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Preparation of Poly(sodium-4-styrenesulphonate) Doped Polypyrrole Film on Platinum Electrode and its Use in Determination of Hydrogen Peroxide

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Abstract

In this study, a new film electrode for determination of hydrogen peroxide was developed. Polypyrrole-poly (sodium-4-styrenesulphonate) film have been prepared on the platinum electrode by the electropolymerization of pyrrole in the presence of poly(sodium-4-styrenesulphonate). Optimum synthesis/preparation/formation of polypyrrole-poly(sodium-4-styrenesulphonate) film was determined. Oxidation of the hydrogen peroxide was examined at a different potential. Therefore, four different potentials (0.4, 0.5, 0.6, and 0.7 V) were determined. Also, concentration of pyrrole and poly(sodium-4-styrenesulphonate) were examined. The sensitivities of electrodes to hydrogen peroxide were determined to clarify the role of PSS concentration. Three different PSS concentrations (0.05, 0.1, 0.15 M) were examined. The effect of the number of cycles in their sensitivity to hydrogen peroxide is investigated. Morphology of electrodes was qualified by means of SEM. Free enzyme work was performed using the prepared film electrode and glucose oxidase. Linear increases were observed in the amperometric response currents of the electrode versus increasing glucose concentrations. It was shown that this film can be used for hydrogen peroxide producing enzymes.

1. INTRODUCTION

The specification of hydrogen peroxide in biological systems and environment is of enormous importance because of its effects on the ecosystem and the human health [1,2]. In spite of the improvements in analytical methods [3,7], it is still a challenge to ascertain new approaches that could improve the simplicity, selectivity, sensitivity and rapid monitoring for detection of H_2O_2 [8].

H_2O_2 is a product created in many reactions catalyzed by oxidase enzyme. The amount of substrate determined via the biosensors prepared with oxidase enzyme is based upon the oxidation of H_2O_2 product at a certain potential [9, 17]. The amperometric detection of H_2O_2 is normally performed anodically [18].

Polymers manifest themselves as favorable materials for the following reasons [19]. Firstly, there is noticeable flexibility in the convenient monomeric structures. Secondly, many of the available monomers are suitable for the circumstances needed for enzyme activity such as neutral aqueous solutions. Thirdly, electroactive polymers are capable of being reversibly doped and undoped, a property which may be exploited to develop enzyme-based biosensors. Finally, these materials can be deposited electrochemically, a method that offers a simple approach for enzyme immobilization. Now there exists a growing literature on the use of electroactive polymers and its derivatives for sensor fabrication [20, 23].

Conducting polypyrrole is a biological compatible polymer matrix wherein number of drugs and enzymes can be incorporated by way of doping. The polypyrrole, obtained as freestanding film by electrochemical polymerization, has gained tremendous recognition as sophisticated electronic measuring device in the field of sensors and drug delivery.

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Doping materials such as Nafion, poly (vinyl alcohol), poly(methyl methacrylate), poly (styrene sulphonate) , poly (vinyl sulphonate), dodecylbenzene sulphonate, and p-toluene sulphonate may enhance the conductivity, stability, and mechanical force of PPy matrix. It was reported that the incorporation of a large-sized dopant anion, like poly(vinyl sulphonate) (PSS), into PPy films during electropolymerization makes PPy film more porous and sulphonate ions of the PPy– PSS composite films supply a charged surface for electrostatic interaction between the enzyme and the surface [24,27].

Poly(sodium-4-styrenesulphonate) (PSS) is a polymer made by the direct sulfonation of polystyrene [28,29]. The sodium salts of PSS are used as superplastifiers in cements, dye-improving agents, proton exchange membranes in fuel cells, and also in ion exchange resins when PSS is crosslinked [30].

Electrochemical sensors, especially amperometric biosensors, hold a leading position among biosensors. In the case of electrochemical biosensors, the effective immobilization of an enzyme on an electrode surface with a high interception of its biological activity is a crucial point for the commercial development of biosensors.

