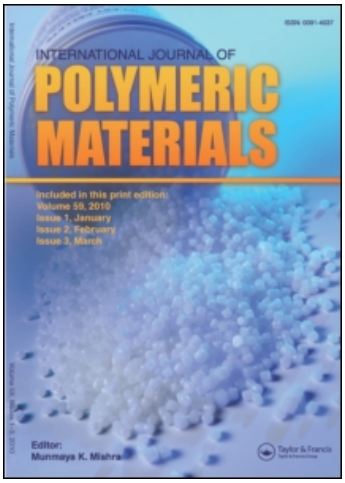

International Journal of Polymeric Materials
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713647664

Poly(Pyrrole)-Poly(N-Methylpyrrole) Composite Matrix for Amperometric Biosensor Design

Santosh B. Kadam[a]; Kunal Datta[a]; Prasanta Ghosh[a]; Mahendra D. Shirsat[a]
[a] Intelligent Materials Research Laboratory, Department of Physics, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India

Online publication date: 03 January 2011

To cite this Article Kadam, Santosh B. , Datta, Kunal , Ghosh, Prasanta and Shirsat, Mahendra D.(2011) 'Poly(Pyrrole)-Poly(N-Methylpyrrole) Composite Matrix for Amperometric Biosensor Design', International Journal of Polymeric Materials, 60: 3, 233 — 243
To link to this Article: DOI: 10.1080/00914037.2010.504173
URL: http://dx.doi.org/10.1080/00914037.2010.504173

International Journal of Polymeric Materials, 60:233–243, 2011

ISSN: 0091-4037 print/1563-535X online
DOI: 10.1080/00914037.2010.504173

Taylor & Francis
Taylor & Francis Group

Poly(Pyrrole)-Poly (N-Methylpyrrole) Composite Matrix for Amperometric Biosensor Design

Santosh B. Kadam, Kunal Datta, Prasanta Ghosh, and Mahendra D. Shirsat

Intelligent Materials Research Laboratory, Department of Physics, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India

Electrochemical copolymerization of pyrrole–N-methyl pyrrole has been carried out galvanostatically on platinum substrate. The polypyrrole–poly (n-methylpyrrole) composite films were subjected to electrical, spectral and morphological characterizations. Glucose biosensor was further fabricated based on the immobilization of glucose–oxidase in the composite films by crosslinking via glutaraldehyde. The effect of phosphate and acetate buffer was investigated. The biosensor exhibited an excellent linear response for a wide range of glucose concentration from 0 mM to 50 mM in both the buffers at pH 7.4. However, the sensitivity of the developed biosensor in the presence of a phosphate buffer was found to be higher.

Keywords electropolymerization, glucose biosensor, poly (n-methyl pyrrole), polypyrrole

INTRODUCTION

In the 1960s, Clark and Lyons [1] demonstrated the first ever enzyme-based electrode and paved the pathway towards a new generation of biosensors. The

Received 1 April 2010; accepted 2 June 2010.
The authors are grateful to CSIR, India and UGC, India for financial assistance. K. Datta is thankful to CSIR, India for providing a research fellowship. P. Ghosh is thankful to UGC, India for providing a research fellowship.
Address correspondence to Mahendra D. Shirsat, Intelligent Materials Research Laboratory, Department of Physics, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431 004, Maharashtra, India. E-mail: mdshirsat_bamu@yahoo.co.in

large spectrum of investigations on biosensors that followed was mostly driven by the excellent specificity and repeatability rendered by enzymes. However, the application of pristine enzymes as a prime sensor material was fundamentally constrained by the fact that they do have poor stability in solution. Numerous efforts, thereafter, have been carried out in pursuit of a way out of this problem, and immobilization of the enzyme in a suitable matrix was globally adapted as a convenient prescription [2–5]. An organic conducting polymer (OCP) matrix is so far the most admired and probably the widest explored solution in its class for immobilization of enzymes [6–10]. Nevertheless, in order to ensure the optimum efficiency of the enzymes, appropriate chemical and morphological tailoring of the base matrix remains a fundamental challenge, and continual striving for better material properties constitutes a prime area of investigation in biosensor research. Composites of OCPs can be interesting in this aspect since they do offer a facile methodology to combine the superior properties of the constituent monomers to overcome individual shortcomings.

