Int. J. Electrochem. Sci., 1(**2006**)425-434

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Immobilization of GOD on Electrochemically Synthesized PANI Film by Cross-linking via Glutaraldehyde for Determination of Glucose

*P.D. Gaikwad, D.J. Shirale, V.K. Gade, P.A. Savale, H.J. Kharat, K.P. Kakde, and M.D. Shirsat**

Optoelectronics and Sensor Research Laboratory, Department of Physics, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004 Maharashtra, India
*E-mail: mdshirsat_bamu@yahoo.co.in

Received: 6 September 2006 / *Accepted:* 26 October 2006 / *Published:* 1 December 2006

The Polyaniline-sulfuric acid-glucose oxidase (PANI-H_2SO_4-GOD) electrode has been investigated in the present work. Platinum electrode was used for the synthesis of Polyaniline-sulfuric acid (PANI-H_2SO_4) film using Potentiometric method with 0.2 M aniline, 1.0 M H_2SO_4 solution, 1.0 pH and 1 mA/cm^2 applied current density. The synthesized PANI-H_2SO_4 films were characterized by electrochemical technique, electrical conductivity, UV-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). Then GOD was immobilized on synthesized PANI-H_2SO_4 film by cross-linking via glutaraldehyde in phosphate and acetate buffer. The sensitivity of PANI-H_2SO_4-GOD electrode in phosphate and acetate buffer has been recorded. It was found that the phosphate buffer gives fast response as compared to acetate buffer in Potentiometric measurements.

Keywords: Polyaniline, Immobilization, glucose oxidase, cross-linking

1. INTRODUCTION

An extensive research has been carried out in the field of bioelectrochemistry in last two decades. The rapid progress of bioelectrochemistry has been integrated into analytical applications, e.g. biosensors working as detectors in clinical and environmental analysis [1-4]. Electrochemically polymerized conducting polymer has received considerable attention. The remarkable switching capability of these electroactive materials between the conducting oxidized (doped) and the insulating-reduced (undoped) state is the basis of many applications. Among others, the poly-conjugated conducting polymers have been recently proposed for biosensing applications because of a number of favorable characteristics, such as: (i) direct and easy deposition on sensor electrode by electrochemical oxidation of monomer, (ii) control of thickness and (iii) Redox conductivity. The polyelectrolyte

characteristic of the polymer is also useful for sensor applications. The conducting polymers have been widely used for the development of various biosensors. The surface morphology and conductivity of the synthesized films are two key factors for sensor applications. The synthesized film should have uniform, porous surface morphology and higher conductivity. The conducting polymers fulfill both these requirements. Sensor systems based on conducting polymers also rely on sensible changes in the optical and electrical features of this kind of materials [5–9].

Conducting polymers have considerable flexibilities in modifying their chemical structures. By chemical modeling and synthesis, it is possible to modulate their electrical and mechanical properties [10]. Moreover, the polymer itself can be modified to bind with protein molecules [11]. Conducting polymers are also known for their ability to be compatible with biological molecules in neutral aqueous solutions. Additionally, conducting polymers have the ability to efficiently transfer the electric charges produced by biochemical reactions to electronic circuits [12-14]. Polyaniline is one of the most important and widely used conducting polymers for biosensor applications [15]. Polyaniline (PANI) is a technologically important conducting polymer due to its unique electrical, electrochemical, and optical properties. Conducting polymer based biosensors have found promising applications in various fields, such as biotechnology, food and agriculture product processing, health care, medicine, and pollution monitoring. The combination of oxidoreductases and amperometric electrodes is most commonly studied biosensor concept. Through various strategies, the enzyme reaction can be easily followed and sensitively measured by electrochemical techniques [16-27].

Among the various matrices, conducting polymers have attracted much attention, due to their ability to bind oppositely charged complex entities in their oxidized conducting state and to release them in their neutral insulating state. The immobilization of enzymes or proteins in such polymers can be achieved by several techniques like physical entrapment, chemical cross-linking, covalent coupling etc.The physical entrapment; covalent coupling and other methods suffer from leaching problems of the enzyme. However this problem can be significantly overcome by using chemical cross-linking method of immobilization via glutaraldehyde.