In recent years, various methods have been developed to determine hydrogen peroxide (H_2O_2) because of its wide and varied applications in many fields, such as in clinical, food, pharmaceutical, and environmental analyses [31-34]. Electrochemical biosensors containing enzyme electrodes which catalyze substrate in sequence are mostly used to determine H_2O_2 [35]. Enzyme electrodes consist of two original parts: a conductive part for electroanalytical purposes and a particular enzyme coat that provides the selectivity [36]. The performance of this kind of biosensors greatly depends on the amount of enzyme adsorption, favorable orientations, and suitable interaction of electrode with redox active prosthetic groups of enzymes [37].

In this study, polypyrrole-poly(sodium-4-styrenesulphonate) film have been prepared on the platinum electrode by the electropolymerization of pyrrole in the presence of poly(sodium-4-styrenesulphonate). Optimum conditions of polypyrrole-poly(sodium-4-styrenesulphonate) film was founded.

2. MATERIALS AND METHODS

2.1. Instrumental Techniques and Reagents

All electrochemical experiments were performed using a BAS Epsilon-EC-Ver 1.40.67 NT electrochemical analyzer. A conventional three-electrode system was carried out with a Pt plate (0.5 cm^2) as the working electrode, an Ag/AgCl (3M KCl) as the reference electrode, and a platinum wire as the counter electrode. The pH values of the buffer solutions were measured with an ORION Model 720A pH-ionmeter. Temperature control was accomplished with a Grant W14 thermostat. The scanning electron micrograph was recorded using a JEOL JSM-6060LV SEM machine. Glucose oxidase (EC 1.1.3.4. from Aspergillus Niger obtained from the microorganism and with an activity of 5204 U/ml) and glucose were purchased from Sigma. Pyrrole and PSS was supplied by Fluka. All the other chemicals were supplied from Sigma. All the solutions were prepared by the use of distilled water. Glucose stock solution was allowed to mutarotate for at least 24 h at room temperature prior to use and stored at 4 ^oC.

2.2. Preparation of Pt/PPy-PSS Film Electrode

The surface of the Pt plate electrode was covered by the electropolymerization of pyrrole and the poly (sodium 4-styrenesulfonate). The electrode was immersed in a 10 mL solution of 0.1M pyrrole and 7.4 mL aqueous solution of the sodium salt of poly(sodium 4-styrenesulfonate) (25%). The solution was purged with argon to remove the oxygen. The electropolymerization of pyrrole upon the electrode surface was accomplished by the cyclic voltammetric scans between -0.8 and 0.8 V at a scan rate of 20mV/s. The electrode was washed with buffer solution after the coating procedure.

2.3.The Sensitivity of PPy–PSS Film Electrode to Hydrogen Peroxide

The response current of electrode covered with PPy-PSS was determined as follows. A buffer solution of pH 7.0 (10 mL, 25 °C) was added to the cell. The solution included 0.1 M phosphate to adjust pH and 0.1 M sodium perchlorate as a supporting electrolyte. The electrodes were maintained at 0.4 V for approximately 4 h and steady current (ia) was saved. The different concentration of hydrogen peroxide was added to the cell. The currents acquired at 0.4 V were recorded every 200 s. The difference in current values was plotted against the hydrogen peroxide concentration. The linear region of current values was determined.

3. RESULTS AND DISCUSSION

In this paper, we described a new film electrode used to determine H_2O_2. The surface of the Pt plate electrode was covered by the electropolymerization of pyrrole and the poly (sodium 4-styrenesulfonate). The parameters affecting the performance of the film electrode were examined.

3.1. The Determination of Working Potential

The working potential for film electrodes prepared using different dopants has been noted in many studies [12, 27]. In this study, after preparing Pt/PPy–PSS electrodes, oxidation of the hydrogen peroxide was examined at a different potential (Figure 1). For this purpose, four different potentials (0.4, 0.5, 0.6, and 0.7 V) were examined. The biggest current value was achieved at 0.70 V. At high potentials, the interferences caused by exogenous substances present in body fluids (e.g., ascorbic acid) [38]. Therefore, 0.4 V was selected as working potential.

