DEVELOPMENT OF A NEW ETHANOL BIOSENSOR BASED ON POLYFLUORENE- g- POLY(ETHYLENEGLYCOL) AND MULTIWALLED CARBON NANOTUBES

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electrodes were prepared by adjusting the concentration of enzyme (0.5 µL, 2 µL, 4 µL, 6 µL and 8 µL of AOx) while keeping the other parameters constant. Amperometric results showed than 4 µL (6.4 U) of AOx amount provided the most stable and highest response as shown in Figure 2.6.

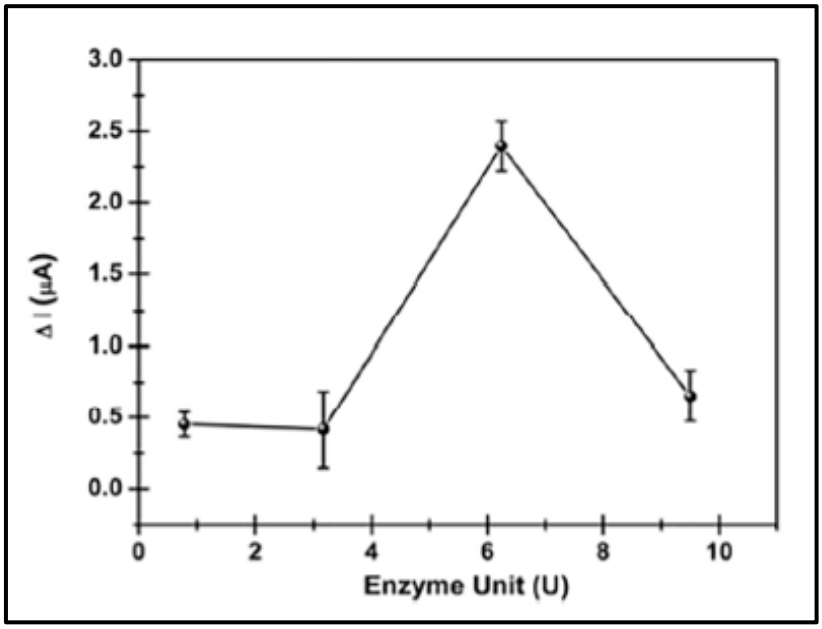

Figure 2.6. Effect of enzyme amount on constructed biosensor response (in 50 mM PBS, pH 7.0, 25 ^{o}C)

2.2.1.4 Optimization of pH

pH of working medium for the fabricated biosensor was optimized to achieve the most sensitive and stable system. For this purpose, 50 mM buffer solutions were prepared in pH range of 3.0 – 10.0 (NaOAc buffer at pH 3.0 and 5.0, PBS buffer at pH 7.0 and Tris buffer at pH 8.0 and 10.0) and the sensor response was recorded at these different pH solutions. As shown in Figure 2.7., maximum and most stable current was obtained at pH 7.0 medium.

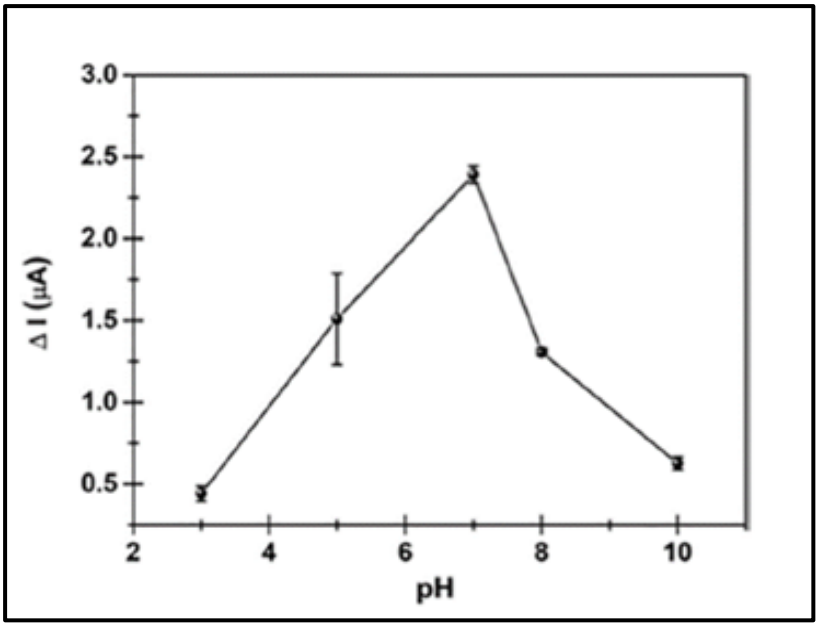

Figure 2.7. Effect of pH on constructed biosensor response (at -0.7 V, 25 ^{o}C)