In the present investigate, we have studied influence of phosphate and acetate buffer on GOD immobilized PANI-H_2SO_4 film by cross-linking via glutaraldehyde

2. EXPERIMENTAL

2.1. Synthesis of PANI-H_2SO_4 film

PANI-H_2SO_4 film was synthesized from an aqueous solution of distilled 0.2 M aniline (S. D. Fine Chemicals, Mumbai) and 1.0 M of sulfuric acid (Rankem, New Delhi) using electrochemical deposition method. It was carried out by Potentiometric technique at 27 ºC in a one compartment, three-electrode glass cell. The platinum foils were used as working and counter electrode and Ag/AgCl was used as reference electrode. The electrolyte solution was prepared in distilled water. The applied current density of 1 mA/cm^2 and the 1.0 pH were kept constant during synthesis of PANI film. After synthesis, polymer coated electrodes were rinsed thoroughly in distilled water and dried in cold air and then use for subsequent characterization.

2.2. Immobilization of GOD on PANI- H_2SO_4 film

The GOD (Aldrich) was immobilized by cross-linking via glutaraldehyde (0.1 %) on PANI-H_2SO_4 films, thus restricting the leaching of the enzyme film. These films were left for 30 min and washed with phosphate and/or acetate buffer. The stock solution of GOD (1 mg/ml) prepared in 0.1 M phosphate and/or acetate buffer was adsorbed onto the surface of PANI-H_2SO_4 film. The enzymatic incorporation was done. This kind of immobilization results in greater physical and chemical stability of the catalytic material due to the cross-linking of enzyme. In this case, the active site of the enzyme could be more accessible for the enzyme reaction. An adequate concentration of GOD was chosen so that it ensures higher enzyme loading and provide excellent Potentiometric response with an efficient retention of the enzyme.

3. RESULTS AND DISCUSSION

The amount of glucose can be determined by measuring the anodic potential of oxidation of hydrogen peroxide, produced in the reaction given below

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

Moreover, formation of hydrogen peroxides can be detected by the Potentiometric method during electrode reaction.

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$

The enzyme electrode formed by glucose oxidase with glutaraldehyde is used for Potentiometric measurement of glucose with an Ag/AgCl electrode [28].The glutaraldehyde plays a significant role in potential response [29].

In order to construct the Potentiometric enzyme sensor, GOD was used as an example of a redox protein. The enzyme catalyses, in the presence of molecular oxygen, lead to the oxidation of glucose into gluconic acid and hydrogen peroxide. The conversion of glucose to gluconic acid involves the transfer of two protons and two electrons from the substrate to the flavin moiety of the enzyme [30]. The electron transfer from the redox cofactor to the sensing electrode is also facilitate by the presence of a polymeric conducting material

3.1 Potentiometric studies of PANI-H_2SO_4 film

The chronopotentiogram of synthesized PANI-H_2SO_4 film is shown in figure 1. The PANI-H_2SO_4 film was synthesized on platinum substrate from 0.2 M concentration of aniline and 1.0 M of H_2SO_4 with 1mA/cm^2 applied current density at 1.0 pH. This has resulted in high conductivity, with uniform and porous surface morphology of synthesized PANI-H_2SO_4 film.

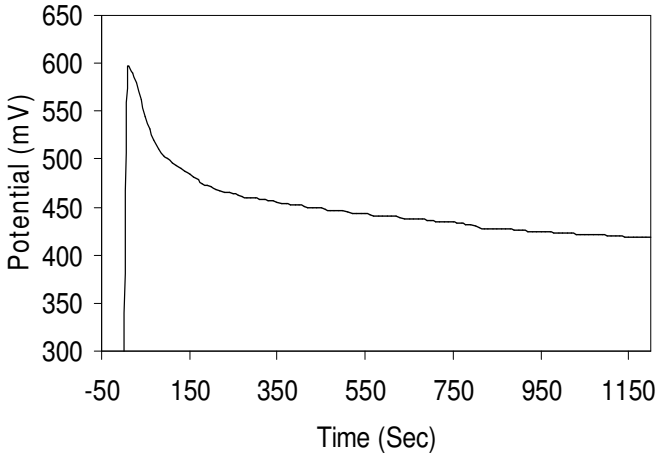

Figure 1. Chronopotentiogram of PANI-H_2SO_4 film synthesized at 1.0 pH, 0.2 M aniline, 1.0 M H_2SO_4 1 mA/cm^2 current density at temp. 27 ºC.

The behavior of the Potentiometric synthesis overshoot during first few second probably indicates difficult formation of dimmers and oligomers. After this, potential remain constant suggesting that building up of the films proceeds according to the same reaction along the full thickness of the polymer. The electrical conductivity of synthesized PANI-H_2SO_4 was measured by four probe technique and it was 0.73 S/cm.