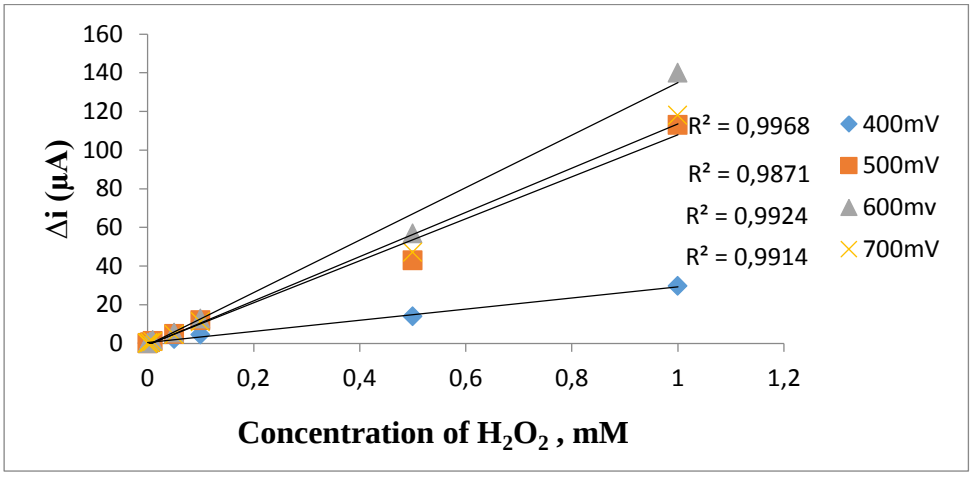

Fig 1. Determination of working potential at Pt/PPy-PSS electrode

3.2. The Pyrrole Concentration Experiments

The electrodes sensitivity to hydrogen peroxide was studied to explain the important of pyrrole concentration (Figure 2). For this reason, several pyrrole concentrations (0.05, 0.1, 0.15 and 0.2 M) were examined [39]. The lowest correlation coefficient of electrode was found at 0.05 M pyrrole concentration. It is shown that Ppy-PSS film synthesized with the pyrrole concentration of 0.20 M is not suitable for the determination of H_2O_2 because it has the lowest sensitivity. The surfaces of these electrodes prepared by 0.15 M pyrrole were smoother and higher sensitivity than those of the other electrodes. Therefore, the pyrrole concentration was used as 0.15 M in the next trial.

