Int. J. Electrochem. Sci., 6 **(2011)** 1317 - 1330

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electrochemical Study of Glycerol of Cooking Palm Oil with Glycerol Dehydrogenase at Polymers Modified Electrodes

Zahraa A Jarjes[1], *Mohammed Razip Samian*[2], *Sulaiman Ab Ghani*[1,*]

[1] Pusat Pengajian Sains Kimia, Universiti Sains Malaysia, 11800 USM P. Pinang, Malaysia
[2] Pusat Pengajian Sains Kajihayat, Universiti Sains Malaysia, 11800 USM P. Pinang, Malaysia
[*]E-mail: sag@usm.my

Received: 10 November 2010 / *Accepted:* 30 March 2010 / *Published:* 1 May 2011

This report described on the cyclic voltammetric behavior of glycerol (obtained at optimum conditions of hydrolysis of cooking palm oil using commercial lipase), glycerol dehydrogenase (GDH) in the presence of nicotinamide adenine dinucleotide (NAD^+) on polymer modified carbon cloth electrodes. Conducting polymers *viz.* polymethylene green, polyaniline, poly (*o*-phenylene diamine) and poly (4-vinylpyridine) were used to regenerate NAD^+ and to shuttle electrons from the NADH to the electrode. In general, the redox processes of glycerol were of quasi-reversible over potential ranges of -0.5 to +0.6 V *vs* Ag/AgCl (3M KCl). The effect of pH, concentrations of NAD^+, and weight of lipase have been studied to achieve a higher current density.

Keywords: Conducting polymers, Cooking palm oil, Glycerol, Glycerol dehydrogenase, Nicotinamide adenine dinucleotide

1. INTRODUCTION

One of the important characteristics of a fuel cell is high energy density. For this reason, researchers have examined the electrochemistry of a number of complex, high energy density fuels like glycerol [1–3]. Glycerol is an attractive fuel for a fuel cell since it is generated in large amounts during the production of both bioethanol [4] and biodiesel [5]. Thus, the ability to oxidize such a higher order polyalcohols like glycerol would have a profound impact on the fuel cell market.

There are several known redox enzymes capable of gaining electrons from glycerol [6] and other alcohols [7]. Alcohol oxidase utilizes oxygen as natural electron acceptor to produce hydrogen peroxide [8, 9]. Alcohol dehydrogenases, on the other hand, use either NAD/NADH couple or other artificial redox mediators for re-oxidation of its active site. Both enzyme types could be applied in the design of biofuel cells (usually the anode part). Glycerol dehydrogenase (GDH) is used to oxidize

glycerol resulting in NADH in a solution containing NAD^+. The NADH can then be directly oxidized at a conventional electrode such as gold, platinum or carbon [10, 11]. However, due to the slow charge transfer kinetics a large overpotential is required. Development of catalytic electrode surfaces for the oxidation of NADH to enzymatically active NAD^+ have focused on the use of redox mediator species [12] and conducting polymers [13]. Conducting polymers are known for their abilities to (i) be compatible with biological molecules in neutral aqueous solutions [14] and (ii) transfer the electric charges produced by biochemical reactions to electronic circuits [15].

Polyaniline (PAni) is one of the most important conducting polymers [16]. It is the first conducting polymer to be commercialized and has applications ranging from batteries [17] to biosensors [18]. Polymethylene green (PMG) is employed for the construction of biosensors for hydrogen peroxide [19] and also for the detection of theophyline [20]. However, among many conducting and electroactive polymers, poly (*o*-phenylene diamine) (PoPD) is one of great interest because of its potential use in various fields of technology like the electro-oxidation of methanol [21] and oxygen reduction [22, 23]. Redox polymers such as poly (4-vinylpyridine) (P4VP) have been used to construct electrochemical sensors [24] and amperometric biosensors [25].

In this work we investigate the capacity of glycerol bioanode for potential application in biofuel cell. The current is generated by the oxidation of glycerol liberated from the hydrolysis of cooking palm oil using commercial lipase (under the optimum conditions). The GDH-immobilized membrane is casted on the surface of polymer modified electrode. The glycerol is oxidized in the presence of GDH and NAD^+. Since the NADH oxidation has poor reaction kinetics and occurs at large over potentials thus the polymer is used to regenerate NAD^+ and to shuttle electrons from the NADH to the electrode. The electrocatalytic properties of the deposited PAni, PMG, PoPD and P4VP are illustrated via cyclic voltammograms shown through the effects of pH, NAD^+ concentrations and weight of lipase on the current density.

2. EXPERIMENTAL

2.1. Chemical

Cooking palm oil was obtained from a local supermarket. *Candida rugosa* lipase type VII, glycerol dehydrogenase from *Enterobacter aerogenes* and β-nicotinamide adenosine dinucleotide were purchased from Sigma-Aldrich, USA. Other chemicals were of analytical grade and purchased from various sources. Aqueous buffer solution was prepared from 0.044 M KH_2PO_4, 0.044 M NaOH and 0.15 M NaCl. All aqueous solutions were prepared using water (resistivity 18.2 MΩcm) from Milli-Q Plus (Millipore Corp., USA).

2.2. Apparatus

Voltammetric experiments were carried out with BAS Epsilon 2 workstation (Bioanalytical System, USA). A three electrode system was employed with a platinum wire counter electrode and Ag/AgCl (3 M KCl) reference electrode. All potentials were measured against Ag/AgCl (in 3 M KCl). The test electrode was 1 cm^2 of carbon cloth B-1 (Clean Fuel Cell Energy LLC, USA).

2.3. Methods

2.3.1. Assay of lipase activity

Lipase activity was assayed following the method of Arima et al [26] with some modifications. An oil emulsion was prepared as follows: 2 g polyvinyl alcohol (PVA) was dissolved in 100 mL boiled water and then filtered. Later 75 ml of the filtered PVA solution and 25 mL oil were then blended in a homogenizer. Then, 5 mL oil emulsion, 4 mL of phosphate buffer at 7.5 and 0.1 g of lipase were incubated in a water bath for 30 min at 37 ºC. The enzyme reaction was completed by addition of 20 mL of acetone-ethanol mixture (1:1 V/V). The released acids were titrated with 0.1 M NaOH in the presence of phenolphthalene as an indicator. Blank containing the same assay mixture was incubated without added enzyme (one unit of lipase activity was defined as the amount of enzyme that librated 1 mmole of free fatty acid per min under test conditions).

