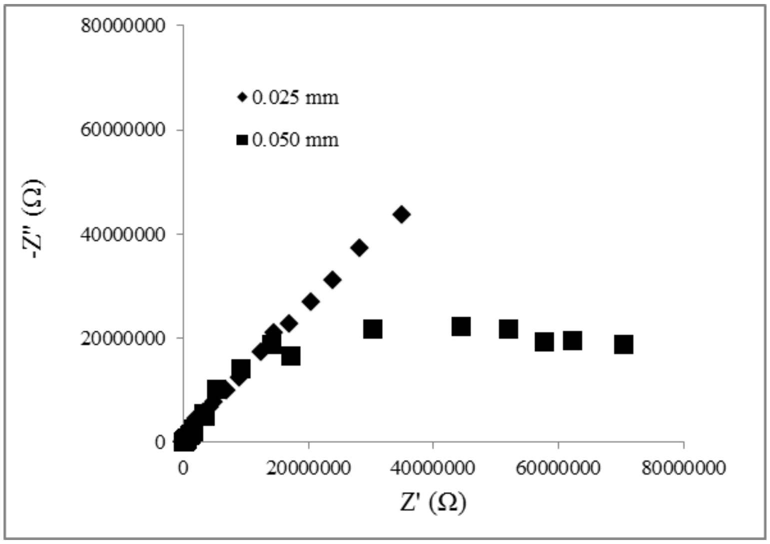

Figure 4: Nyquist spectrums of 5 µM H_2O_2 detected on the electrode diameter of (♦) 25 µm and (▪) 50 µm in PBS solution pH 7.4.

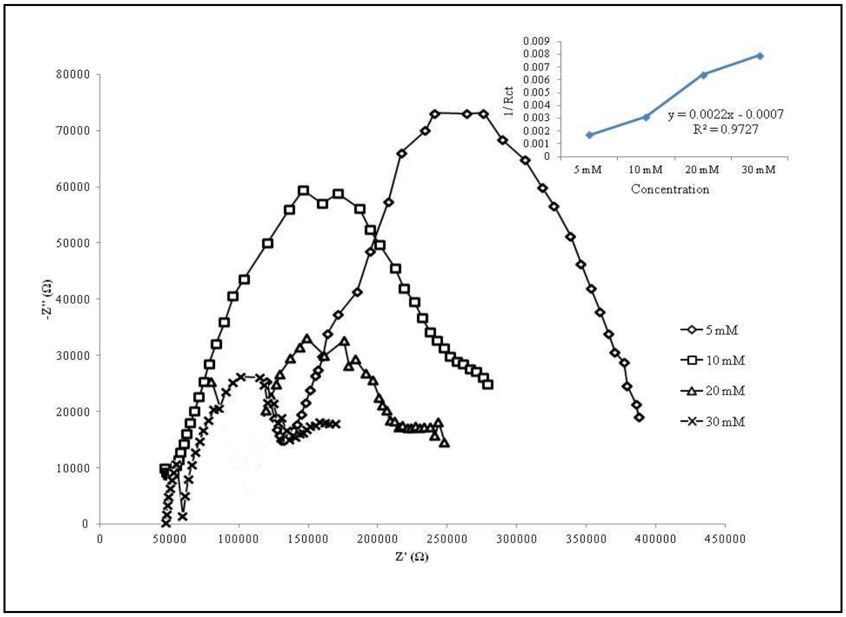

Figure 5: Nyquist plot with corresponding to various concentrations of the standard H_2O_2; inset: Calibration graph of $1/R_{ct}$ Vs Concentration (mM).

Equivalent circuit

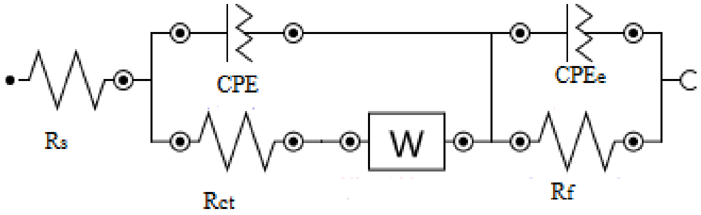

Figure 6: Modified equivalent circuit model for fitting EIS spectra dealing with Pt/PPD micro-electrode immersed in H_2O_2.

R_S is the solution resistance. The constant phase element, CPE is then related to the space charge capacitance at the surface electrode–electrolyte interface. R_{ct} is related to the charge transfer resistance at the surface electrode-electrolyte interface. The Warburg impedance (W) is the mass transfer to the electrode surface.

We proposed this modified Randless circuit in order to fit the impedance spectrum. The equivalent circuit in Figure 6 consisted of a constant phase element (CPE) that is parallel to a resistance and was found to give the best fit to the experimental data. The surface of Pt/PPD was non-homogenous as the crater look feature was described from the previous study [16], therefore standard C_{dl} could not describe the charge properties of the interface sufficiently. In addition, element of Q or CPE was commonly applied to the polymer coating system in real application even this is still not clearly understood [6,25]. The other CPE_e and R_f elements are the CPE values of the electrode and resistance of the film respectively, and as an additional to fit with the Nyquist spectrum. [26-27]. Frequency Resonance Analyzer (FRA) software was used to obtain the chi-square goodness-of-fit (χ^2) which authorized the fitted circuit (χ^2= 0.06) obtained. The lowest figure obtained demonstrating the most fitted circuit for the system.

Sensor Performance

This sensor was practically used in determination of H_2O_2 in antiseptic mouthwash which contains 6% w/v H_2O_2 with original concentration, 1.96 M for study purposes. Various concentrations of sample (0.2 – 1.2 M) was calibrated before the samples were spiked in 300 mM PBS solution at pH 7.4. From the trends of the Nyquist plot (Figure not shown), it can tell that similar characteristics happened with standard H_2O_2 whereby at higher frequency the interaction between the electrode-analyte interfaces behaved as capacitor while at lower frequency the diffusion behavior was taken control on the electrode. Then, the linear calibration of 1/ R_{ct} was plotted with R^2= 0.9566. To evaluate the accuracy of the sensor applicability, standard addition analysis was carried out by using CV technique. As the results found, the concentration of H_2O_2 found in real sample was 1.8 M which is close to the original concentration as tabulated in Table 1.

Table 1: The results of the H_2O_2 in real sample analysis obtained using Pt/PPD microelectrode assembly by the oxidation peak of CV in pH 7.4 PBS, with n (number of replicates) = 4

Concentration founded (M)	1.8 ≈ 1.9 M (original concentration of H_2O_2 in antiseptic mouthwash)
% Recovery	97 % ± 0.14

Stability and repeatability

The electrode was kept at 4^oC when not in used. The response time of this sensor decreased 43 % towards 10 µM H_2O_2 after continuous usage for 4 weeks. This is due to the degradation of the PPD layer on the platinum surface. Nevertheless, even though the response time was decreased, but the stability of the sensor is in agreement with other reported work [1,2,28].

Conclusion

The PPD modified Platinum microelectrode of 50 µm in diameter (Pt_{50}/PPD) has been successful characterized on its electrochemical performance towards H_2O_2 using CV, Chronoamperometry and EIS. This electrode was successfully applied in H_2O_2 detection at a lower operating potential (+0.4V) as well as succeeds in brain implantable needle type microelectrode [29]. Interestingly, with several more developments, this electrode is suggested to be applicable as an enzymatic biosensor in future planning.

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