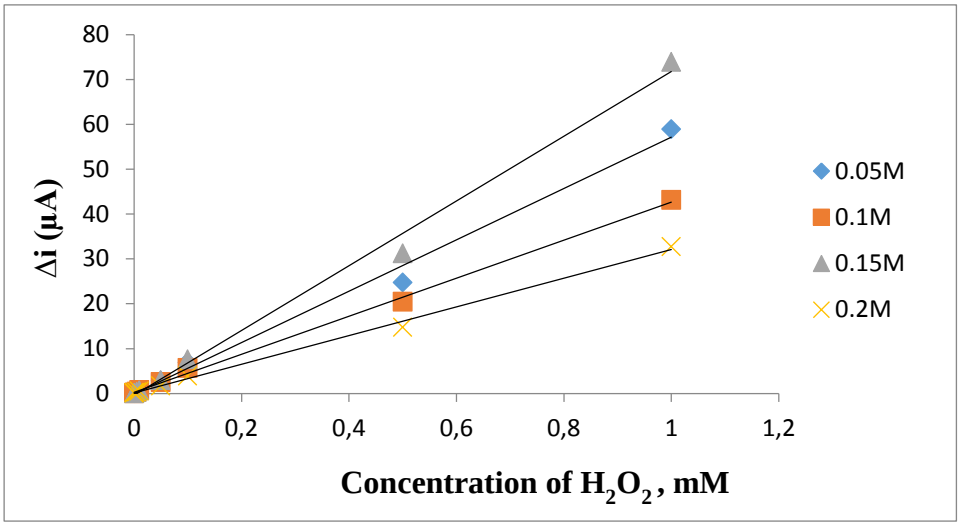

Fig 2. The effect of pyrrole concentration on the response of hydrogen peroxide

3.3. The Effect of PSS Concentration

Studies investigating the dopant effect on the sensitivity of film electrodes prepared with hydrogen peroxide sensitivity are reported in the literature [39,12]. In this study, PSS was used as an anionic dopant. The sensitivities of electrodes to hydrogen peroxide were determined to clarify the role of PSS concentration. Three different PSS concentrations (0.05, 0.1, 0.15 M) were examined (Figure 3). Figure 3 shows that the correlation coefficients of film electrodes covered in 0.0125 and 0.15 M PSS were lower than 0.10 M. The best linear response appeared with the use of 0.025 M sodium PSS. All of the experiments were carried out with Pt/PPy–PSS electrodes prepared by maintaining the PSS concentration at 0.025 M.

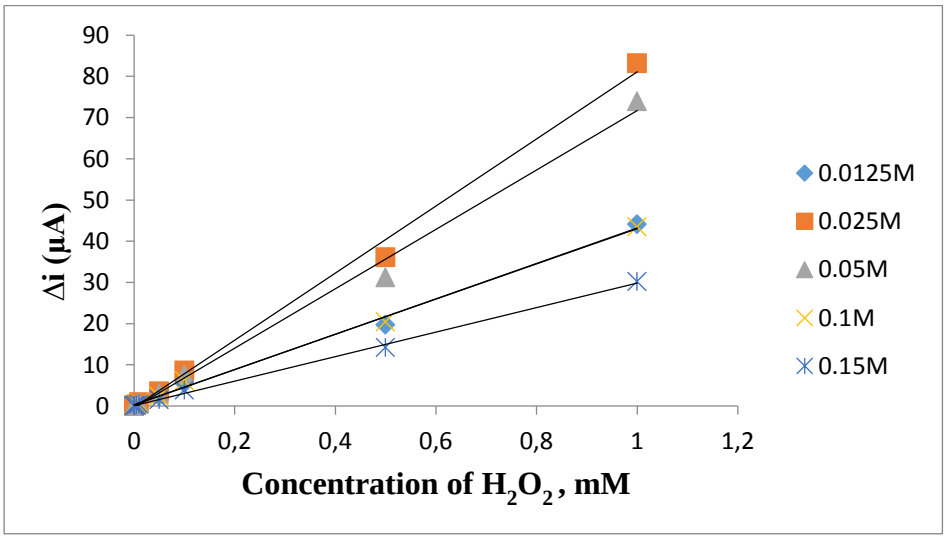

Fig 3. Effect of concentration PSS on Pt / PPy-PSS electrode (0.15 M pyrrole concentration)

3.4. The Effect of Cycle Number of Ppy–PSS Film

Studies investigating the effect of the number of cycles on the sensitivity of prepared film electrodes to hydrogen peroxide are reported in the literature [39]. Film thickness was determined using cycle numbers. The effects of film cycle number on the sensitivity of the electrodes against hydrogen peroxide concentration were examined for different cycles (Figure 4). Figure 4 indicates that the electrodes prepared by 2, 4, 6, and 12 cycles have high linearity. When cycle numbers increase, the film thickness grows up. Sensitivity decreases with increasing film thickness because it is proportional to conductivity. The highest response currents were obtained for electrodes prepared by 4 cycles. Moreover, this electrode has much more mechanical strength and reproducibility. The observed higher value of amperometric response current may be attributed to the thin film. Pt surface was too thick for the higher cycles. Therefore, amperometric response currents were poor. For the electrodes prepared by 4 cycles, the surface of the electrode was smoother and more durable than those of the others.