2.3.2. Preparation of the modified electrodes

The electropolymerization of aniline, 4-vinyl pyridine, *o*-phenylene diamine and methylene green were performed as described elsewhere [27 – 29]. The electrodes were rinsed and then allowed to dry overnight prior to further modification. The enzyme solution was prepared using 2.5 mg glycerol dehydrogenase in 5 mL of phosphate buffer (pH 7). Casting solution for making the mixture cast membranes of 5% Nafion and quaternary ammonium bromide were prepared as described elsewhere [30]. The enzyme and Nafion casting solution consisting of 50 µL modified Nafion suspension, 50 µL GDH and 50 µL of 6 mM of NAD were vortexed before pipetting it on the surface of electrode and allowed to dry in a low humidity environment prior to electrical measurements.

2.3.3. Preparation of oil emulsion for the voltammetric experiments

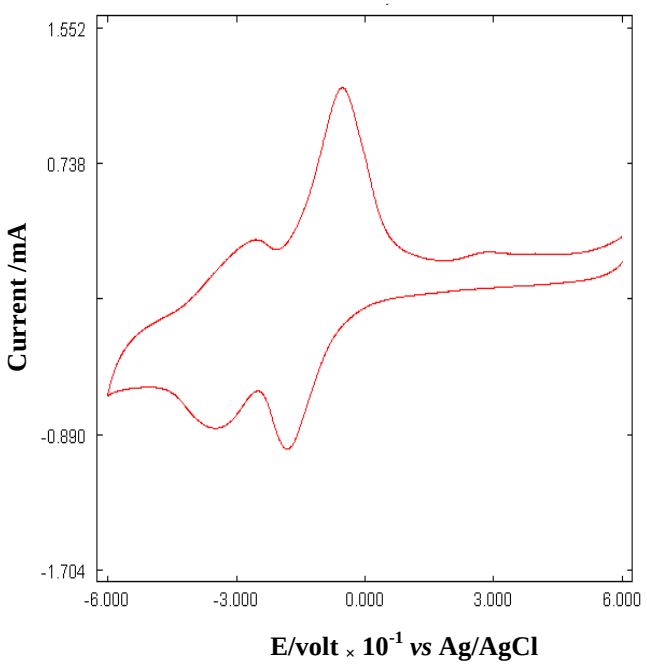
An oil emulsion was prepared from 2 g cooking palm oil and 0.1 g lipase in 25 mL aqueous buffer solution. The oil and aqueous phases were mixed and homogenized at room temperature for 30 min and then incubated at 37 ºC for 60 min prior to use.

3. RESULTS AND DISCUSSION

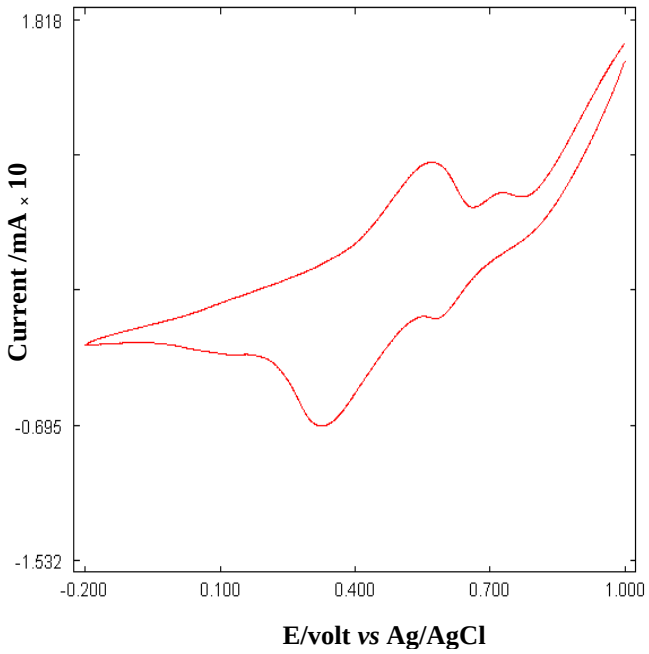
3.1. Electropolymerization of the monomers

The cyclic voltammogram (CV) of PMG on the surface of carbon cloth electrode (Fig. 1a) indicates two redox couples; cathodic peak potentials (E_{pc}) at – 0.19 V and – 0.05 V and anodic peak potentials (E_{pa}) at – 0.35 V and – 0.25 V. Akkermans et al. [31] has suggested the reaction mechanism is a single-step involving two-electron transfer reaction producing intermediates for the polymerization. The large oxidative current above 0.05 V with the respective reductive current

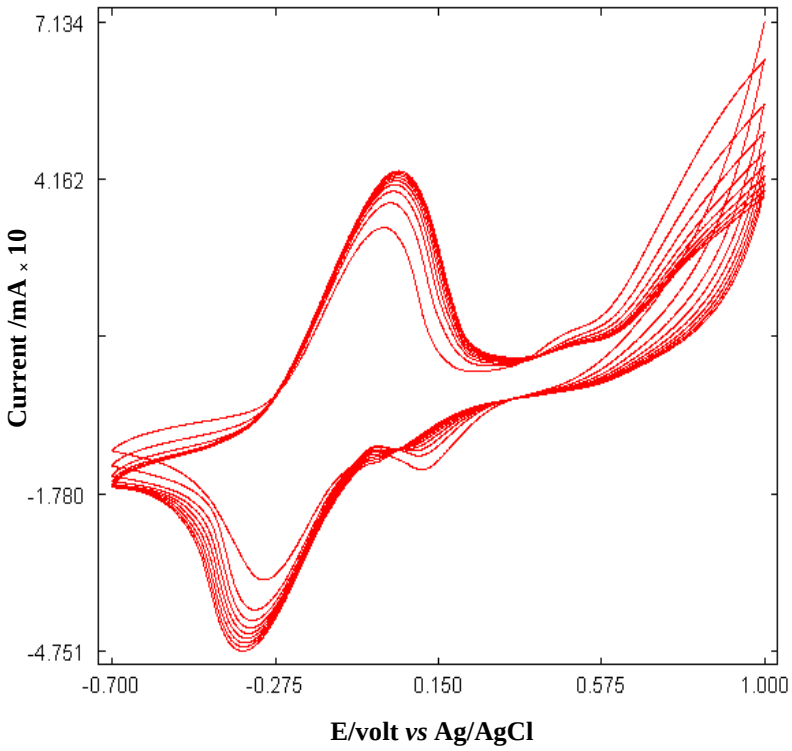
suggests that a reversible electrode process has occurred on the carbon cloth electrode. The surface morphology study of PMG by scanning electron microscope (SEM) shows the presence of several protuberances and hollow structures (Fig. 2a).



a)



b)



c)