2.2.1.5 Effect of Combination of Polymer and Carbon Nanotube

To obtain the highest sensor response, effect of polymer and multiwalled carbon nanotube combination was also examined. Under optimized conditions, different combined electrodes (PF-g-PEG/AOx and MWCNT/PF-g-PEG/AOx) were prepared. Amperometric responses of different modified electrodes were shown in Figure 2.8. MWCNT/PF-g-PEG/AOx) biosensor system has higher amperometric signal and short response time towards ethanol. It is seen that MWCNT and CP combination increased stability of the sensor and provided faster electron transfer than CP/AOx.

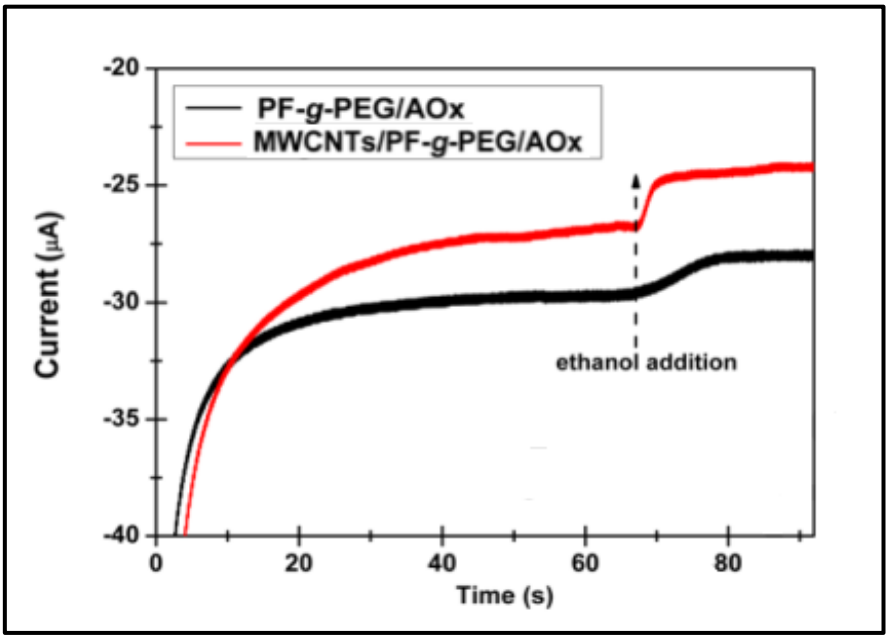

Figure 2.8. Comparison of the amperometric responses of the alcohol sensors for 4.25 mM ethanol

In Figure 2.9 amperometric measurements of these two modified electrodes were given at different ethanol concentrations. PF-g-PEG/AOx modified electrodes response towards ethanol with low sensitivity and longer response time. Modification of multiwalled carbon nanotube increased the stability of proposed sensor and decreased the response time in comparison with PF-g-PEG/AOx sensor. Besides this modification increased the sensitivity of the sensor. As a result, MWCNT/CP/AOx fabricated system gave rise to more precise and increased amperometric signal in comparison to CP/AOx system