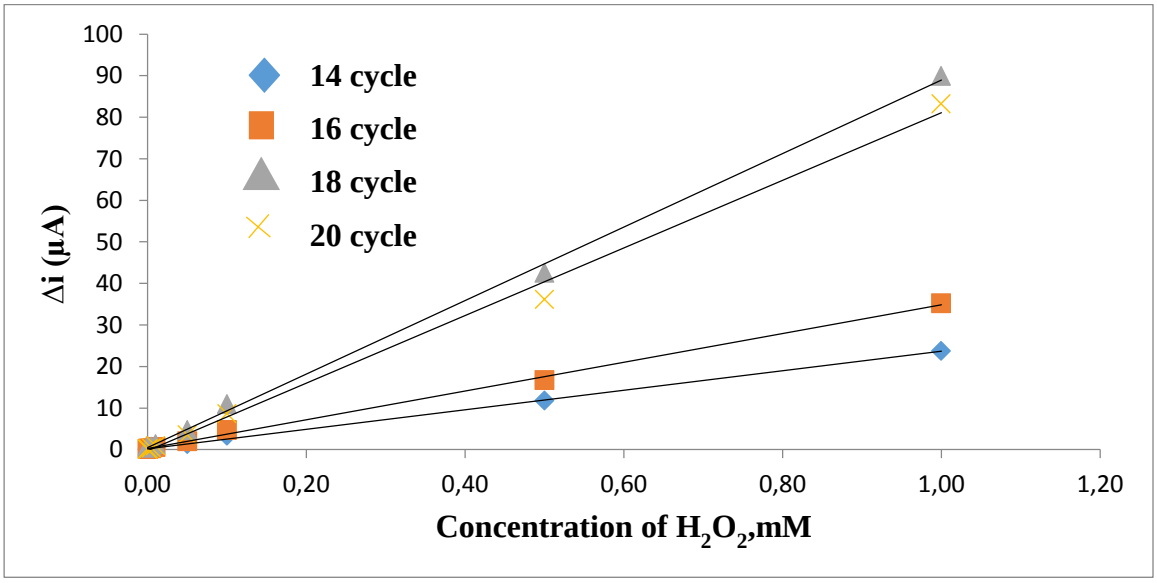

Fig 4. Effect of cycle numbers on Pt / PPy-PSS electrode (0.15 M pyrrole concentration and the PSS concentration at 0.025 M).

3.5. Evaluation of SEM Data

Scanning electron microscopy (SEM) was used to surface morphology PPy-PSS film. The PPy/PSS electrode was dried on a moist atmosphere before the analysis. When the SEM photograph of the prepared film electrode is examined, it can be seen that the structure of the cauliflower of polypyrrole has changed (Figure 5).