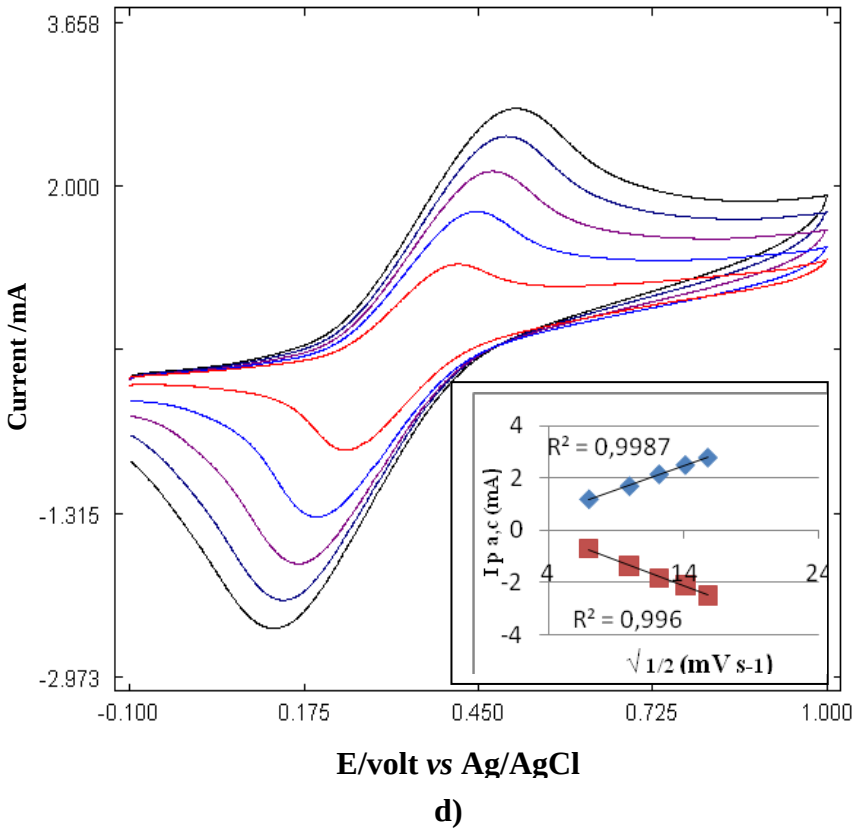


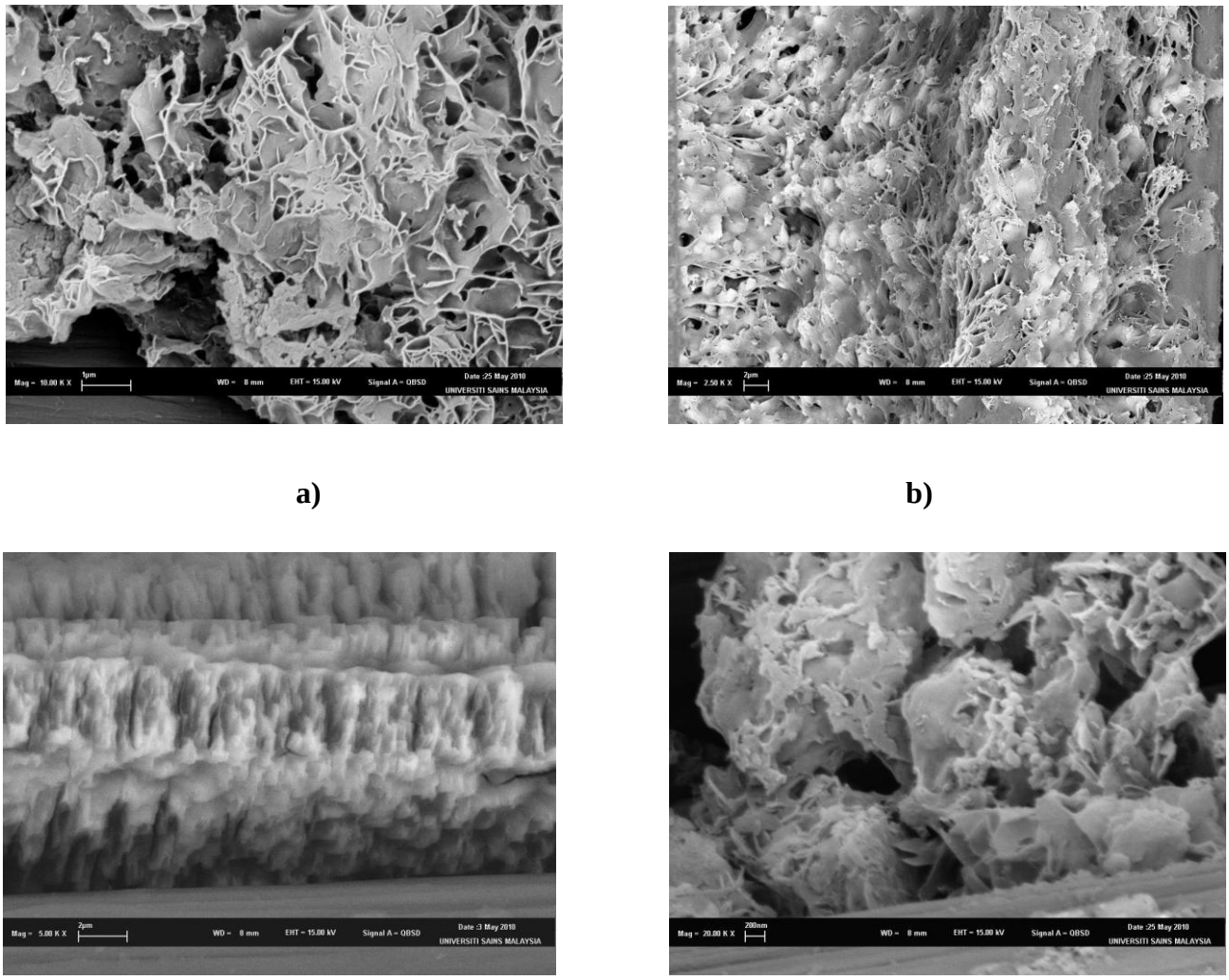
Figure 1. Cyclic voltammogram of **(a)** 0.4 mM methylene green and 0.1 M sodium nitrate in 10 mM sodium tetraborate, **(b)** 50 mM aniline in 0.2 M *p*-toluene sulphonic acid and 0.5 M KCl, **(c)** 50 mM *o*-phenylenediamine in 1 M H_3PO_4 and 0.5 M $CaCl_2$ (scan rate 100 mVs^{-1}) and **(d)** 3 mM 4-vinylpyridine in 0.1 M tetrabutyl ammonium perchlorate in acetonitrile, pH 3.0. Unless otherwise stated all experiment are at scan rate of 50 mVs^{-1} (Inset: relationships between cathodic and anodic peak current and the square root of scan rate.)