The present investigation deals with the fabrication of a glucose biosensor based on redox properties of glucose oxidase (GODx) [11–12] immobilized on a poly(pyrrole)[P(Py)]/poly(n-methylpyrrole)[P(NMP)] composite matrix and evaluation of the performance of the fabricated sensor for a wide range of glucose concentration (0–50 mM). Pyrrole was a spontaneous choice for the base backbone due to its biocompatibility [13,14], thermal stability [15], high conductivity [16] and ease of synthesis. At the same time, the tendency of pyrrole to degrade in the presence of oxygen and water [17] was a serious point of concern. A composite with N-methylpyrrole emerged as an attractive solution since it is less reactive to oxygen and water [18]. In addition, the mechanical strength of n-substituted polymers of the pyrrole family (i.e., N-methylpyrrole) [19–20] was an added advantage over the low mechanical strength of pyrrole aroused due to its infusibility and insolubility.

The P(Py)/P(NMP) composite film was synthesized by chronopotentiometric technique with $NaNO_3$ as a supporting electrolyte. Elsewhere communicated optimized process parameters were employed to ensure an optimum thickness and surface morphology of the produced films. GODx was immobilized on P(Py)/P(NMP)/$NaNO_3$ by crosslinking via glutaraldehyde at pH 7.4. The P(Py)/P(NMP)/$NaNO_3$/GODx electrodes were tested and compared for phosphate and acetate buffer, respectively. The sensitivity of the biosensor in the presence of phosphate buffer was found to be better.

EXPERIMENTAL

Preparation of P(Py)/P(NMP)/$NaNO_3$ Film

P(Py)/P(NMP)/$NaNO_3$ film was synthesized from an aqueous solution (20 ml) of 0.1 M Py(Alfa Aesar, 98+%), 0.1 M NMP (Acros Organics, 98%)

and 0.1 M $NaNO_3$ (Rankem, India). The electropolymerization was carried out by chronopotentiometric technique with a CHI 660C electrochemical workstation. A standard three-electrode set-up was employed in one compartment electrochemical cell. Planar platinum foils were used as the working and counter electrode. The reference electrode was an Ag/AgCl electrode. The current density, deposition time and pH were kept constant at 1.0 mA/cm^2, 10 min and 1.0, respectively (optimized values). All depositions were carried away at room temperature. As a post-synthesis treatment, polymer-coated platinum substrates were rinsed with double de-ionized water and dried in an ambient atmosphere.

The P(Py)/P(NMP) composite films were subjected to in situ electrochemical characterization carried out during synthesis. FTIR spectral study was carried out to confirm the formation of P(Py)/P(NMP) composite using a Testscan Shimadzu FTIR 800 series in the region between 400–4000 cm^{-1}. Scanning electron micrographs were recorded at various magnifications using a JEOL JSM-6360 A Analytical Scanning Electron Microscope.

Immobilization of GODx on P(Py)/P(NMP)/NaNO3 Film

The stock solution of GODx (Aldrich) (1 mg/ml) was prepared in 0.1 M phosphate buffer (pH 7.4) and/or 0.1 M acetate buffer (pH 7.4) and allowed to mutarotate over 24 h before further use. Finally, the GODx stock was adsorbed onto the surface of P(Py)/P(NMP)/$NaNO_3$ films. Immobilization was carried out by crosslinking via (0.1%) glutaraldehyde (Loba Chemie, India) on P(Py)/P(NMP)/$NaNO_3$ films. A 30 min drying time was allotted followed by repeated washing with phosphate and/or acetate buffer to ensure restriction of leaching of enzyme from film. Adequate concentrations of GODx and glutaraldehyde in the crosslinking mixture were chosen to ensure higher enzyme loading and good retention of the enzyme [21].