3.2. UV-Visible studies of PANI- H_2SO_4 film

The UV-visible spectrum of synthesized PANI-H_2SO_4 film recorded using UV-visible spectrophotometer 1601 is shown in Fig. 2.

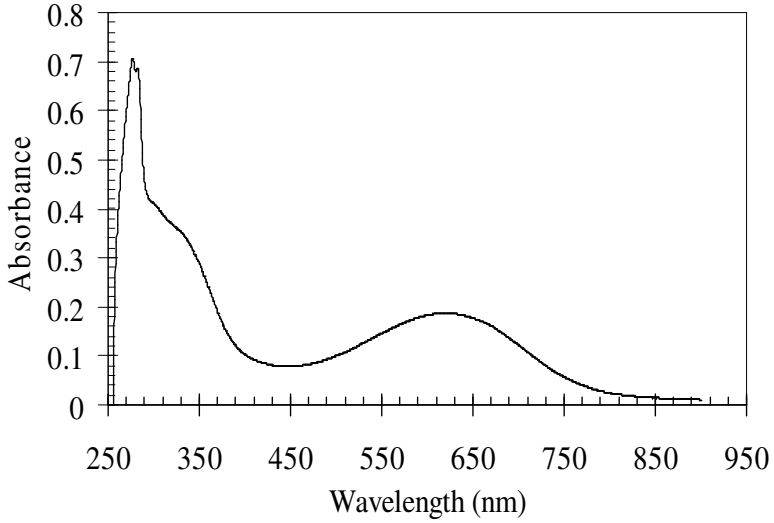

Figure 2. UV-visible spectra of PANI-H_2SO_4 film synthesized at 1.0 pH, 0.2 M aniline, 1.0 M H_2SO_4, 1 mA/cm^2 current density at temp. 27ºC.

A green colored film showed two absorption peaks for PANI-H_2SO_4 film. The peak at 338 nm is because of π-π^* transition and a broad peak at 629 nm is due to excitation formation of quinoid ring corresponds to the semi-conducting phase for PANI-H_2SO_4 film.

3.3 FTIR studies of PANI-H_2SO_4 film

The FTIR spectra were recorded (Testscan Shimadzu FTIR-8400 series) in the region 4000 cm^{-1}- 200 cm^{-1}. The principal transmittance of synthesized PANI film observed in the FTIR spectrum is shown in Fig. 3. The broad peak at 3442.7 cm^{-1} corresponding to -NH_2 stretching, peak observed at 2995.5 cm^{-1}due to C-H stretching and C-N stretching is observed at 1311.5 cm^{-1}. Thus, the FTIR spectral results confirm the formation of Polyaniline in H_2SO_4 medium.

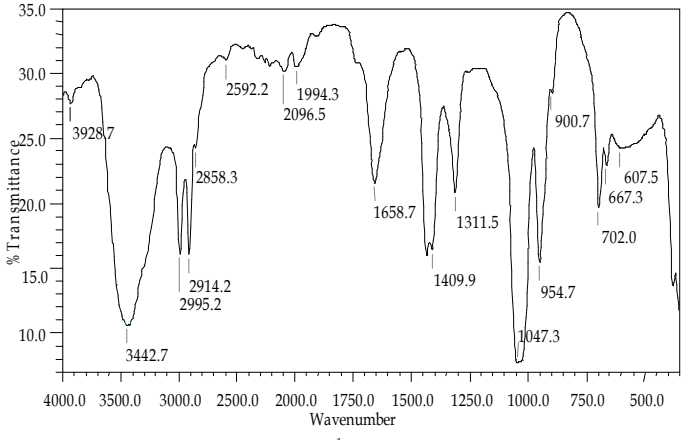

Figure 3. FTIR spectra of PANI-H_2SO_4 film synthesized at 1.0 pH, 0.2 M aniline, 1.0 M H_2SO_4, 1 mA/cm^2 current density at temp. 27ºC.

3.4. SEM studies of PANI- H_2SO_4 film

The scanning electron micrograph was recorded using JEOL, JSM-6360A SEM machine. The SEM micrograph of synthesized PANI-H_2SO_4 film is shown in figure 4.