Figure 1b shows the CV during the formation of PAni film. It has already been established [32] that the protonation of the PAni plays a significant part in the observed behavior. The fully reduced leucoemeraldine is an insulator. Upon oxidation in acidic solution the leucoemeraldine gives off the protonated emeraldine - an electronic conductor. Further oxidation produces pernigraniline which is also an insulator but is unstable with respect to hydrolysis and chain fission to further provide quinone and imine functional groups [33]. This is demonstrated by the two larger redox peaks obtained in the potential range of 0.33 to 0.57 V. The used of supporting electrolyte KCl helps to increase acidic ability of *p*-toluene sulphonic acid (PTSA) in aqueous medium and also in the mobility of radical cations towards electrode surface for the formation of PAni [27]. Figure 2b shows the surface of PAni film indicating its growth as granular-like structures which then fuse together to form aggregates.

Figure 1c shows the CV for the growth of PoPD in 1 M H_3PO_4 and 0.5 M $CaCl_2$ (scan rate 100 mVs^{-1}). The initial CVs show a redox couple at E_{pc} and E_{pa} of – 0.34 V and 0.08 V, respectively, which corresponds to the reduction and oxidation of the PoPD film [34]. The E_{pc} peak at 0.15 V is not observed in the successive cycles due to the growth of polymer film on the electrode surface. The SEM

image of synthesized PoPD (Fig. 2c) shows a ladder type and a dense surface coverage of PoPD on carbon cloth.

The CV of P4VP (Fig. 1d) shows that the best potential range to be used for the electro polymerization is between – 0.1 V and 1 V. A pair of redox peaks is observed at scan rate of 20 – 250 mVs^{-1}. As the scan rate increases the E_{pa} shifted towards positive and the E_{pc} moves towards negative. The plot of I_{pc} and I_{pa} *vs* $\upsilon^{1/2}$ is a straight line and the linear regression , R^2, of 0.99 indicates that the electrode process is a diffusion-controlled. The P4VP is a universal mono-functional surface modifier, that is, only one type of functionality (pyridyl group) is required for the adhesion to surfaces because the pyridyl group has a strong affinity to metals [35]. The SEM on samples obtained at 50 mVs^{-1} shows that the surface is spiky with small spherical shaped objects within the spiky structures (Fig. 2d).



a) **b)**

c) **d)**

Figure 2. SEM micrographs of **(a)** polymethylene green, **(b)** polyaniline, **(c)** poly (*o*-phenylene diamine) and **(d)** poly (4-vinylpyridine)

3.2. Glycerol oxidation

Lipase, EC 3.1.1.3, is the enzyme responsible for the hydrolysis of the acylglycerides to produce free fatty acids and glycerol [36]. The production of glycerol from inexpensive biological substrate like cooking palm oil will make it ideal for power generation in biofuel cell [37]. In this study the lipolysis (using commercial lipase from the yeast *Candida rugosa*) of cooking palm oil is at optimum when pH at near to neutral, temperature at 37 °C, incubation times 60 min and the enzyme and substrate concentrations of 0.1 and 2 g, respectively. However, the liberated glycerol will be oxidized by GDH enzyme immobilized on quaternary ammonium salt modified Nafion membrane on the carbon cloth electrode. Nafion membrane is known as a benchmark material for good performances on an electrode [38]. The usage of film of quaternary ammonium salts and Nafion have been reported [39] to increase the mass transport of small analytes through the films and decreased the selectivity of the membrane against anions. Figure 3 shows the schematic reaction of cooking palm oil lypolysis. The glycerol produced is then directly oxidized by GDH in the presence of NAD^+ as the electron acceptor resulting in the formation of dihydroxyacetone, NADH.

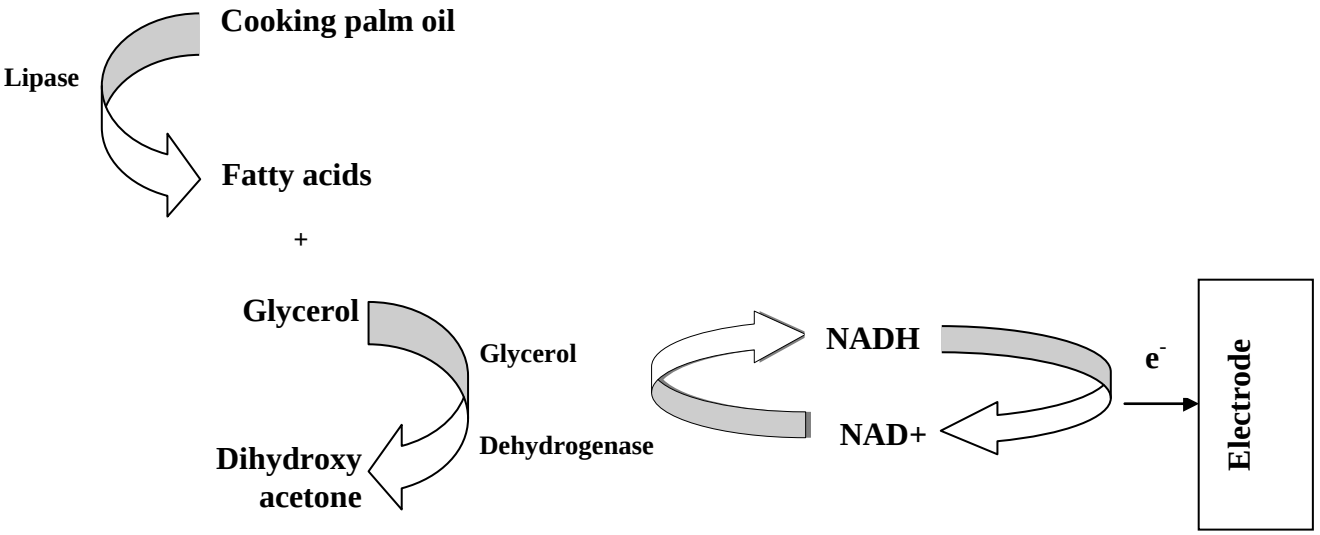


Figure 3. Schematic reactions for the oxidation of glycerol.

