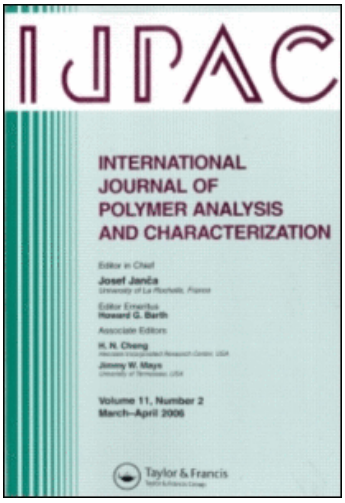

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Glucose Oxidase Immobilized on Galvanostatically Synthesized Poly(*N*-Methylpyrrole)/Polyvinyl Sulfonate Film for Determination of Glucose

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Glucose Oxidase Immobilized on Galvanostatically Synthesized Poly(*N*-Methylpyrrole)/Polyvinyl Sulfonate Film for Determination of Glucose

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Abstract: Glucose oxidase immobilized on electrochemically polymerized poly(*N*-methylpyrrole) (P(NMP)) film with polyvinyl sulfonate (PVS) dopant has been investigated. It was found that the galvanostatically synthesized P(NMP) matrix is suitable for the development of poly(*N*-methylpyrrole)/polyvinyl sulfonate/glucose oxidase (P(NMP)/PVS/GODx) electrode. Cross-linking via glutaraldehyde is adopted for the immobilization of GODx. Incorporation of the glucose in the presence of phosphate and acetate buffer was studied. The effect of phosphate buffer and acetate buffer for the development of biosensors has also been investigated. The kinetics parameters determined for the P(NMP)/PVS/GODx electrode developed in phosphate buffer and acetate buffer at pH 7.4 were 12.5 mM and 16.6 mM for k_m and 20 μA and 24 μA for I_{max} respectively. The sensitivity of P(NMP)/PVS/GODx electrode in phosphate and acetate buffer has been studied. It was found that the sensitivity for the phosphate buffer is

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higher than that for the acetate buffer. The stability of synthesized P(NMP)/PVS/GODx electrode in phosphate buffer was found to be more than that in acetate buffer. The characterization of electrochemically polymerized P(NMP) film was done by FTIR and scanning electron micrography (SEM).

Keywords: Biosensor; Cross-linking; Glucose oxidase; Immobilization; Poly(*N*-methylpyrrole)

INTRODUCTION

Enzymes are well known as a biological sensing material in the development of biosensors due to their specificity and selectivity. The first enzyme-based electrode developed by Clark and Lyons[1] has been used in a number of clinical, environmental, agricultural, and biotechnological applications.[2–4] However, in order to improve its response, the conducting polymer matrix is widely used as a medium for immobilization of enzymes.[5–10] In recent years, a lot of attention has been devoted to the synthesis of conducting polymers[11–18] for the immobilization of a biocomponent. It has been reported that a conducting polymer matrix provides better stability and longer lifetime for the enzymes. Many researchers are extensively working to develop biosensors with better response so that they can be used for the detection of various analytes such as cholesterol, glucose, and urea and will be useful for saving many human lives. Diabetes is one of the leading causes of death by disease. Without care, high levels of blood sugar associated with diabetes can slowly damage both small and large vessels in the body, resulting in a variety of complications. With an appropriate care, these complications can be prevented. The requirement of frequent and continuous monitoring of glucose in diabetics has motivated the scientific community to work extensively in the field of glucose sensors and it is still essential to continue research to fabricate sensors with better response. Since enzymes have poor stability in solution, they need to be immobilized.[19–23] The stability of the enzymes determines the sensitivity and reliability of the biosensor signals. Good operational stability in the polymer matrix can be achieved by synthesizing the polymer with polyvinyl sulfonate (PVS).[24–26] Charge neutrality is an important factor for the immobilization of biocomponents. The polymer composite with PVS can maintain charge neutrality during reduction.[27] It is reported that the polymer film synthesized with polyelectrolyte gives good operational stability in the polymer matrix with increased growth rate and higher compactness. It is also useful for improving the conductivity.[28] However, the immobilization of glucose oxidase (GODx) by cross-linking via glutaraldehyde on

the galvanostatically deposited poly(*N*-methylpyrolle)-polyvinyl sulfonate (P(NMP)/PVS) matrix has not been studied so far.