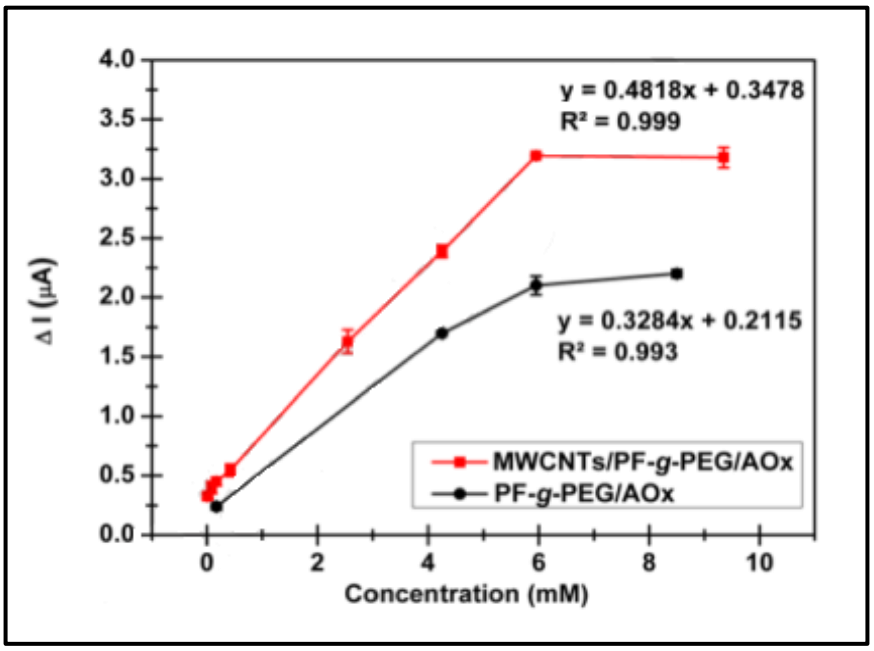

Figure 2.9. Calibration curve of the biosensors (MWCNTs/PF-g-PEG/AOx and PF-g-PEG/AOx to increasing ethanol concentrations in 50 mM pH 7.0 PBS

2.2.2 Investigation of Interference

One of the important desired features of the biosensor was its specificity towards ethanol. To investigate the effect of different interferants on the proposed biosensor system, 0.1 mM of common substances (ethanol, urea, glucose, ascorbic acid, and citric acid) were injected 50 mM buffer solution at ph 7.0, and their behavior was investigated. Results showed that the proposed biosensor did not show a notable response in the presence of interferants. (Figure 2.10)

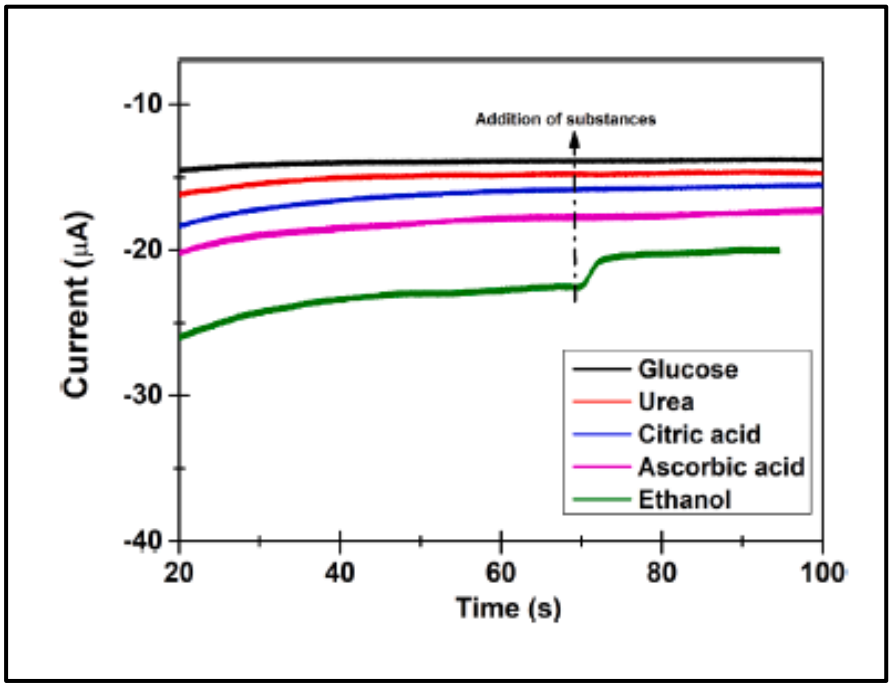

Figure 2.10. Interference effect of interferants on biosensor response (in 50 mM PBS pH 7.0, -0.7 V)

2.2.3 Investigation of Substrate Specificity

To investigate the substrate selectivity of the fabricated biosensor, various alcohols (methanol, ethanol, and 2-propanol) were investigated. 4.25 mM of alcohol solutions were injected to the buffer solution. Response of the methanol was accepted as 100 % (as reference). The responses of the biosensor to other alcohol solutions showed good correlation. (Figure 2.11) Results revealed that fabricated biosensor can analyze ethanol content correctly.