Figure 4 shows the CV of GDH unmodified electrode (prepared by immobilization of GDH in dodecyl trimethyl ammonium/Nafion membrane which is then casted directly on the surface of carbon cloth) in oil emulsion in the presence of 1 mM of NAD (Fig. 4a) and in buffer solution pH 7 only (Fig. 4b). The redox couple at E_{pa} +0.37 and E_{pc} +0.27 in oil emulsion indicates that no peak observable when the CV is recorded in buffer phosphate background. This suggests that NADH, formed from NAD^+ the cofactor for GDH which catalyze the oxidation of glycerol, is directly oxidized at the electrode surface.

Further modification with PMG (Fig. 5a2) a negative shift is observed as compared to the electrooxidation at GDH unmodified electrode (Fig. 5a1) under the same condition. An enhancement in the kinetics of the oxidation of glycerol and reduction of NAD $^{+}$ have also been achieved as compared to the CV of GDH/PMG modified electrode recorded in phosphate buffer (pH 7.0) containing 1 mM NAD^{+} (Fig. 5a3).

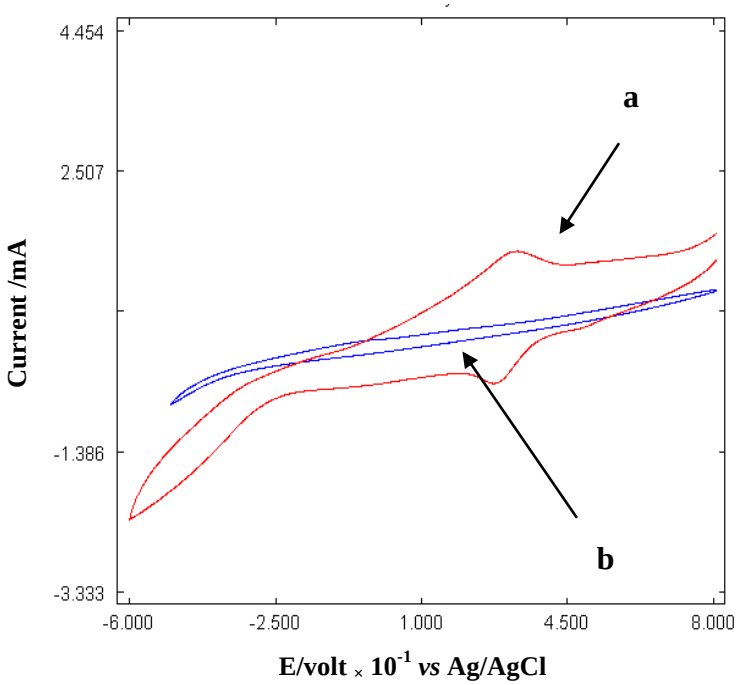
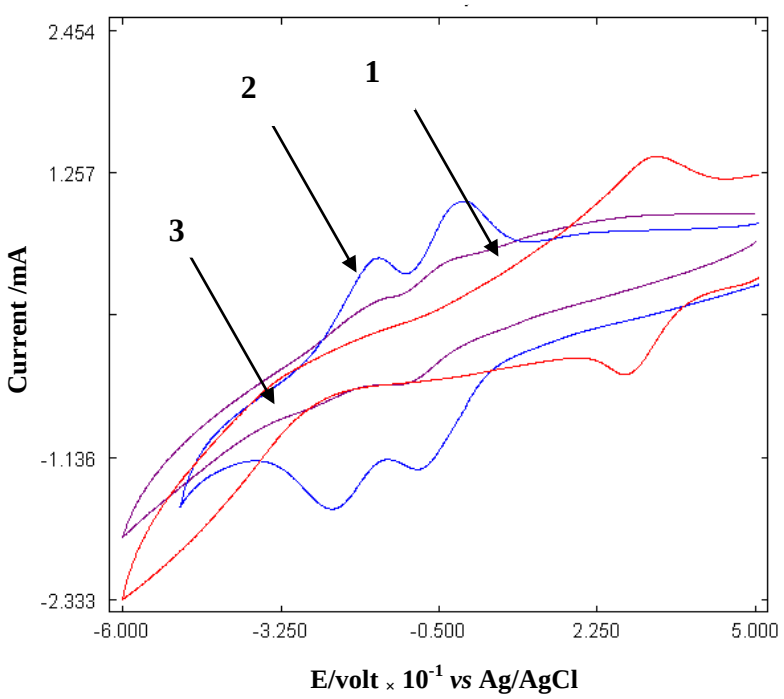


Figure 4. Cyclic voltammograms of GDH/carbon cloth electrode in **(a)** oil emulsion in the presence of 1 mM of NAD^{+} and **(b)** phosphate buffer solution (pH = 7). The scan rate is 100 mVs^{-1}.



a)