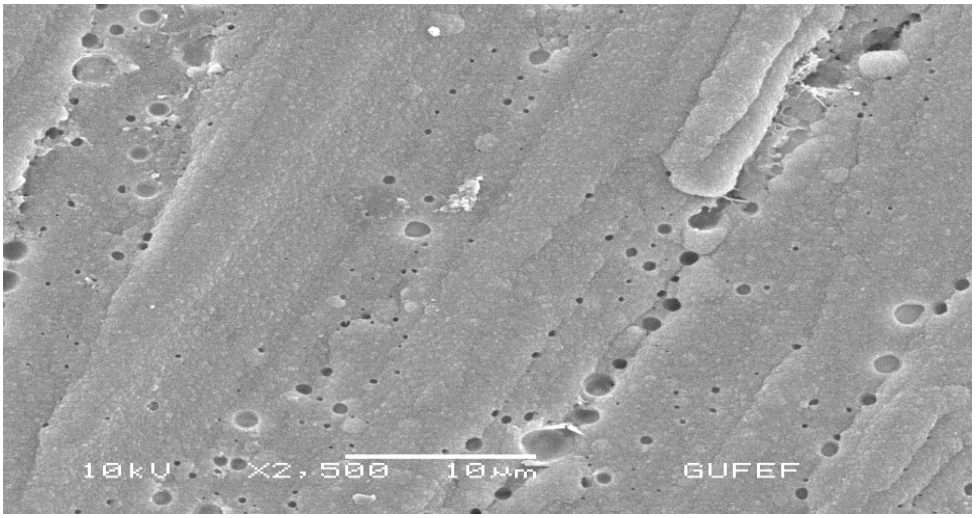

Fig 5. The SEM picture of Pt /PPy-PSS electrode

3.6. Free Enzyme Studies of Pt/Ppy–PSS Film

In this phase, free enzyme in hydrogen peroxide sensitive film electrode has been investigated. The glucose oxidase was selected as the enzyme. The hydrogen peroxide occurs as a result of the reaction of glucose and glucose oxidase enzyme in the presence of oxygen. The prepared film electrode 0.1 M and 9 mL phosphate buffer, pH 7.0, and 1 mL of 1M sodium perchlorate (supporting electrolyte) were dipped into the solution at 0.40 V. Then 50 µL of glucose oxidase enzyme was added to stabilize the enzymatic medium and the equilibrium current was recorded. Glucose solution additions were then made at increasing concentrations. After each addition, the concentrations were stirred for 300 seconds and the current values were read and recorded after 200 seconds at 0.40 V constant potential. The Δi values for each concentration are calculated by taking the difference between the read current values and the equilibrium current. The resulting flow differences (Δi) were plotted against glucose concentration (Figure 6).

4. CONCLUSION

In this study, a new film electrode for the determination of hydrogen peroxide was developed for the first time. All the parameters were examined. For the electrode prepared in this study, the concentration of pyrrole was 0.15M, the concentration of PSS was 0.025M and the number of cycles was found as fourteen cycles. Sodium PSS was used as an anionic dopant. The experimental results showed clearly that the film electrode showed good performance in the determination of hydrogen peroxide. Herein the prepared film electrode can be used for enzymes that produce hydrogen peroxide in the reaction. Free enzyme assay was performed with the prepared film electrode. Glucose oxidase was used for this purpose. It can be said that amperometric response currents increase against increasing glucose concentrations, and therefore the film electrode is susceptible to glucose. This film electrode (PPy–PSS) can supply a biocompatible and electrochemical microenvironment for immobilization of the enzyme, making this material a good candidate for the fabrication of highly sensitive and selective for glucose oxidase and similar enzymes that produce hydrogen peroxide. Consequently, the film electrode prepared for use in biosensor preparation has superior properties.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

[1] Ahmed, W., Sidhu, J.P.S., Toze S., "Speciation and frequency of virulence genes of Enterococcus spp. isolated from rainwater tank samples in southeast Queensland, Australia." Environmental Science and Technology, 46(12):6843-6850, (2012).

[2] Wang, Z., Liu, F., Teng, X., Zhao, C., Lu C. "Detection of hydrogen peroxide in rainwater based on Mg-Al-carbonate layered double hydroxides-catalyzed luminol chemiluminescence", Analyst, 136:4986-4990, (2011).

[3] Campelo, G., Souza, S.D., Bezerra, P.A., Paula, A., Paim, S., Marques, D., Paim, A.P.S., Lavorante, A.F. "A multicommuted flow system for fast screening/sequential spectrophotometric determination of dichromate, salicylic acid, hydrogen peroxide and starch in milk samples",Food Control, 46:127-135, (2014).

[4] Hu, A., Liu, Y., Deng, H., Hong, G., Liu, A. "Fluorescent hydrogen peroxide sensor based on cupric oxide nanoparticles and its application for glucose and L-lactate detection", Biosens. Bioelectron., 61: 374-378,2014.

[5] Lu, S., Song, J., Campbell-Palmer, L. "A modified chemiluminescence method for hydrogen peroxide determination in apple fruit tissues", Sci. Hortic., 120: 336-341, (2009).

[6] Nakamura, H., Mogi, Y., Akimoto, T., Naemura, K., Kato, T., Yano, K., Karube, I. "An enzyme-chromogenic surface plasmon resonance biosensor probe for hydrogen peroxide determination using a modified Trinder's reagent", Biosens. Bioelectron. 24: 455-460, (2008).

[7] Rodrigues, S.S.M., Ribeiro, D.S.M., Molina-garcia, L., Medina, A.R., Prior, J.A.V., Santos, J.L.M. 2014. "Fluorescence enhancement of CdTe MPA-capped quantum dots by glutathione for hydrogen peroxide determination", Talanta, 122:157-165, (2014).

[8] Baghayeria, M., Veisib, H., "Fabrication of a facile electrochemical biosensor for hydrogen peroxide using efficient catalysis of hemoglobin on the porous Pd@Fe3O4-MWCNT nanocomposite", Biosensors and Bioelectronics, 74:190-198, (2015).