Amperometric Determination of Glucose

The stock solution of D-Glucose was prepared in a phosphate (0.1 M, pH 7.4) and acetate buffer (0.1 M, pH 7.4), respectively, and left for 24 h before testing. The P(Py)/P(NMP)/$NaNO_3$/GODx biosensor was maintained at + 700 mV vs. Ag/AgCl reference electrode in phosphate and acetate buffer solutions, respectively, in order to yield a stable background current [22]. The biosensor was tested for amperometric response towards gradually increasing concentrations (0–50 M) of glucose. The steady state anodic current (usually reached after ca.10 s. of spiking a glucose solution) was recorded by a CHI 660C electrochemical workstation.

RESULTS AND DISCUSSION

Sensing Mechanism

In an amperometric enzyme sensor, GODx acts as a redox protein. Figure 1 depicts the proposed mechanism for the PPy/PNMP/ $NaNO_3$/GODx biosensor. The added glucose solution is oxidized by the immobilized GODx in the presence of dissolved O_2 according to the following reaction:

$$\text{Glucose} + O_2 \xrightarrow{\text{GODx}} \text{GluconicAcid} + H_2O_2 \quad (1)$$

The generated H_2O_2 is electrocatalytically oxidized at the PPy/PNMP conducting composite resulting an anodic current in accord to the following reaction

$$H_2O_2 \xrightarrow{\text{PPy/PNMP}} O_2 + 2H^+2e^- \quad (2)$$

Basically, the conversion of glucose to gluconic acid involves transfer of two protons and two electrons from the substrate to the flavin moiety of the enzyme. The electron transfer from the redox co-factor to the sensing electrode is facilitated by the presence of conducting composite.

Electrochemical Synthesis of P(Py)/P(NMP)/$NaNO_3$ Film

P(Py)/P(NMP)/$NaNO_3$ composite films were synthesized galvanostatically with optimized process parameters already mentioned above. Chronopotentiograms revealed low polymerization potential that ensures higher conductivity and uniform surface morphology as per earlier reported literature [23]. Synthesized films exhibited uniform morphology and good adhesivity. Chronopotentiogram recorded during synthesis of P(Py)/P(NMP)/$NaNO_3$ composite film with optimized process parameters is given in Figure 2.

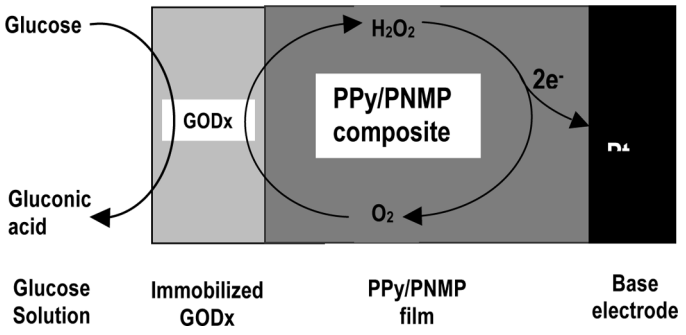

Figure 1: Proposed mechanism of the P(Py)/P(NMP)/$NaNO_3$/GODx electrode.

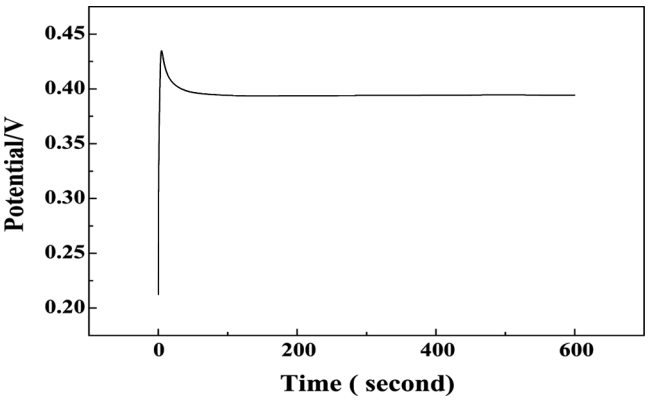

Figure 2: Chronopotentiogram recorded during synthesis of P(Py)/P(NMP)/$NaNO_3$ composite film with optimized process parameters.