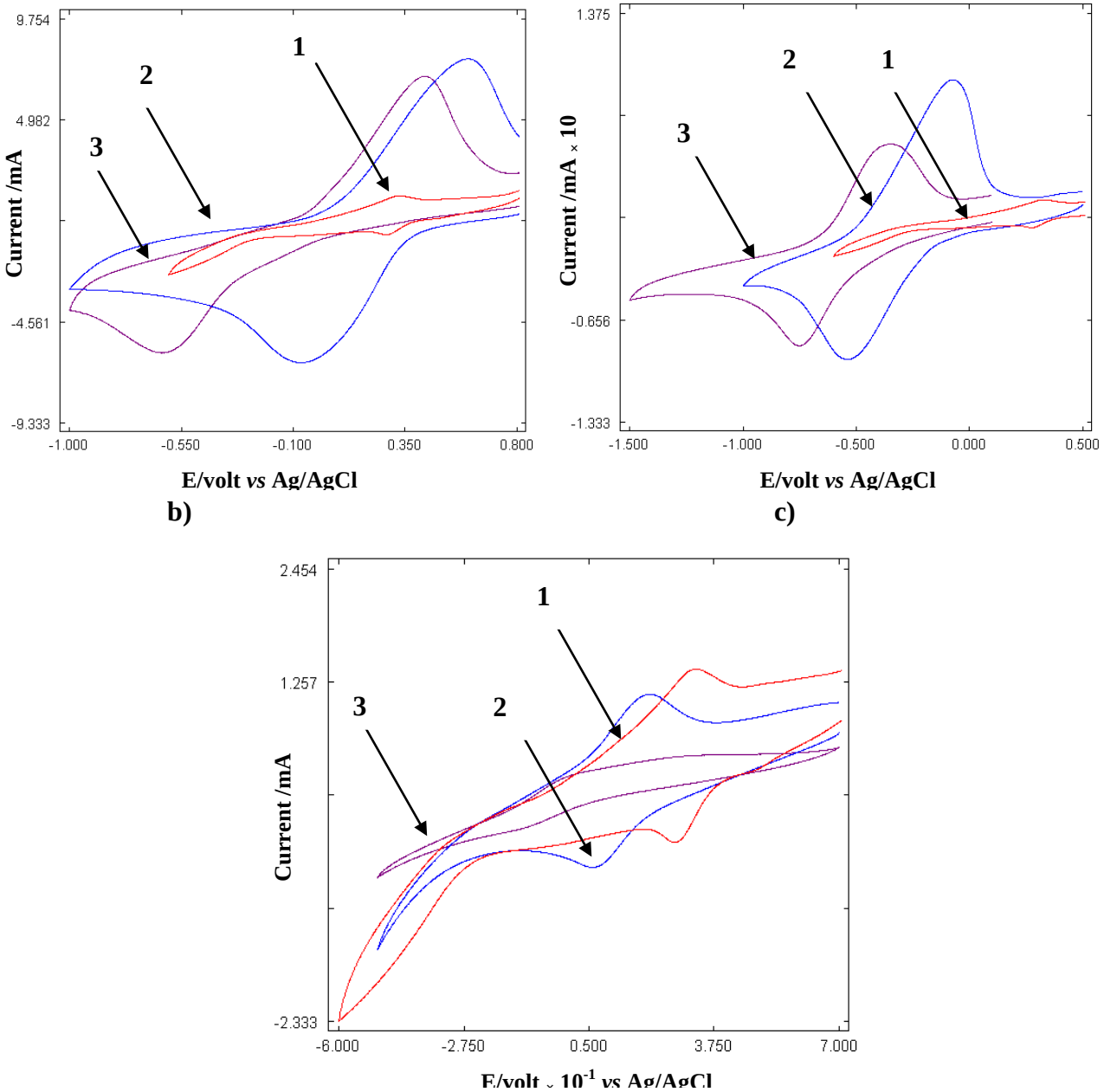


Figure 5. Cyclic voltammograms of **(a1)** GDH/carbon cloth electrode in oil emulsion in the presence of 1 mM of NAD^+ **(a2)** GDH/PMG modified electrode in oil emulsion in the presence of 1 mM of NAD^+, **(a3)** GDH/PMG modified electrode in phosphate buffer pH 7 and in the presence of 1 mM of NAD. **(b1)** GDH/carbon cloth electrode in oil emulsion in the presence of 1 mM of NAD^+**, (b2)** GDH/PAni modified electrode in oil emulsion and in the presence of 1 mM of NAD^+, **(b3)** GDH/PAni modified electrode in phosphate buffer pH 7 and in the presence of 1 mM of NAD^+ . **(c1)** GDH/carbon cloth electrode in oil emulsion in the presence of 1 mM of NAD^+ **(c2)** GDH/PoPD modified electrode in oil emulsion and in the presence of 1 mM of NAD^+,**(c3)** GDH/PoPD modified electrode in phosphate buffer pH 7 and in the presence of 1 mM of NAD^+, **(d1)** GDH/carbon cloth electrode in oil emulsion in the presence of 1 mM of NAD^+ , **(d2)** GDH/P4VP modified electrode in oil emulsion and in the presence of 1 mM of NAD^+, **(d3)** GDH/P4VP modified electrode in phosphate buffer pH 7 and in the presence of 1 mM of NAD^+.

These results indicate that GDH/PMG modified electrode catalyzed the oxidation of glycerol and the reduction of NAD^+. It is also evident that the NADH generated by the enzymatic reaction is reoxidized by the GDH/PMG modified electrode. The oxidations of NADH at PMG modified electrodes have been reported [40, 41]. Figure 5b2 shows well-defined E_{pc} and E_{pa} at 0.59 V and – 0.07 V, respectively of GDH/PAni modified electrode. The anodic peak current, I_{pc}, has increased and shifted towards a positive direction as compared to the electrooxidation at GDH modified electrode (Figure 5b1) under the same conditions. It is observed that there is an increase in anodic peak currents, I_{pa}, compared to the GDH/PAni modified electrode obtained in phosphate buffer (pH 7.0) in the presence of 1 mM NAD (Fig. 5b3). The results indicate that GDH/PAni modified electrode has also improved the kinetics of the oxidation of glycerol. The oxidation of NADH at PANI modified electrodes has also been studied [42].

The CV of GDH/PoPD modified electrode with E_{pc} and E_{pa} at −0.537 V and −0.075 V, respectively, is shown in Figure 5c2. The inclusion of these polymers has obviously improved kinetics. Moreover, a negative shift is observed at GDH/PoPD modified electrode as compared to GDH unmodified electrode (Fig. 5c1). Furthermore, a significant increase in the anodic current and shifting towards a positive direction compared to GDH/PoPD modified electrode has been recorded in phosphate buffer (pH 7.0) containing 1 mM NAD (Figure 5c3) indicating a good catalytic activity towards glycerol. The electrocatalysis of PoPD towards the oxidation of NADH has been reported [21]. The electrocatalytic behavior of the GDH/P4VP modified electrode (Fig. 5d2) shows a pair of redox peaks appeared at 0.057 V and 0.2 V. A decrease in the electrooxidation overvoltage is observed when compared with electrooxidation at GDH unmodified electrodes (Fig. 5d1). The CV of GDH/P4VP modified electrode recorded in phosphate (pH 7.0) containing 1 mM NAD (Fig. 5d3) shows only one broad reduction peak associated with decreased in I_{pc}. However, the electrode still displays good catalytic activity towards glycerol.

3.3. Effect of NAD concentrations

NAD^+ is essential in a reaction catalyzed by GDH. The effect of NAD^+ concentration on the response of enzyme electrode to glycerol in oil emulsion (pH 7) is then studied. Figure 6 shows the effect of different NAD^+ concentrations on the oxidative current density. For every polymer tested it appears that as and when the concentration of NAD^+ increases exceeding 1mM the current decreases. This behavior indicates that NAD^{+}` acts as a reversible competitive inhibitor for the oxidation of NADH on the polymer film, presumably by competition for the active sites within the film.

3.4. Effect of pH

Enzyme activity depends on the pH of the background electrolyte in the environment in which the electrode is used. Hence, it may have an important effect on its response. Thus, the influence of oil emulsion pH from pH 5 to 9.0 is investigated. Figure 7 shows the pH profiles of the oxidative current density of these polymers. The maximum oxidative current is found in pH range of 7- 8 which is the optimum pH for the activity of GDH [43, 44]. Similar to effect on NAD concentration, peak currents

remain nearly constant and lower for PMG and P4VP modified electrodes at various pH. Hence, the mediation of these polymers is not affected by the pH of the medium. It is also possible that GDH has little affinity towards these polymers.