In the present work, we have immobilized the GODx on the galvanostatically synthesized P(NMP) film in the presence of polyelectrolyte PVS. The major cause of poor stability is the desorption (leaching out) of enzyme from immobilization materials. Therefore, the cross-linking method via glutaraldehyde has been chosen for the immobilization of GODx. The P(NMP)/PVS matrix provides greater stability of the immobilized enzymes. The stability of the optimized P(NMP)/PVS/GODx electrode was also investigated. Since human blood pH is normally 7.4, the GODx was immobilized on the synthesized P(NMP)/PVS film at pH 7.4. The P(NMP)/PVS/GODx electrode was tested and compared for phosphate buffer (pH 7.4) and acetate buffer (pH 7.4).

EXPERIMENTAL SECTION

Preparation of Poly(*N*-Methylpyrrole)/Polyvinyl Sulfonate (P(NMP)/PVS) Film

P(NMP)/PVS film was synthesized from an aqueous solution of distilled 0.05 M *N*-methylpyrrole (NMP) (Spectrochem) and 0.025 M sodium salt of polyvinyl sulfonate (25% by weight) (Aldrich) using the electrochemical deposition method.[29] The synthesis was carried out by the galvanostatic technique at 27°C in a one-compartment, three-electrode glass cell. The indium tin oxide (ITO) coated glass plate was used as a working electrode, platinum foil as a counter electrode, and Ag/AgCl as a reference electrode. The electrolyte solution was prepared in deionized water. The applied current density of 1 mA/cm^2 and the pH 1.5 were kept constant during synthesis of composite films. After synthesis, the polymer-coated electrodes were rinsed by deionized water, dried in cold air, and then used for subsequent characterization.

Immobilization of GODx on Poly(*N*-Methylpyrrole)/Polyvinyl Sulfonate (P(NMP)/PVS) Film

The stock solution of GODx (1 mg/ml) (SISCO) prepared in 0.1 M phosphate buffer and/or 0.1 M acetate buffer (pH 7.4) was adsorbed onto the surface of P(NMP)/PVS films. The enzyme GODx was immobilized by cross-linking via (0.1%) glutaraldehyde (Loba Chemie) on P(NMP)/PVS PVS films, left for 30 min, and washed two or three times with phosphate buffer and/or acetate buffer, thus restricting the leaching of the enzyme from the film.

The enzymatic incorporation was done in glutaraldehyde media. This kind of immobilization results in greater physical and chemical stability of the catalytic material due to the cross-linking formed with the glutaraldehyde and enzyme. In this case, the active sites of the enzyme could be more accessible for the enzymatic reaction. The lifetime of the biosensor was studied when it was kept at 4°C in phosphate and acetate buffers. Adequate concentrations of GODx and glutaraldehyde in the cross-linking mixture were chosen to ensure higher enzyme loading and provide excellent amperometric response with an efficient retention of the enzyme.

Determination of Glucose

The stock solution of D-glucose for different concentrations was prepared in phosphate buffer (0.1 M, pH 7.4) and acetate buffer (0.1 M, pH 7.4) and left for 24 hours before testing. Steady-state current (usually reached after 20 seconds) was used to measure the glucose concentration. The electrode was then removed from the solution and washed with fresh respective buffers in order to be used for another assay.

The synthesized P(NMP) film was charcterized by Fourier transform infraded (FTIR) spectra (FTIR-8400 Shimadzu) and scanning electron microscopy (JEOL JSM-6360A Analytical SEM). The P(NMP)/PVS/GODx was also characterized by using SEM.