It is Cauliflower structure, it show very good uniformity and porosity, which is suitable for immobilization of biocomponent**.**

3.5. Potentiometric response of PANI-H_2SO_4-GOD electrode

The change in response potential of the active device glucose oxidase is the parameter of interest for sensor applications. The response potential of the device depends on several factors such as (1) the contact resistance between the metal electrode and the polymer film, (2) the geometric factor of the film and (3) the film conductivity. The film conductivity is depends on several factors, such as

analyte pH, temperature, polymer film potential, substrate concentration and enzyme loading. The GOD was immobilized on electrochemically synthesized PANI -H_2SO_4 film by cross-linking via glutaraldehyde. The potential-time relationship of PANI- H_2SO_4-GOD electrode when the current of the enzyme was set 0.5 mA in phosphate and acetate buffer is as shown in figure 5 and figure 6 respectively.

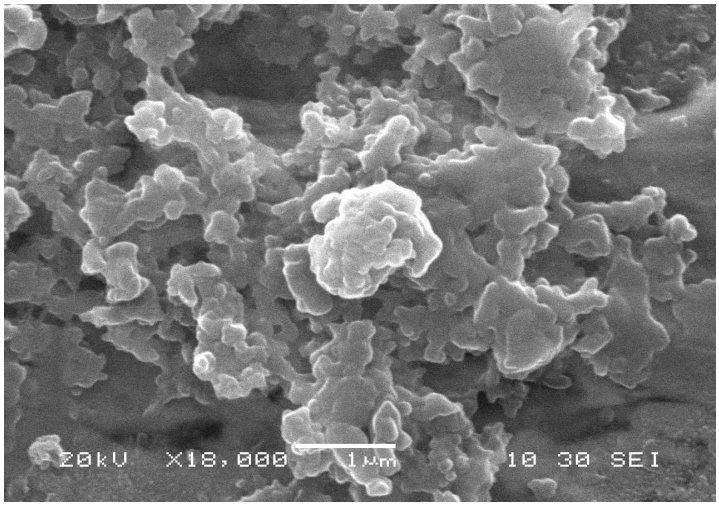

Figure 4. SEM micrograph of PANI-H_2SO_4 film synthesized at 1.0 pH, 0.2 M aniline, 1.0 M H_2SO_4, 1 mA/cm^2 current density and temp 27 ºC.

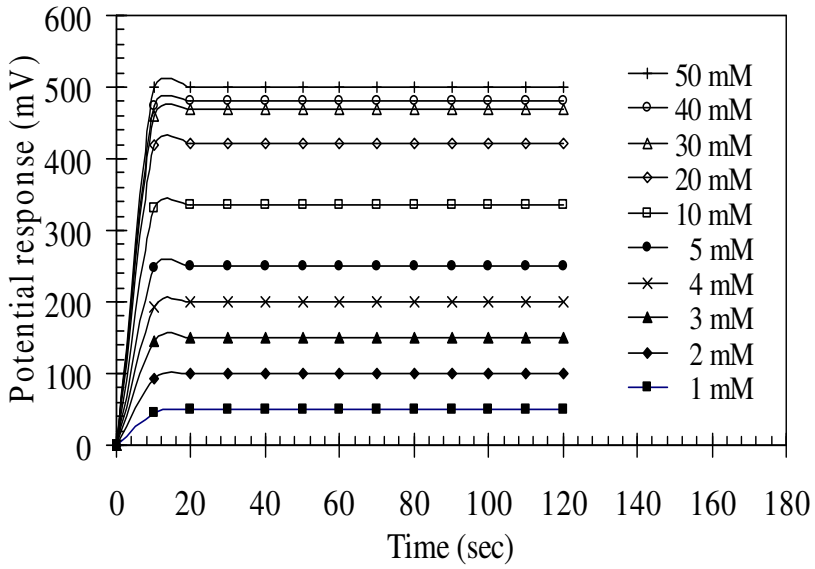

Figure 5. Potential-time curves for the PANI-H_2SO_4-GOD electrode for various glucose concentrations in 0.1M Phosphate buffer, pH 5.8