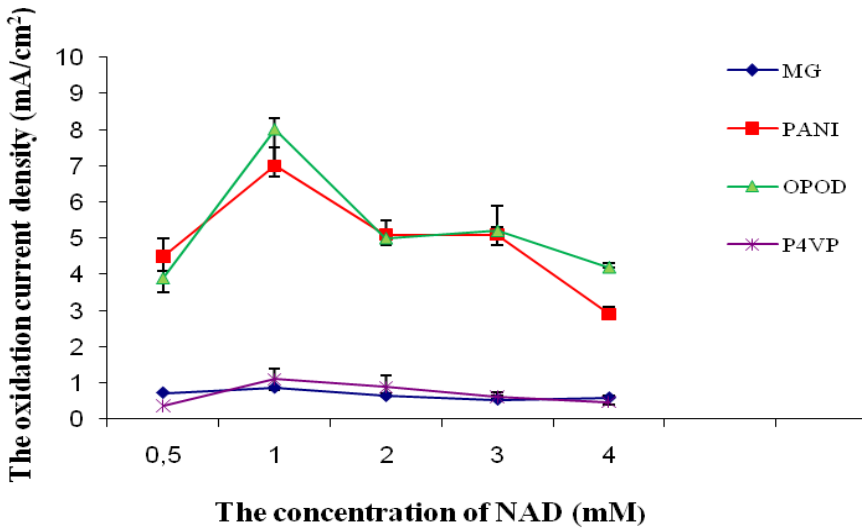


Figure 6. The oxidative current density at GDH/polymers modified carbon cloth in oil emulsion at various concentrations of NAD^+, (n = 3).

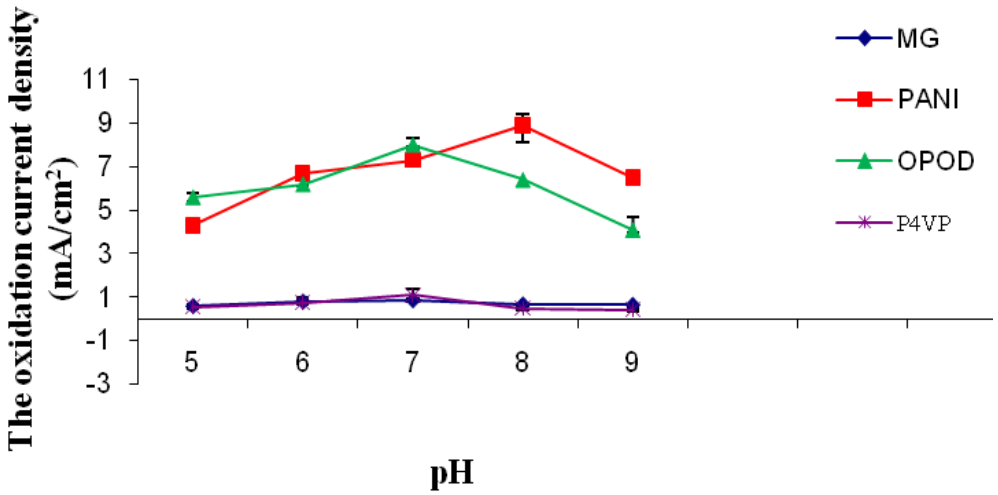


Figure 7. The oxidative current density at GDH/polymers modified carbon cloth in oil emulsion at various pH, (n = 3).

3.5 Effect of weight of lipase

The enzyme GDH catalyzes the oxidation of glycerol liberated from the first enzymatic reaction (lipolysis). Thus various amounts (0.01 – 0.2 g) of lipase in the cooking palm oil emulsion are

studied for their relationship with the oxidative current density (Fig. 8). The highest current density is obtained at 0.05 – 0.1 g lipase which agrees well with the optimum concentration of lipase enzyme activity. It shows that lipase allows a much higher percentage of glycerol to be utilized by GDH leading to an enhance performance of the enzyme. Thus, the optimal amount of lipase used ensures a good balance between the levels of hydrolysis and oxidation of the glycerol.

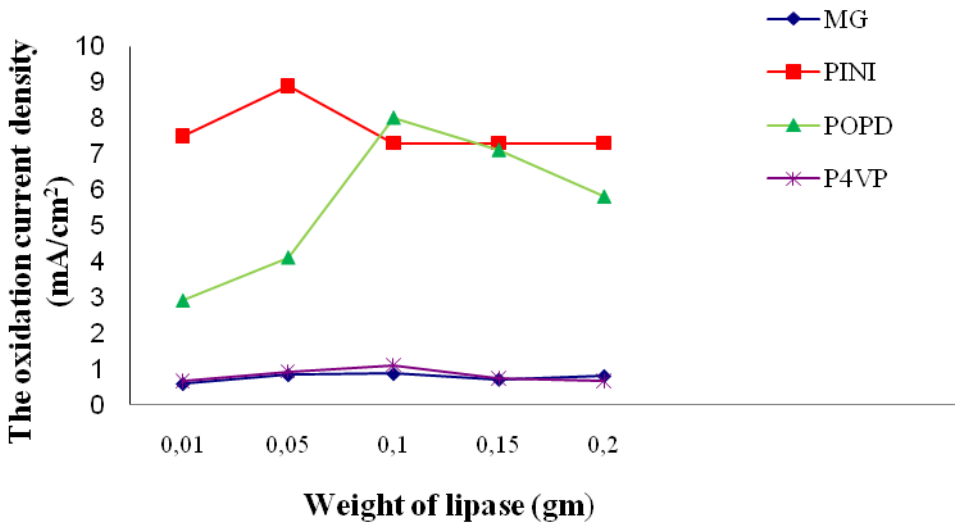


Figure 8. The oxidative current density at GDH/polymers modified carbon cloth in oil emulsion at various weight of Lipase, (n = 3).

4. CONCLUSIONS

The electrochemical studies of carbon cloth electrodes modified by GDH immobilized on either of these polymers i.e. PMG, PAni, PoPD and P4VP, have been carried out via CV of NAD^+ and glycerol released from the lipolysis of cooking palm oil under optimum condition. The enzymatically produced NADH is oxidized at these polymers which also act as electrocatalysts. The performances of these electrodes except those modified with PMG and P4VP are dependent on the pH of medium, the concentration of NAD^+ and also the weight of lipase. The performances of PAni and PoPD modified electrodes are relatively superior. These results could provide the basis for future development of a novel glycerol bioanode for use in biofuel cell.