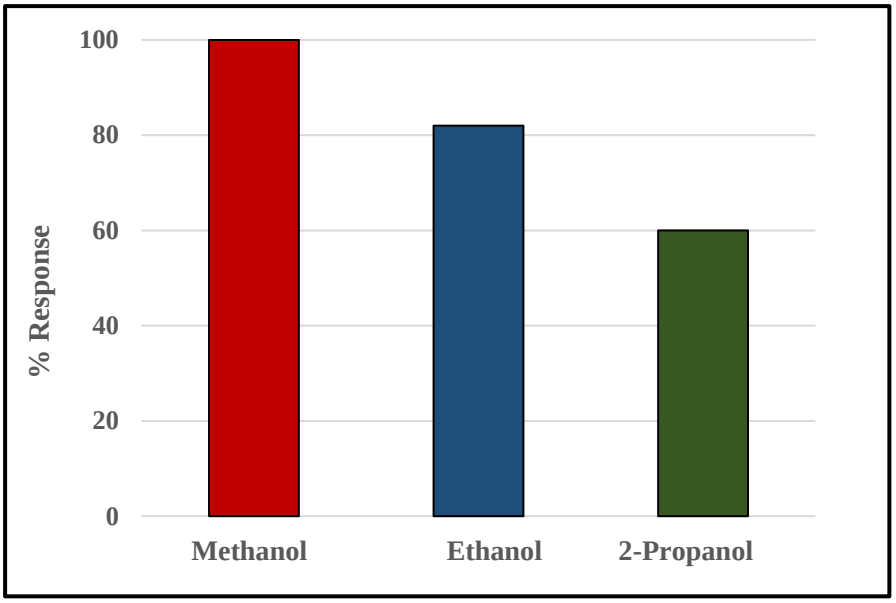

Figure 2.11 Comparison of derivatives of alcohol using proposed biosensor (50 mM PBS, pH 7.0, 25 ^{o}C)

2.2.4 Characterization

2.2.4.1 Analytical Characterization of Proposed Biosensor

Linear relationship between the change in current and concentration of ethanol was achieved in the range of 0.0085 – 5.95 mM, and that range was used as the dynamic linear range in further analytical characterization. (Figure 2.12)

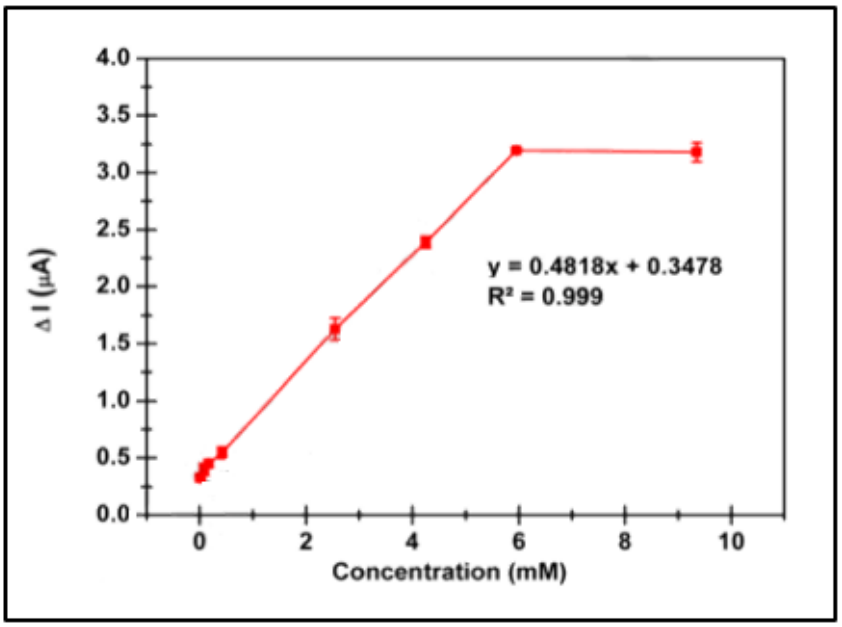

Figure 2.12. Calibration curve of proposed biosensor (50 mM PBS, ph 7.0)

Represented equation for calibration curve is $y = 0.4818x + 0.3478$ with $R^2 = 0.999$. When concentration of ethanol exceeds 5.95 mM, saturation was observed, and a deviation from linearity was achieved. The lowest concentration for detection which is the limit of detection was found as 0.11 mM. Also, sensitivity value was calculated as 7.99 $\mu A\ mM^{-1}\ cm^{-2}$. Obtained results are compared with other ethanol sensing studies, and it is clearly seen that fabricated MWCNT/PF-g-PEG/AOx system has wider dynamic range and low detection limit. (Table 2.1)