FTIR Spectra of P(Py)/P(NMP)/$NaNO_3$ Film

The Principal absorption band observed in the FTIR spectrum of the P(Py)/P(NMP)/$NaNO_3$ composite film is shown in Figure 3. The broad peak at 3435 cm^{-1} corresponds to N-H stretching. The absorption band observed at 2850–2862 cm^{-1} is due to C-H vibrations, and the C=O stretching is observed at 1639 cm^{-1}. The band observed at 1563 cm^{-1} is due to C=C stretching. The peak at 2923 cm^{-1} is attributed to the CH_3 stretching. The sharp absorption band observed at 1384 cm^{-1} is due to the ring stretch of n-methyl pyrrole.

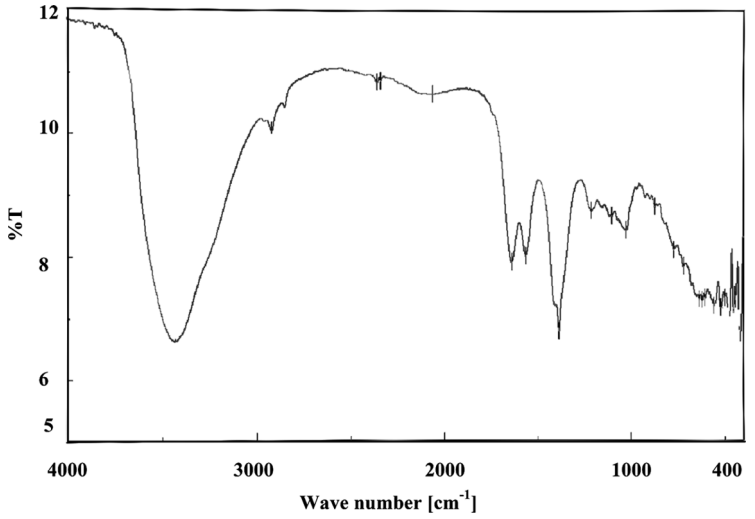

Figure 3: FTIR spectrum of P(Py)/P(NMP)/$NaNO_3$ composite film with optimized process parameters.

The FTIR spectral result confirms the formation of poly pyrrole P(Py) and poly n-methyl pyrrole P(NMP).

SEM Study of P(Py)/P(NMP)/$NaNO_3$ Film

The SEM image (Figure 4) for pristine P(Py)/P(NMP)/$NaNO_3$ composite film reveals uniform and granular surface morphology. Such morphology is highly suitable for the entrapment of enzyme thus rendering higher stability to the biosensor.

Sensing Response of P(Py)/P(NMP)/$NaNO_3$/GODx Electrode

Fast and linear response of the P(Py)/P(NMP)/$NaNO_3$/GODx biosensor was observed in an acetate as well as phosphate buffer. However, comparison reveals the phosphate buffer to be more efficient towards sensitive fingerprinting of glucose concentration. The current-time relationship of the P(Py)/P(NMP)/$NaNO_3$/GODx electrodes in the presence of acetate and phosphate buffer (with the electrode poised at +700 mV vs. Ag/AgCl reference electrode) is shown in Figure 5 and Figure 6, respectively.

The response current was found to reach a steady state easily. The linear relationship between response current and glucose concentration (0–10 mM) in 0.1 M acetate buffer (pH 7.4) is shown in Figure 7 (the linear regression equation is $y = 1.05x + 4.0873$ and the linear regression coefficient is $R^2 = 0.9422$). The inset indicates a linear response from 10 to 50 mM of glucose

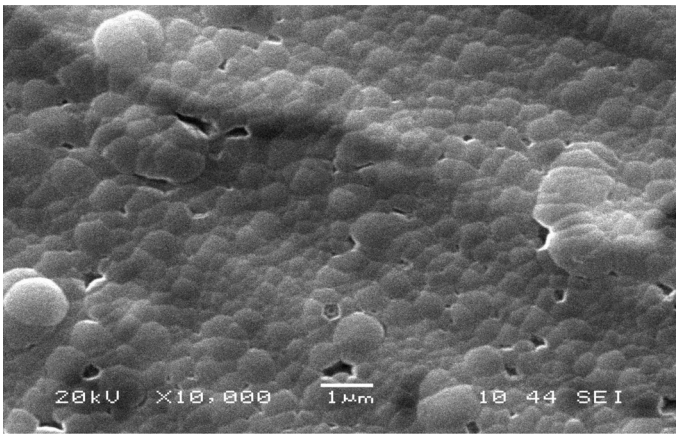

Figure 4: SEM image of P(Py)/P(NMP)/$NaNO_3$ composite film with optimized process parameters.