RESULTS AND DISCUSSION

The activity of the immobilized GODx can be easily evaluated by electrochemical analysis because this activity is related with the amperometric current, which is proportional to the concentration of the H_2O_2 produced by the GODx on the anode. It can be stated as

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

Thus, with the sensing current, the activity of immobilized GODx can be determined.

Galvanostatic Studies of P(NMP)/PVS Film

Process parameters for the synthesis of P(NMP) film were optimized.[29] The potential-time curve of the synthesized P(NMP) film with optimized process parameters, 1 mA/cm^2 current density, pH 1.5, 0.05 M *N*-methylpyrrole, and 0.025 M PVS at 27°C, is shown in Figure 1. The behavior of

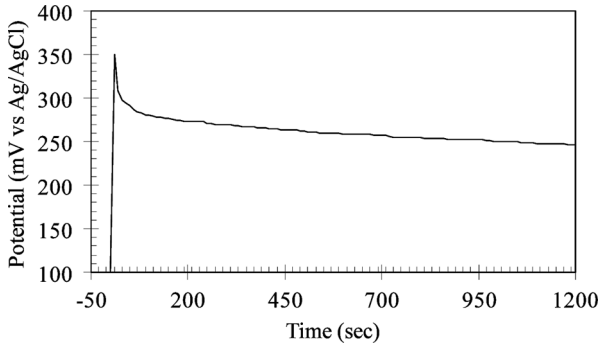

Figure 1. Potential-time curve of synthesized P(NMP)/PVS film with optimized process parameters.

the galvanostatic synthesis during the first few seconds probably indicates the difficult formation of dimers and oligomers. After this, the potential becomes almost constant, suggesting that building up of the film proceeds according to the same reaction along the full thickness of the polymer. Since the anion of PVS has a large size, it does not leave the polymer matrix easily. This results in the synthesized P(NMP) film with PVS having good operational stability. Similarly, the sulfonate ions provide a charged surface for electrostatic interaction between the enzyme and the surface, which is useful for the immobilization of a biocomponent.

FTIR Study of P(NMP)/PVS Film

The FTIR spectrum of the synthesized P(NMP)/PVS film with optimized process parameters is shown in Figure 2. The peak observed at 2923 cm^{-1} corresponds to the CH_3 stretching of P(NMP). Peaks 1444.6 and 1265.2 cm^{-1} are due to the ring stretching of P(NMP). Peak 1033.8 is due to the C−H in plane deformation. C−H out of plane deformation is observed at 887.2 cm^{-1}. The peaks observed at 1375 and 1450 cm^{-1} are due to the C−H (methyl). N−H stretching is observed in the range 1590–1655 cm^{-1}. The peak at 1045 cm^{-1} corresponds to the stretching of SO_3^- group. The FTIR spectrum shows good agreement with earlier reported work.[30,31]

SEM Study of P(NMP)/PVS Film

The SEM image of synthesized P(NMP)/PVS without immobilization of GODx (P(NMP)/PVS) is shown in Figure 3. It represents the porous

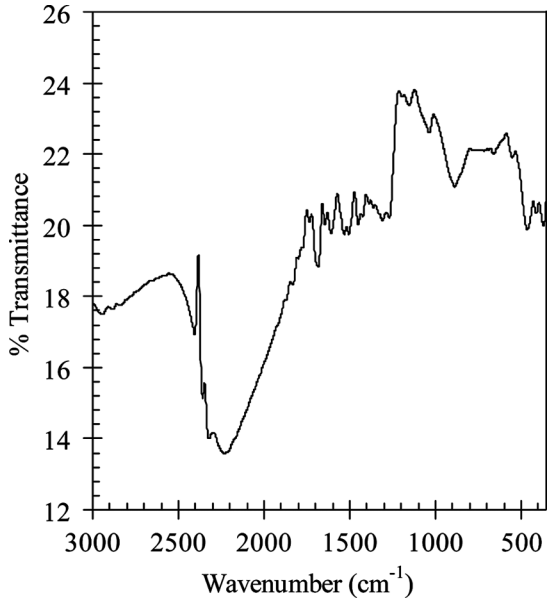

Figure 2. FTIR spectrum of synthesized P(NMP)/PVS film with optimized process parameters.