[9] Choi, M.M.F., Yiu, T.P. "Immobilization of beef liver catalase on eggshell membrane for fabrication of hydrogen peroxide biosensor", Enzym. Microb. Technol., 34:41-47, (2004).

[10] Pei, J., Li, X., "Xanthine and hypoxanthine sensors based on xanthine oxidase immobilize on a CuPtCl6 chemically modified electrode and liquid chromatography electrochemical detection", Anal. Chim. Acta, 414: 205-213, (2000).

[11] Laudet, E., Botting, N.P., Crayston, J.A., Dale, N., "A three-enzyme microelectrode sensor for detecting purine release from central nervous system", Biosens. Bioelectron., 18: 43-52, (2003).

[12] Çete, S., Bal, O., "Preparation of Pt/polypyrrol-para toluene sulfonate hydrogen peroxide sensitive electrode for the utilizing as a biosensor" Artifical Cells, Nanomedicine, and Biotechnology. 41(6), 414-420, (2013).

[13] Liu, S., Ju, H., "Reagentless glucose biosensor based on direct electron transfer of glucose oxidase immobilized on colloidal gold modified carbon paste electrode" Biosensors and Bioelectronics. ,19: 177-183, (2003).

[14] Wang, J.X., Sun, X.W., Wei, A., "Zinc oxide nanocomb biosensor for glucose detection" Appl. Phys. Lett., 88: 233106, (2006) .

[15] Amin, G., Asif, M.H., Zainelabdin, A., Zaman, S., Nur, O., Willander, M., "Influence of pH, precursor concentration, growth time, and temperature on the morphology of ZnO nanostructures grown by the hydrothermal method" J. Nanomater. 2011:9 (2011).

[16] Kumar, S.A., Chen, S.-M., "Nanostructured Zinc Oxide Particles in Chemically Modified Electrodes for Biosensor Applications", Anal. Lett., 41: 141-158, (2008).

[17] Lee, C., Chiu, Y., Ho, S., Lee, Y., "Investigation of a photoelectrochemical passivated ZnO-based glucose biosensor" Sensors. 11: 4648-2655, (2011).

[18] Yıldırımoğlu, F., Arslan, F., Çete, S., Yaşar, A., "Preparation of a polypyrrole-polyvinylsulphonate composite film biosensor for determination of cholesterol based on entrapment of cholesterol oxidase", Sensors 9: 6435-6445, (2009).

[19] Guiseppi-Elie, A, Wallace, GG, Matsue, T., In: Skotheim T, Elsenbaumer, R, Reynolds, JR., "Handbook of Conductive Polymers" New York: Marcel Dekker, (1995).

[20] Kim, J.H., Cho, J.H., Sig Cha, G., Lee, C.W., Kim, H.B., Paek, S.H., "Conductimetric membrane strip immunosensor with polyaniline-bound gold colloids as signal generator" Biosens. Bioelectron., 14: 907-915, (2000).

[21] Shin, M.C., Yoon, H.C., Kim, H.S., "In situ biochemical reduction of interference in an amperometric biosensor with a novel heterobilayer configuration of polypyrrole/glucose oxidase/horseradish peroxidase", Anal. Chim. Acta., 329:223–230 (1996).

[22] Soylemez, S., Kanik, F.E., Nurioglu, A.G., Akpinar, H., Toppare, L., "A novel conducting copolymer: investigation of its matrix properties for cholesterol biosensor applications" Sens. Actuators B: Chem., 182:322–329, (2013).

[23] Soylemez, S., Kanik, F.E., Tarkuc, S., Udum, Y.A., Toppare, L., "A sepiolite modified conducting polymer based biosensor" Colloids Surf. B Biointerfaces., 111:549–555, (2013).

[24] Arora, K., Prabhakar, N., Chand, S., Malhotra, B.D.,"Immobilization of single stranded DNA probe onto polypyrrole-polyvinyl sulfonate for application to DNA hybridization biosensor", Sensors and Actuators B., 126:655, (2007).