Biosensors	Linear Range (mM)	LOD (mM)	Reference
Hydrogel/Platinum electrode/AOx	0.02-3.75	NR	**[35]**
PNR/carbon film electrode	0 – 0.8	0.044	**[36]**
DPP/Mercury Electrode	0.2 – 2.0	4.3	**[37]**
SPCE/MWCNT/AuNP/PNR/AOx/GA	0.178 – 1.0	0.05	**[38]**
PMCCH/AOx	NR	8.3	**[39]**
MWCNTs/PF-g-PEG/AOx	**0.0085 – 5.95**	**0.11**	**This Work**

Table 2.1. Comparison of analytical parameters of ethanol biosensor in literature

To examine the precision of the proposed biosensor, 10 sequential measurements were recorded. Standard deviation (SD) and relative standard deviation (RSD) for these 10 consecutive measurements were found as ± 0.09 and 3.91% respectively.

Km^{app} for the enzyme in the proposed biosensor system was calculated using Lineweaver-Burk plot as 0.015 mM. These results confirm the high affinity of immobilized alcohol oxidase towards to ethanol.

2.2.4.2 Surface Characterization of Biosensor

Effective surface areas of modified electrodes (MWCNT/PF-g-PEG/AOx and MWCNT/PF-g-PEG and bare electrode) were calculated using Randles-Sevcik equation. Experiments were performed in 50 mM $Fe(CN)_6^{-3/-4}$ solution that is containing 0.1 M KCl. Cyclic voltammograms were achieved at 100 mV/s scan rate (Figure 2.13) and peak currents were found as 0.43 cm^2 and 0.193 cm^2 for MWCNT/PF-g-PEG and MWCNT/PF-g-PEG/AOx, respectively. Immobilized enzyme decreased the current and electroactive surface area due to its adsorption onto the probe.

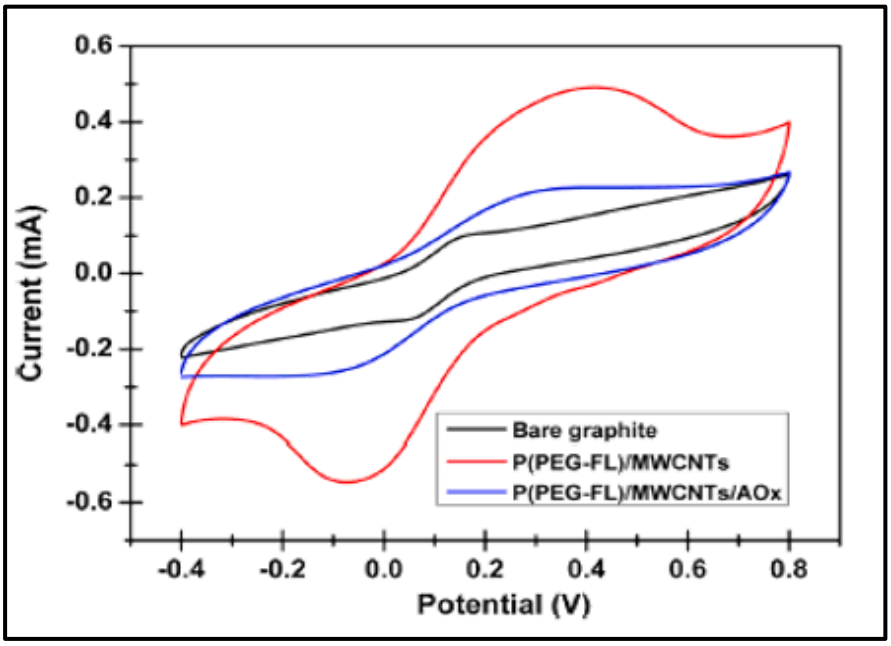

Figure 2.13 Cyclic voltammogram of bare graphite electrode, MWCNTs/ PF-g-PEG and MWCNTs/PF-g-PEG/AOx in 5mM $Fe(CN)_6^{3-/4-}$

To analyze the changes in morphological surface, scanning electron microscopy (SEM) technique was used. (Figure 2.14) MWCNT/PF-g-PEG image is more homogeneous to compare with PF-g-PEG film. Immobilization of the enzyme on modified MWCNT/PF-g-PEG surface made surface more uniform indicating the coverage of the active surface by the enzyme.