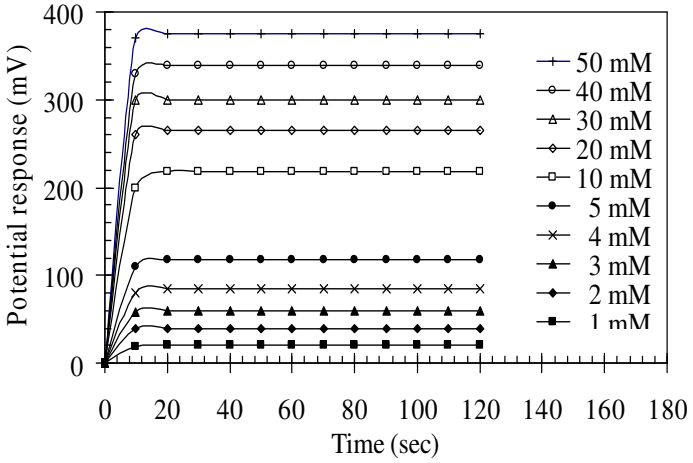

Figure 6. Potential-time curves for the PANI - H_2SO_4-GOD electrode for various glucose concentrations in 0.1M Acetate buffer, pH 5.8

It was found that the response potential of the enzyme electrode easily reached to steady state. The relationship between response potential and glucose concentration in 0.1 M phosphate buffer and acetate buffer, at 5.8 pH, is shown in figure 7.

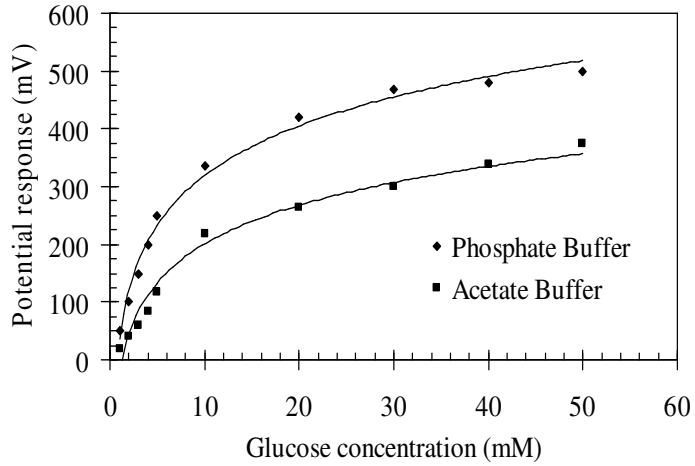

Figure 7. The relationship between potential response and glucose concentration for the PANI-H_2SO_4-GOD electrode in 0.1 M Phosphate buffer and Acetate buffer, pH 5.8

It was found that potential increases with increasing glucose concentration in the range 1 mM-50 mM. In the present case assuming that the enzyme is uniformly distributed throughout the film, the reaction takes place predominantly on the surface of the film in the lower concentration. However, at higher concentration the reaction on the surface of the film and the diffusion occurring simultaneously results in delay in the response time. With increasing concentration of glucose, the response potential also increases and finally reached to the steady state value.

3.6. *Michaelis-Menten Constant (K'_m)*

The apparent Michaelis-Menten constant *(K'_m)* was calculated for the immobilized enzyme by Potentiometric method [32]. The relationship between reciprocal potential against reciprocal glucose concentration in 0.1 M phosphate and acetate buffer is shown in figure 8. The values of response potential (V_{max}) and K'_m for phosphate buffer are 500 mV and *11.11* mM respectively. Similarly for acetate buffer the value of response potential (V_{max}) and K'_m are 650 mV 16.66 respectively (table 1). The value of the K'_m depends on the immobilization of enzyme lesser K'_m gives faster response to glucose [33].

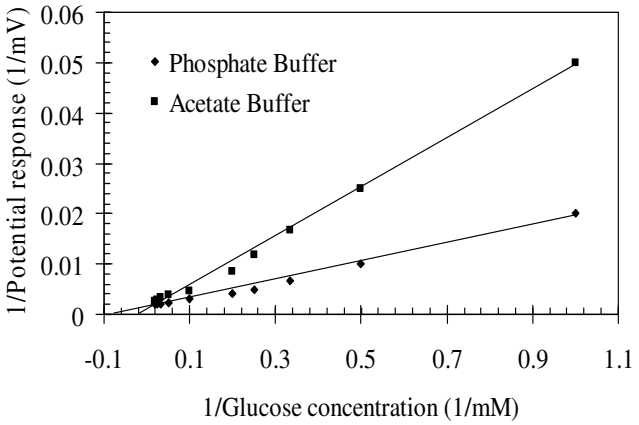

Figure 8. Determination of apparent Michaelis-Menten Constant (Km) for PANI-H_2SO_4-GOD electrode in 0.1M Phosphate buffer and Acetate buffer, pH 5.8

Table 1. Comparison of the analytical performance of PANI-H_2SO_4-GOD Electrode for phosphate and acetate buffer at pH 5.8.