matrix, suitable for the incorporation of the enzyme. The porous matrix certainly enhances the sensitivity of the glucose biosensor because it can

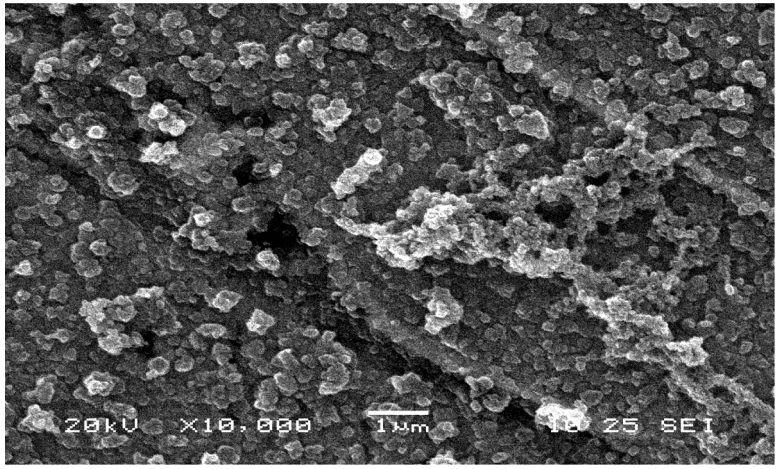

Figure 3. SEM image of synthesized P(NMP)/PVS film with optimized process parameters.

entrap the biocomponent/enzyme easily and can hold it for longer duration.

Current Response of P(NMP)/PVS/GODx Electrode

Current response of P(NMP)/PVS/GODx electrode for different concentrations of glucose (1 mM–50 mM) in phosphate buffer (pH 7.4) and acetate buffer (pH 7.4) with time is shown in Figures 4 and 5 respectively. It was observed that for phosphate buffer and acetate buffer, the response current increases with increase in the concentration of glucose and finally reaches a steady-state value. A steady-state current was used for the calibration of the glucose concentration. The relationship between the response current and glucose concentration for the potential of 0.7 V in phosphate buffer and acetate buffer is shown in Figure 6. It was observed that the current increases with increasing glucose concentration in the range of 1 to 50 mM. It exhibits good linearity for sensing the glucose in the range of 1 to 10 mM.

Determination of Michaelis-Menten Constant

The Michaelis-Menten constant (K_m) was calculated for the immobilized enzyme. The plot of the reciprocal current versus reciprocal glucose concentration at the potential of 0.7 V in phosphate buffer and acetate buffer is shown in Figure 7. In phosphate buffer (pH 7.4), the maximum current

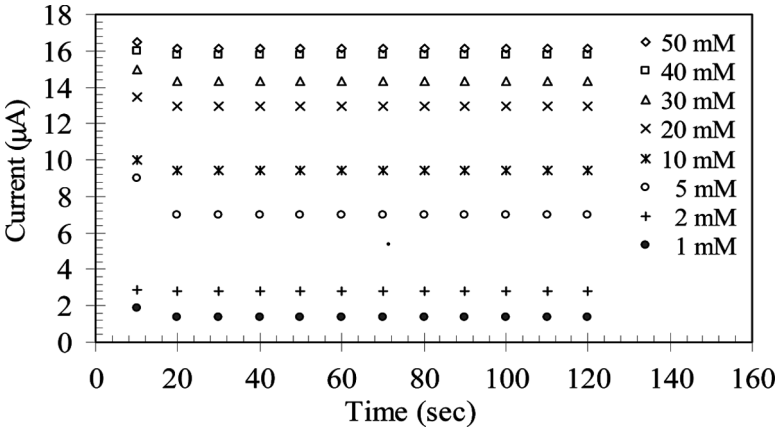

Figure 4. Current-time curves for the P(NMP)/PVS/GODx electrode in phosphate buffer (7.4 pH) for different glucose concentrations.