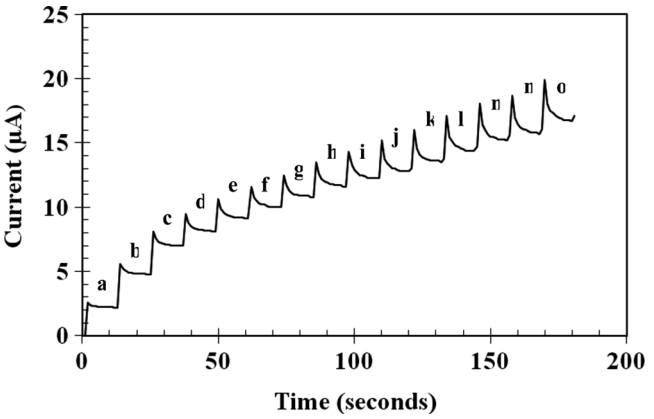

Figure 5: Current-time relationship (sensing response) of P(Py)/P(NMP)/$NaNO_3$/GODx electrode in 0.1 M acetate buffer (pH7.4) for different glucose concentrations of 0–50 mM: a) 0 mM; b) 1 mM; c) 2 mM; d) 3 mM; e) 4 mM; f) 5 mM; g) 6 mM; h) 7 mM; i) 8 mM; j) 9 mM; k) 10 mM; l) 20 mM; m) 30 mM; n) 40 mM; o) 50 mM.

concentration (The linear regression equation is $y = 0.08x + 13.06$ and the linear regression coefficient is $R^2 = 0.9919$).

Similarly, the relationship between response current and glucose concentration (0–10 mM) in 0.1 M phosphate buffer (pH 7.4) is shown in Figure 8 (the linear regression equation is $y = 0.4841x + 1.8786$ and the linear regression coefficient is $R^2 = 0.943$). The inset indicates a linear response from 10 to 50 mM of glucose concentration (the linear regression equation is $y = 0.037x + 6$ and the linear regression coefficient is $R^2 = 0.9923$).

As evident from the response characteristics, in both cases, the current increases with increasing glucose concentration from 1 to 50 mM. For each

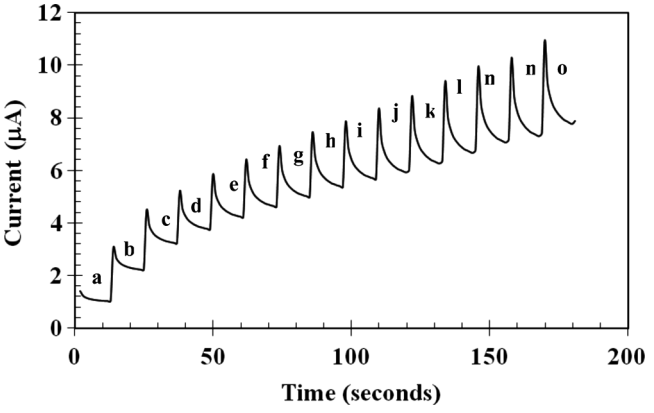

Figure 6: Current-time relationship (sensing response) of P(Py)/P(NMP)/$NaNO_3$/GODx electrode in 0.1 M phosphate buffer (pH7.4) for different glucose concentrations of 0–50 mM: a) 0 mM; b) 1 mM; c) 2 mM; d) 3 mM; e) 4 mM; f) 5 mM; g) 6 mM; h) 7 mM; i) 8 mM; j) 9 mM; k) 10 mM; l) 20 mM; m) 30 mM; n) 40 mM; o) 50 mM.