ACKNOWLEDGEMENTS
The authors are indebted to the Ministry of Higher Education, Malaysia and the University for (i) RU grant 1001/PKIMIA/811044 and (ii) Postgraduate Incentive grant 1001/PKIMIA/821014. One of us (Z.A. Jarjes) is thankful to the University for the Postgraduate Fellowship awarded.

References

1. M.L. Avramov-Ivic, J.M. Leger, C. Lamy, V.D. Jovic and S.D. Petrovic, *J. Electroanal. Chem.*, 308 (1991) 309
2. G. Yildiz, F. Kadirgan, *J. Electrochem. Soc*, 141 (1994) 723
3. K. Matsushita, T.Yamashita, N. Aoki, H.Toyama and O. Adachi, *Biochemistry*, 38 (1999) 6111
4. A.S. Aldiguier, S. Alfenore, X. Cameleyre, G. Goma, J.L. Uribelarrea, S.E. Guillouet and C. Molina-Jouve, *Bioprocess Biosyst. Eng.*, 26 (2004) 217
5. J.C. Thompson, B.B. He, *Appl. Eng. Agric.*, 22 (2006) 261
6. I.Lapenaite, A. Amanaviciene and A. Ramanavicius, *Crit. Rev. Anal. Chem*., 36 (2006) 13
7. D. Ivnitski, B. Branch, P. Atanassov and C. Apblett, *Electrochem. Commun*., 8 (2006) 1204
8. A. Ramanavicius, A. Malinauskas, *Electrochim. Acta*, 51(2006) 6025
9. A. Malinauskas, J. Malinauskiene and A.Ramanavicius, *Nanotechnology*, 16 (2005) 51
10. S.A. Wring, J. P. Hart, *Analyst*, 117 (1992) 1215
11. M. Hedenmo, A. Narvaez, E. Dominguez and I. Katakis, *Analyst*, 121 (1996) 1891
12. L. Gorton, *J. Chem. Soc., Faraday Trans.*, 82 (1986) 1245 (and references therein)
13. P.N. Bartlett, E. Simon, *J. Am. Chem. Soc*. 125 (2003) 4014 (and references therein)
14. M. Gerard, A. Chaubey and B.D. Malhotra, *Biosens. Bioelectron.*, 17 (2002) 345
15. S. De Taxis du Poet, P. Miyamoto, T. Murakami, J. Kimuraand I. Karube, *Anal. Chim. Acta*, 235 (1990) 255
16. D.C. Trivedi in H.S. Nalwa (Ed.), *Handbook of Organic Conductive Molecules and Polymers*, vol. 2, John Wiley, New York (1997)
17. O. Osama, O. Kimura and T. Kabata, *Electron. Commun. Jpn*, 75 (1992) 1123
18. J.H. Kim, J.H. Cho and G.S. Cha, *Biosens. Bioelectron.*, 14 (2000) 907
19. C. Lei, J. Deng, *Anal. Chem.*, 68 (1996) 3344
20. Y.V. Ulyanova, A.E. Blackwell and S.D. Minteer, *Analyst*, 131 (2006) 257
21. S.M. Golabi, A. Nozad, *J. Electroanal. Chem*., 521 (2002) 161
22. T. Ohsaka, T. Watanabe, F. Kitamura, N. Oyama and K. Tokuda, *Chem. Commun*., 16 (1991) 1072
23. Y.J. Li, R. Lenigk, X.Z. Wu, B. Gruendig, S.J. Dong, J. Shao and R. Renneberg, *Electroanalysis*, 10 (1998) 671
24. F. Bakhtiarzadeh, S. Ab Ghani, *J. Electroanal. Chem.*, 624 (2008) 139
25. F, Ahmad, S. Ab Ghani, *Int. J. Environ. Anal. Chem.*, 85 (2005) 781
26. K.H. Arima, T. Wen and T. Beppu, *Agric. Biol. Chem.*, 11 (1972) 1913
27. A. Parsa, S. Ab Ghani, *J. Electrochem. Soc.*, 156 (2009) E105
28. A. Parsa, S. Ab Ghani, *Polymer*, 49 (2008) 3702
29. L. Nick, L. Akers, M. Christine, D. Moore and D. Shelley, *Electrochim. Acta*, 50 (2004) 2521
30. M. Moore, N.L. Akers, A.D. Hill, Z.C. Johnson and S.D. Minteer, *Biomacromolecules*, 5 (2004) 1241
31. R.P. Akkermans, S.L. Roberts, F. Marken, B.A. Coles, S.J. Wilkins, J.A. Cooper, K.E. Woodhouse and R.G. Compton, *J. Phys. Chem. B*, 103 (1999) 9987
32. G. Inzelt, in A. J. Bard (Ed.), *Electroanalytical Chemistry*, Marcel Dekker, New York (1994)
33. W.S. Huang, B.D. Humphery and A.G. MacDiarmid, *J. Chem. Soc., Faraday Trans*., 82 (1986) 2385
34. D. Jang, Y. Yoo and S.M. Oh, *Korean Chem. Soc*., 16 (1995) 392
35. S. Malynych, I. Luzinov and G. Chumanov, *J. Phys. Chem.*, 106 (2002) 1280
36. R.A. Sheldon, *Biotechnol*., 76 (2001) 3
37. M.E. Youssef, K.E. Al-Nadi and M.H. Khalil, *Int. J. Electrochem. Sci.*, 5 (2010) 267
38. C.M. Bautista-Rodríguez, A. Rosas-Paleta, J.A. Rivera-Márquez1 and O. Solorza-Feria, *Int. J. Electrochem. Sci.*, 4 (2009) 60

39. M.J. Schrenk, R.E. Villigram, N.J. Torrence, S.J. Brancato and S.D. Minteer, *J. Membr. Sci.*, 205 (2002) 4383
40. L. Gorton, A. Torstensson, H. Jaegfeldt and G. Johansson, *J. Electroanal. Chem*., 161 (1984) 103
41. F.F. Ni, L. Gorton and T.M. Cotton, *Langmuir*, 6 (1990) 66
42. P.N. Bartlett, P.R. Birkin and E.N.K. Wallace, *J. Chem. Soc., Faraday Trans*., 93 (1997) 1951
43. Y.C. Kong, J.W. May and J.H. Marshall, *J. Gen. Microbiol.*, 131 (1985) 1571
44. J.E. Strichland, D.N. Millen, *Biochim. Biophys. Acta*, 159 (1968) 221