Sr. No	Parameters	Buffers	
		Phosphate	Acetate
1	V_{max} (mV)	500	650
2	K'_m (mM)	11.11	16.66
3	Linearity (mM)	0-5	0-5
4	Sensitivity (mV/mM)	10	13

3.7. Influence of pH

In an optimized polymerization the pH of the reaction medium, allow an efficient entrapment of the enzyme. It also prevents the loss of the enzyme activity under polymerization conditions [34]. Therefore, enzyme sensor response depends on the working pH of the sample solution. The effect of

pH on the behavior of the enzyme electrode was studied with 0.1 M phosphate and acetate buffer solution with 5 mM glucose. The steady state current at 0.5 mA as a function of the pH for phosphate and acetate buffer is shown in figure 9.

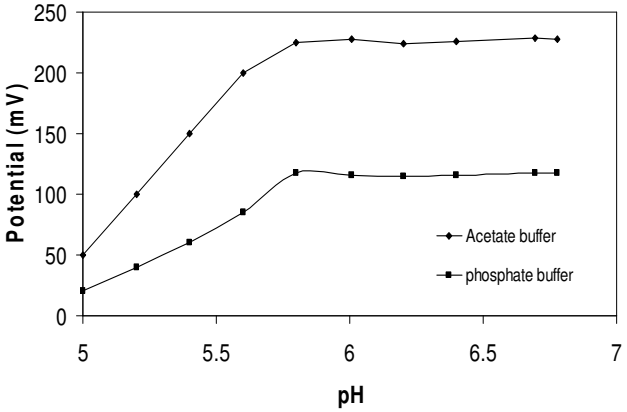

Figure 9. Effect of pH on the PANI- H_2SO_4-GOD electrode response of steady potential measurement at 0.5mA steady current in 5mM glucose solution with 0.1M phosphate and acetate buffer.

The pH of the reaction medium has been varied from 5.0 to 7.0. The response is maximum at pH 5.8 for enzyme solution in both buffers. However, after pH 5.8 the response becomes constant.

4. **Conclusions**

We have successfully developed of PANI-H_2SO_4-GOD biosensor for determination of glucose. It was found that the conducting PANI-H_2SO_4 film having amine functional group can be utilized as a suitable matrix for immobilization of GOD by cross-linking via glutaraldehyde. This efficient cross-linking via glutaraldehyde of the functionalized porous H_2SO_4 doped PANI film lead to the enzyme electrode to exhibit a good performance in terms of dynamic range of detection and short response time. The cost effectiveness and simple method of development of PANI-H_2SO_4-GOD electrode is an additional advantage as compared with conventional electrodes. The PANI-H_2SO_4-GOD electrode in phosphate buffer gives fast response as compared to acetate buffer in Potentiometric measurement.

ACKNOWLEDGEMENTS

Authors are thankful to the University Grants Commission, New Delhi, for financial assistance. They also thank the Departments of Chemistry and Physics, University of Pune, India, for extending FTIR and SEM facilities.

References

1. B. Haghighi, L Gorton, T. Ruzgas and L. J. Jönsson, *Anal. Chim*. Acta 224 (2003) 1.
2. D. J. Shirale, V. K. Gade, P. D. Gaikwad, H. J. Kharat, K. P. Kakde, P. A .Savale, S. S. Hussaini, N. R. Dhumane and M. D. Shirsat. *Transactions of the SAEST* 40 (2005) 128