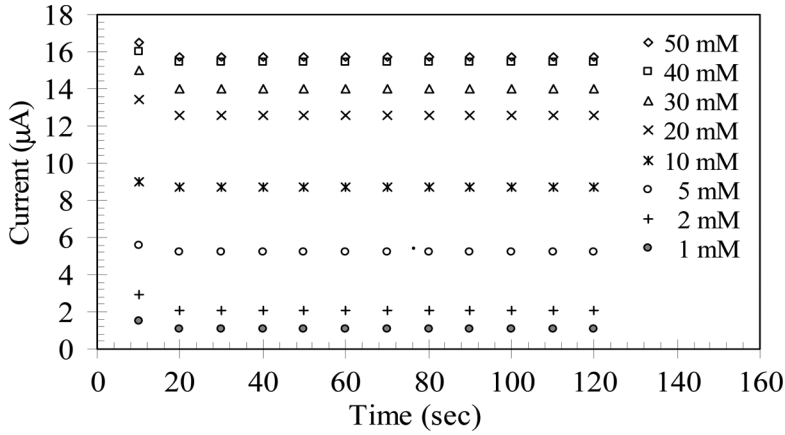

Figure 5. Current-time curves for the P(NMP)/PVS/GODx electrode in acetate buffer (7.4 pH) for different glucose concentrations.

(I_{max}) is 20 μA with K_m 12.5 mM and for the acetate buffer (pH 7.4), the maximum current (I_{max}) is 24 μA with K_m16.6 mM. Moreover, it has been found that the sensitivity in phosphate buffer is higher than that in acetate buffer (Table I). The value of K_m depends on the immobilization of the enzyme. Lower value of K_m gives higher affinity between the substrate and enzyme, which ultimately gives faster response.[32] Hence, the phosphate buffer should be preferred for the immobilization of GODx.

Effect of the Potential

The effect of the potential for the glucose sensor is shown in Figure 8. It was observed that the response current for both buffers increases rapidly with increasing potential from 0.5 to 0.7 V. However, above 0.7 V, it becomes almost constant. Since higher potential causes a decrease in the activity of GODx electrode, the potential of 0.7 V was preferred for the fabrication of P(NMP)/PVS/GODx electrode as a glucose sensor.

Stability of the P(NMP)/PVS/GODx Electrode

An attempt has been made to test the stability of the synthesized P(NMP)/PVS/GODx electrode for both buffers (Figure 9). It was found that, in the beginning, the current response decreases rapidly and becomes more stable later. It was observed that the current response of synthesized P(NMP)/PVS/GODx electrode in acetate buffer decreases

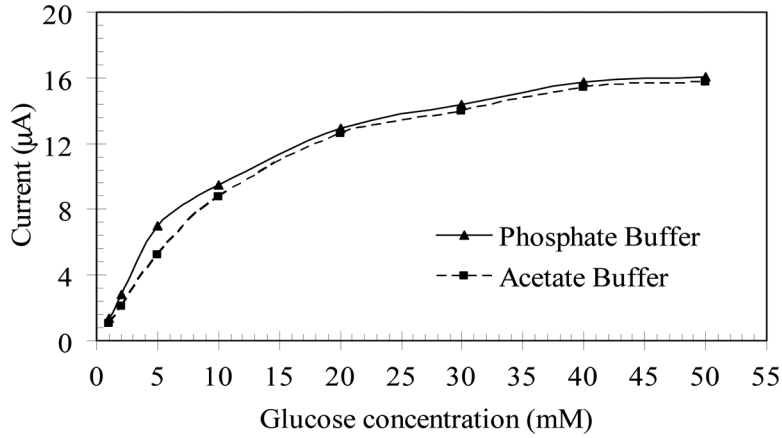

Figure 6. Relationship between response current and glucose concentration for the P(NMP)/PVS/GODx electrode in phosphate and acetate buffer (7.4 pH) at potential 0.7 V.

much more rapidly than in phosphate buffer. The long-term stability test was carried out for 21 days for both buffers. It was found that the reported P(NMP)/PVS/GODx electrode for the phosphate buffer has good stability for 18–20 days, while for acetate buffer, the observed stability is about 16–17 days.