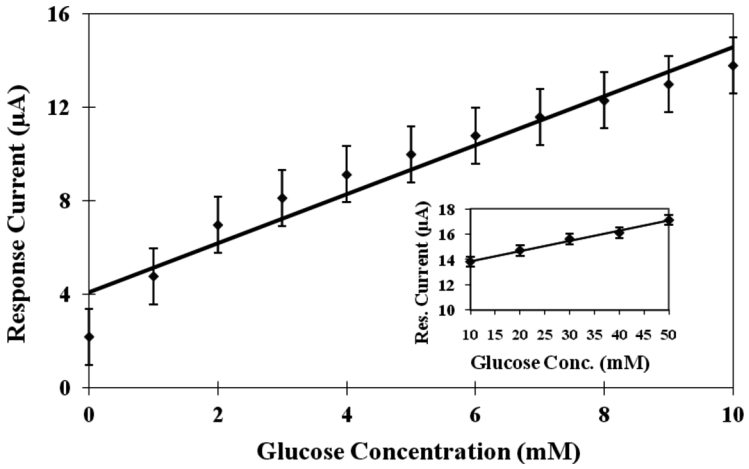

Figure 7: The relationship between response current and glucose concentration for P(Py)/P(NMP)/$NaNO_3$/GODx electrode in 0.1 M acetate buffer (pH7.4). The inset indicates the response from 10 to 50 mM.

spike of glucose, within a response time of ca.10 s, a sharp rise in the current was observed. During successive additions of 1 mM of glucose, a well-defined response was observed. In a nutshell, we have observed almost linear sensing responses over the entire range (1 to 50 mM) of glucose concentration in acetate (the linear regression equation is $y = 0.2244x + 8.1634$ and the linear regression coefficient is $R^2 = 0.6366$) as well as in phosphate buffer (the linear regression equation is $y = 0.1032x + 3.758$ and the linear regression coefficient is $R^2 = 0.6359$). The sensitivity of the biosensor in acetate and phosphate

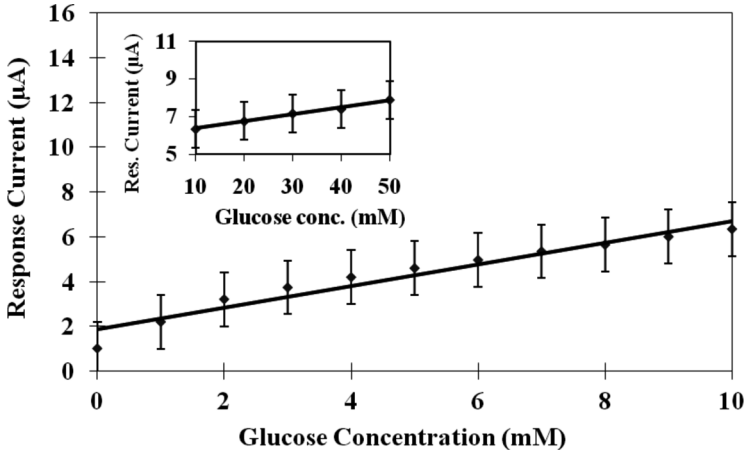

Figure 8: The relationship between response current and glucose concentration for P(Py)/P(NMP)/$NaNO_3$/GODx electrode in 0.1 M phosphate buffer (pH7.4). The inset indicates the response from 10 to 50 mM.

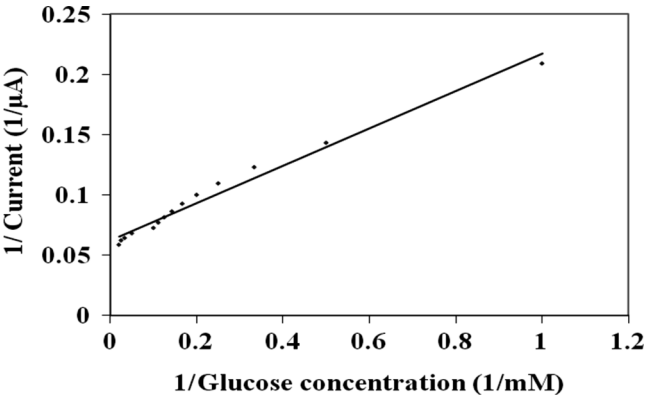

Figure 9: The determination of apparent Michaelis–Menten constant (K'_m) for P(Py)/P(NMP)/$NaNO_3$/GODx electrode in 0.1 M acetate buffer (pH7.4).

buffer is found to be 1.15 μA/mM and 2.48 μA/mM. The detection is sufficient for medical diagnostic purposes since the normal clinical range for glucose in blood is between 3.5 to 6.1 mM, whereas abnormal glucose levels can reach as high as 20 mM (24).