[25] Gade, V.K., Shirale, D.J., Gaikwad, P.D., Savale, P.A., Kakde, K.P., Kharat, H.J., Shirsat, M.D., 2006. "Immobilization of GOD on electrochemically synthesized Ppy – PVS composite fi lm by cross-linking via glutaraldehyde for determination of glucose",Reactive & Functional Polymers, 66: 1420-1426, (2006).

[26] Newman, J.D., White, S.F., Tothill, I.E., Tumer, A.P.F., "Catalytic materials, membranes, and fabrication technologies suitable for the construction of amperometric biosensors", Anal. Chem., 67:4594-4599, (1995).

[27] Çolak, Ö., Yaşar, A., Çete, S., Arslan, F., "Glucose biosensor based on the immobilization of glucose oxidase on electrochemically synthesized polypyrrole-poly(vinyl sulphonate) composite film by cross-linking with glutaraldehyde", Artificial Cells, Blood Substitutes, and Biotechnology., 40: 354-361, 2012.

[28] Gibson, H.W., Bailey, F.C., "Chemical modification of polymers. 13. Sulfonation of polystyrene surfaces", Macromolecules, 1: 34-41, (1980).

[29] Asthana, H., Erickson, B.L., Drzal, L.T., "Sulfonation of polymer surfaces - II. Chemical changes on polypropylene and polystyrene surfaces after gas phase sulfonation" Journal of Adhesion Science and Technology, 11(10): 1269-1288, (1997).

[30] Girard, J., Brunetto, P. S., Braissant, O., Rajacic, Z., Khanna, N., Landmann, R., Daniels, A. U., Fromma, K. M., "Development of a polystyrene sulfonate/silver nanocomposite with self-healing properties for biomaterial applications" Comptes Rendus Chimie., 16(6): 550-556, (2013).

[31] Luo, X.L., Xu, J.J., Zhao, W., Chen, H.Y., "A novel glucose ENFET based on the special reactivity of MnO2 nanoparticles", Biosens. and Bioelect., 19: 1295-1300, (2004).

[32] Cui, S, Guo, X, Chang, F, Cui, Y, Ma, L, Sun, Y, Sun, D., "Apoplastic calmodulin receptor-like binding proteins in suspension-cultured cells of Arabidopsis thaliana" J. Biol Chem, 280:31420–31427, (2005).

[33] Liu, J., Agarwal, M., Varahramyan, K., "Glucose sensor based on organic thin film transistor using glucose oxidase and conducting polymer", Sens. Actuators B: Chem., 135:195-199, (2008).

[34] Lu, X., Zhou, J., Lu, W., Liu, Q., Li, J., "Carbon nanofiber-based composites for the construction of mediator-free biosensors", Biosensors and Bioelectronics, 23: 1236–1243, (2008).

[35] Mao, X., Rutledge, G. C., Hatton, T. A.,"Nanocarbon-based electrochemical systems for sensing, electrocatalysis, and energy storage", Nano Today., 9: 405-432, (2014).

[36] Walcarius, A., Minteer, S.D., Wang, J. , Lin, Y., Merkoçi, A., "Nanomaterials for bio-functionalized electrodes: Recent trends", Journal of Materials Chemistry B., 1 (38):4878-4908, (2013).

[37] Sun, X., Li, X., Wang, Y., Li, X., Zhao, C., Jiao, K. "Electrochemistry of myoglobin in Nafion and multi-walled carbon nanotubes modified carbon ionic liquid electrode", Bioelectrochemistry, 75: 170–175, (2009).

[38] Zhang, Y., Wen, G., Zhou, Y., Shuang, S., Dong, C., Choi, M.M., "Development and analytical application of an uric acid biosensor using an uricase-immobilized eggshell membrane", Biosens. Bioelectron., 22: 1791–1797, (2007).

[39] Özer, B.O., Çete, S. "Development of a novel biosensor based on a polypyrrole–dodecylbenzene sulphonate (PPy–DBS) film for the determination of amperometric cholesterol", Artificial Cells, Nanomedicine, and Biotechnology, 45(4), 824-832, (2017).