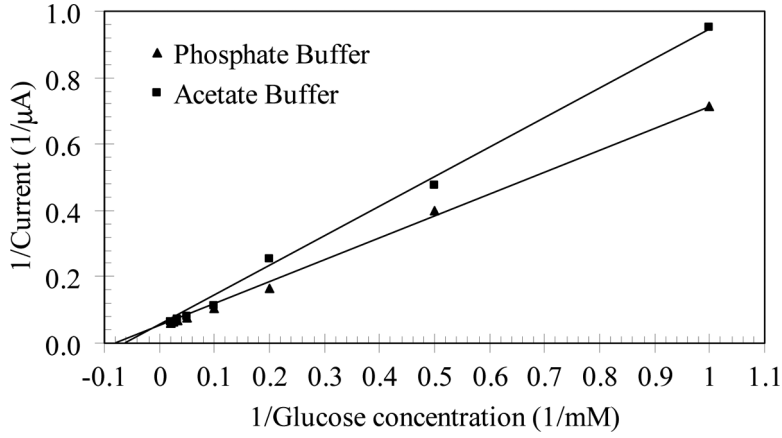

Figure 7. Determination of Michaelis-Menten constant (K_m) for P(NMP)/PVS/GODx electrode in phosphate and acetate buffer (7.4 pH) at potential 0.7 V.

Table I. Comparison of the analytical performance of P(NMP)/PVS/GODx electrode for phosphate and acetate buffer at pH 7.4

		Buffers	
Sr. no.	Parameters	Phosphate	Acetate
1	I_{max} (μA)	20	24
2	K_m (mM)	12.5	16.6
3	Linearity (mM)	1–10	1–10
4	Sensitivity (μA/mM)	1.4	1.05
5	Lifetime (days)	18–20	16–17

SEM Study of P(NMP)/PVS/GODx Electrode

The SEM image of synthesized P(NMP)/PVS film with immobilized GODx in phosphate buffer (pH 7.4) is shown in Figure 10. The occurrence of branche-like structures can be seen in Figure 10, thus indicating the presence of GODx in P(NMP)/PVS matrix. The SEM image of P(NMP)/PVS with immobilized GODx is in good agreement with earlier reported work.[33]

CONCLUSIONS

The immobilization of GODx on the galvanostatically synthesized P(NMP) film with PVS by cross-linking via glutaraldehyde has been

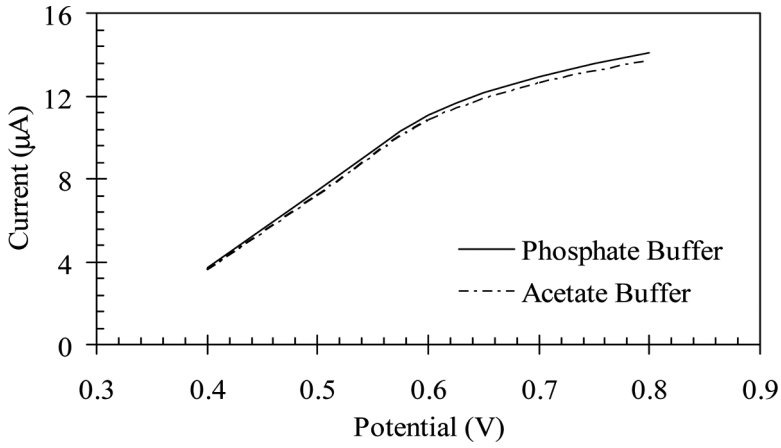

Figure 8. Current-potential curve for the P(NMP)/PVS/GODx electrode in phosphate and acetate buffer (7.4 pH).