Michaelis–Menten Constant (K'_m)

The apparent Michaelis–Menten Constant (K'_m) was calculated for the immobilized enzyme by the amperometric method suggested by Shu and Wilson (25). The relationship between 1/current against 1/glucose concentration for an acetate and phosphate buffer is shown in Figure 9 (the linear regression equation is $y = 0.1545x + 0.0625$ and the linear regression coefficient

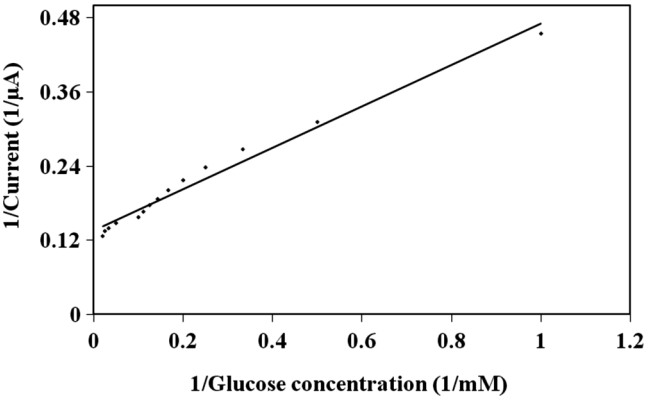

Figure 10: The determination of apparent Michaelis–Menten constant (K'_m) for P(Py)/P(NMP)/$NaNO_3$/GODx electrode in 0.1 M phosphate buffer (pH7.4).

Table 1: Comparison of the analytical performance of P(Py)/P(NMP)/$NaNO_3$/GODx electrode for acetate and phosphate buffer at pH 7.4

		Buffer	
Sr. no.	**Parameters**	**Acetate**	**Phosphate**
1	I_{max} (μA)	7.69	16.66
2	K'_m (mM)	3.3	3.2
3	Linearity (mM)	1–10	1–10
4	Sensitivity (μA/mM)	1.15	2.48

is $R^2 = 0.9808$) and Figure 10 (the linear regression equation is $y = y = 0.3357x + 0.1358$ and the linear regression coefficient is $R^2 = 0.9807$), respectively.

The maximum current (I_{max}) and Michaelis–Menten Constant (K'_m) for acetate and phosphate buffer is found to be 7.69 μA; 3.3 mM and 16.66 μA; and 3.2 mM, respectively. A smaller K'_m ensures a faster response of the electrode to glucose (26). Therefore, the phosphate buffer is more efficient for the immobilization of GODx.

A comparative figure of the analytical parameters of the fabricated sensor in the acetate and phosphate buffer is furnished in Table 1.

CONCLUSION

The study shows the feasibility and practicality of composite-conducting polymer structures for real time sensing applications. P(Py)/P(NMP) composite films with optimized electrochemical process parameters have been synthesized successfully. The adhesivity of the films was good and surface morphology was efficient for biosensing applications. Glucose–oxidase was successfully immobilized on the synthesized films towards materialization of glucose sensor. The efficient crosslinking (via glutaraldehyde) on the uniform and granular P(Py)/P(NMP)/$NaNO_3$ structure lead to an enzyme electrode exhibiting a good performance in terms of dynamic range of detection, short response time and excellent linearity. The sensor was found to have a linear and fast-sensing response to glucose concentration of 0–50 mM, which is suitable for application in medical diagnostics. The sensor was evaluated both in an acetate and phosphate buffer atmosphere, and the phosphate buffer was found to be more efficient for practical applications.