3. V. K. Gade, D. J. Shirale, P. D. Gaikwad, P. A. Savale, K. P. Kakde, H. J. Kharat and M. D. Shirsat, *Reactive & Functional Polymers* (2006) Available online(www.sciencedirect.com)
4. M.Trojanowicz, O.Geschke, T.Krawczynski and K.Cammann, *Sens. Actuators B* 28 (1995) 191
5. D. J. Shirale, V. K. Gade, P. D. Gaikwad, H. J. Kharat, K. P. Kakde, P. A. Savale, S. S. Hussaini, N. R. Dhumane and M. D. Shirsat, *Mat. Lett*. 60 (2006) 1407
6. S. Mu and H. Xue, *Sens. Actuators B* 31 (1996) 155
7. P. D. Gaikwad, D.J. Shirale, V. K. Gade, P. A. Savale, H. J. Kharat, K. P. Kakde, S. S. Hussaini, N. R. Dhumane and M. D. Shirsat, *Bull of Mater. Sci*. 29 (2006) 169
8. V. K. Gade, D. J. Shirale, P. D. Gaikwad, H. J. Kharat, K. P. Kakde, P. A. Savale and M.D. Shirsat, in: M.D. Shirsat (Ed), *Microwaves and Optoelectronics*, Anshan Tunbridge Wells, UK,(2005) 459
9. P. D. Gaikwad, P. A. Savale, D. J. Shirale, H. J. Kharat, K. P. Kakde, V. K. Gade and M.D. Shirsat, in: M.D. Shirsat (Ed), *Microwaves and Optoelectronics,* Anshan Tunbridge Wells, UK, (2005) 450
10. S. Cosnier, *Biosens. Bioelectron* 14 (1999) 443
11. M. Gerard, A .Chaubey and B. D. Malhotra, *Biosens. Bioelectron* 17 (2002) 345
12. T. Situmorang, D. B. Hilbert and J .J. Gooding, *Electroanalysis* 12 (2000) 111
13. P. A. Savale, D. J. Shirale, P. D. Gaikwad, H. J. Kharat, K. P. Kakde, V. K .Gade and M D Shirsat, in: M.D. Shirsat (Ed), *Microwaves and Optoelectronics*, Anshan Tunbridge Wells, UK, (2005) 409
14. De Taxis du Poet, S. Miyamoto, P. Murakami, T. Kimura and J. Karube, *Anal. Chem. Acta* 235 (1990) 255
15. D. J. Shirale, A. S. Bhalerao, H. J. Kharat, P. D. Gaikwad, K. P. Kakde, P. A. Savale, V. K. Gade and M. D. Shirsat, in: M.D. Shirsat (Ed), *Microwaves and Optoelectronics*, Anshan Tunbridge Wells, UK, (2005) 455
16. D.C.Trivedi In: H.S. Nalwa, (Ed.), *Handbook of Organic Conductive Molecules and Polymers*, vol. 2. John Wiley. (1997)
17. A.Yoshizawa, M.Takeda, Y.Oura, T.Takemoto and K.Naoi, *Electrochemistry* 67 (1999) 45
18. E. Naudin, P. Gouerce and D. J. Belanger, *Electroanal. Chem*. 459 (2000) 1
19. N. Leventis and Y. C. Chung, *Electrochem. Soc*. 137 (1990) 3321
20. P. N. Bartlett, P. R. Birkin and E. N. K. Wallace, *J.Chem.Soc.Faraday Trans*. 93 (1997) 1951
21. K. R. Rogers, in: R.A. Meyers (Ed.), *Encyclopedia of Environmental Analysis and Remediation,* Wiley, New York, 755. (1998)
22. V. Lopez-Avila and H. H. Hill, *Anal. Chem*. 69 (1997) 289
23. M. P. Marco and D. Barcelo, *Meas. Sci. Technol*. 7 (1996) 1547
24. G. Harsanyi, R. Dobay and C. S. Visy, *Electroanalysis* 11 (1999) 804
25. M. Umana and J. Waller, *Anal. Chem*. 58 (1986) 2979
26. P. Pal, D. Nandy and T. N. Misra, *Thin Solid Films* 223 (1994) 138
27. G. Fortier and D. Belanger, *Biotechnol. Bioeng*. 37 (1991) 854
28. L. D. Wingard, C. C. Lio, S. K. Wolfsen and S. J. YaO, *Drash Diabetes care* 5 (1982) 199
29. L. D. Wingard, Jr, *Proc.fed Am.Soc.Exp.Biol* 42 (1983) 288
30. A. Haouz, C. Twist, C. Zents , P. Yauc and Alpert. *Eur. Biophy.J*. 27 (1998) 19
31. B. Sari, M. Talu, and F. Yildirim, *Russian Journal of Electrochemistry*, 38 (2002) 707
32. A. F. Diaz and J. A. Logan, *J. Electroanal. Chem*. 111 (1980) 111
33. G.S. Akundy, R. Rajagopalan and J.O. Iroh, *J. Applied Polymer Science* 83 (2002) 1970
34. S. Fabiano, C. Tran-Minch, B. Piro, L.A. Dang, M.C. Pharm and O. Vittori. *Mat. Sci. Eng*. 21 (2002) 61