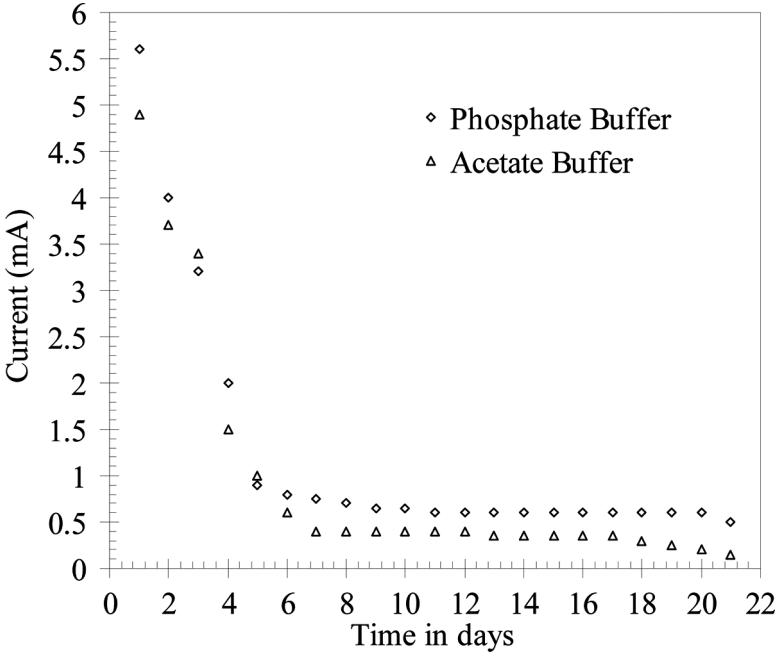

Figure 9. Stability of synthesized P(NMP)/PVS/GODx electrode in phosphate and acetate buffer (7.4 pH) for 5 mM of glucose concentration.

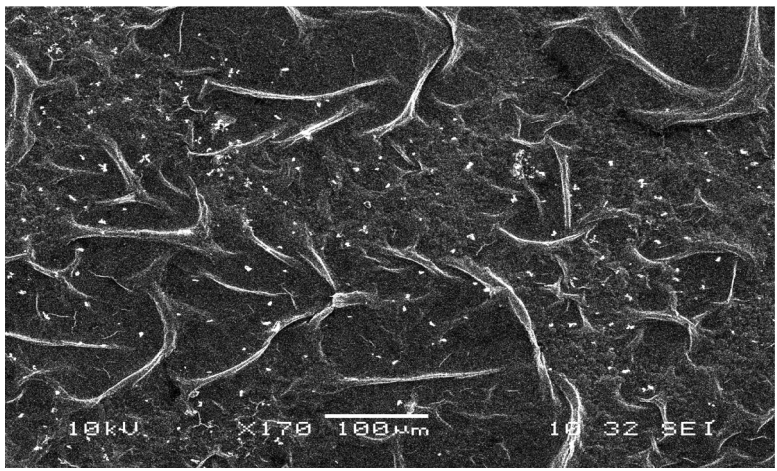

Figure 10. SEM image of synthesized P(NMP)/PVS with immobilized GODx film in phosphate buffer (7.4 pH).

successfully carried out. The sensitivity for P(NMP)/PVS/GODx electrode in phosphate buffer was found good, as were the kinetics parameters. The FTIR spectrum indicates the formation of P(NMP) film with PVS. The SEM image of the P(NMP)/PVS film shows a porous structure that is suitable for penetration of the GODx, and the SEM image of the P(NMP)/PVS/GODx electrode shows a branche-like structure that indicates the presence of GODx in the matrix. The synthesized P(NMP)/PVS/GODx electrode in phosphate buffer (7.4) exhibits good response and good stability for 18–20 days.

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