REFERENCES

[1] Clark, L. C., and Lyons, C. *Ann. N. Y. Acad. Sci.* **102**, 29 (1962).

[2] Malhotra, B. D., and Choubey, A. *Sens. Actuators B.* **91**, 117 (2003).

[3] Gowda, M. D., Kumar, M. A., Thakur, M. S., and Karanth, S. G. *Biosens. Bioelectron.* **17**, 503 (2002).

[4] Olea, D., Moreau, P., and Faure, C. *J. Electroanal. Chem.* **605**, 125 (2007).

[5] Chen, C., Jiang, Y., and Kan, J. *Biosens. Bioelectron.* **22**, 639 (2006).

[6] Degani, Y., and Heller, A. *J. Am. Chem. Soc.* **111**, 2357 (1989).

[7] Cosnier, S. *Biosens. Bioelectron.* **14**, 443 (1999).

[8] Li, J., and Lin, X. *Biosens. Bioelectron.* **22**, 2898 (2007).

[9] Gaikwad, P. D., Shirale, D. J., Gade, V. K., Savale, P. A., Kakde, K. P., Kharat, H. J., and Shirsat, M. D. *Int. J. Electrochem. Sci.* **1**, 425 (2006).

[10] Savale, P. A., Kharat, H. J., Datta, K., Ghosh, P., and Shirsat, M. D. *Int. J. Polym. Mater.* **57**, 1 (2008).

[11] Albareda-Sirvent, M., Merkoci, A., and Alegret, S. *Sens. Actuators B.* **69**, 153 (2000).

[12] Bartlett, N. P., and Astier, Y. *Chem. Commun.* **105**, (2000).

[13] Zang, Z., Roy, R., Dugre, F. J., Tessier, D., and Dao, L. H. *J. Biomed. Mater. Res.* **57**, 63 (2001).

[14] Jakubiec, B., Marois, Y., Zang, Z., Roy, R., Sigot-Luizard, M. F., Dugre, F. J., King, M. W., Dao, L., Larche, G., and Guidoin, R. *J. Biomed. Mater. Res.* **41**, 519 (1998).

[15] Sakkopoulos, S., Vitoratos, E., and Dalas, E. *Synth. Met.* **92**, 63 (1998).

[16] Gade, V. K., Shirale, D. J., Gaikwad, P. D., Savale, P. A., Kakde, K. P., Kharat, H. J., and Shirsat, M. D. *Int. J. Polym. Anal. Charact.* **56**, 167 (2007).

[17] Naoi, K., Hirabayashi, T., Tsubota, I., and Osaka, T. *Bull. Chem. Soc. Jpn.* **60**, 1213 (1987).

[18] Yee, L. M., Kassim, A., Mahmud, H. N. M. E., Shariff, A. M., and Haron, Md. J. *The Malayasin Journal of Analytical Sciences*, **11**, 133 (2007).

[19] Singh, R., Narula, A. K., Tandon, R. P., Rao, S. U. M., Panwar, V. S., Mansingh, A., and Chandra, S. *Synth Met.* **79**, 1 (1996).

[20] Su, W., and Iroh, J. O. *Electrochem. Acta.* **71**, 1293 (1996).

[21] Shirale, D. J., Gade, V. K., Gaikwad, P. D., Savale, P. A., Kakde, K. P., Kharat, H. J., and Shirsat, M. D. *Int. J. Polym. Anal. Charact.* **11**, 369 (2006).

[22] Ming Uang, Y., and Chuan Chou, T. *Biosens. Bioelectron.* **19**, 141 (2003)

[23] Shirale, D. J., Gaikwad, P. D., Gade, V. K., Savale, P. A., and Shirsat, M. D. *Mater. Lett.* **61**, 1372 (2007).

[24] Forzani, Erica S., Zhang, H., Nagahara, Larry A., Amlani, I., Tsui, R., and Tao, N. *Nano Letters* **4**, 1785 (2004).

[25] Shu, R., and Wilson, G. S., *Anal. Chem.* **240**, 2209 (1965).

[26] Shirsat Mahendra, D., Too Chee, O., and Wallace Gordon, G. *Electroanalysis.* **20**, 150 (2008).