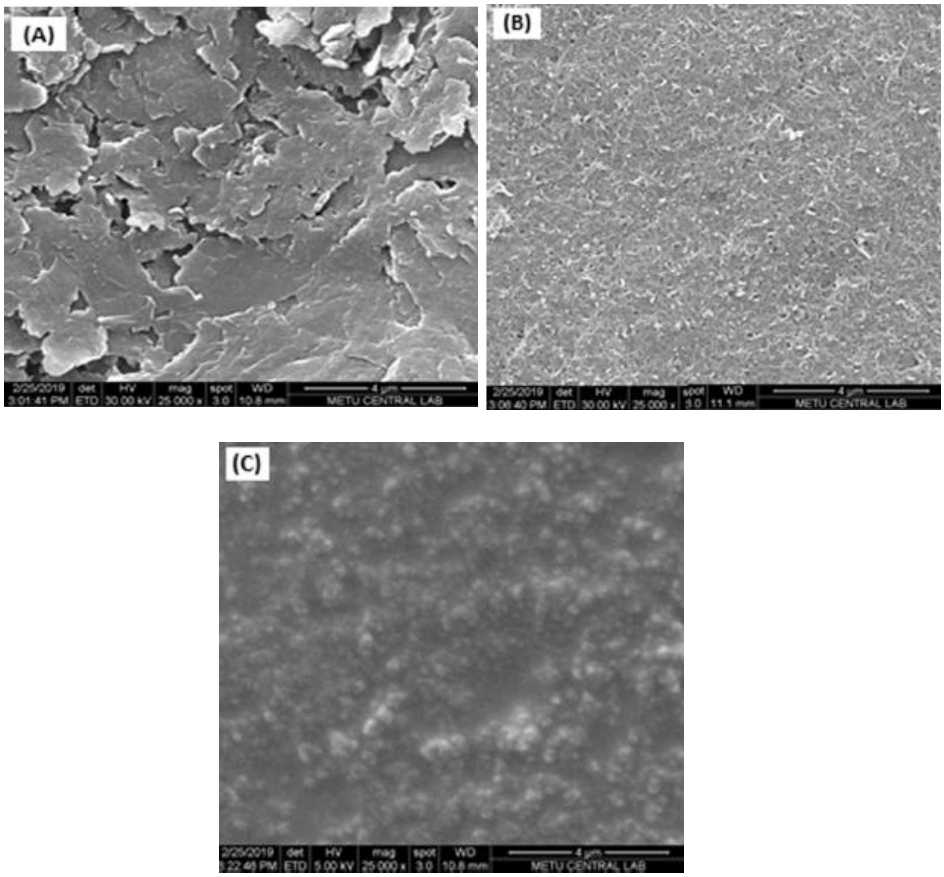

Figure 2.14. SEM images of A) PF-g-PEG B) MWCNT/PF-g-PEG C) MWCNT/PF-g-PEG/AOx

2.2.5 Applicability of Biosensor

To test the accuracy of the proposed biosensor, commercial samples were analyzed using proposed biosensor by injecting appropriate volume for each sample. Ethanol concentration in each sample was determined using previously constructed the calibration. Experimentally obtained ethanol concentrations were compared with the concentration given in the product labels of beverages. (Table 2.2) Results proved that the fabricated biosensor can be used to detect ethanol content accurately in real samples.

Sample	Product Label (%)	MWCNTs/PF-g-PEG/AOx (%)	Percent Error (%)
Rum	37.5	35.04	6.56
Raki	45.0	42.07	6.51
Vodka	37.5	39.25	4.66

Table 2.2. Ethanol detection in beverages using proposed biosensor

CHAPTER 3

CONCLUSION

In this thesis study a novel amperometric biosensor based on conjugated polymer and multiwalled carbon nanotube was designed for detection of alcohol. Multiwalled carbon nanotube solution was cast on cleaned bare graphite electrode surface. Then, poly(ethylene glycol) with fluorene functionality (PEG-FL) monomer was synthesized in one step procedure and electrochemically polymerized with cyclic voltammetry technique onto multiwalled carbon nanotube modified graphite electrode surface. Combination of carbon nanotube and monomer on graphite surface enabled the suitable immobilization of alcohol oxidase enzyme (AOx). Enzyme was immobilized onto the modified surface with physical adsorption technique. Synthesized conjugated polymer; PF-g-PEG, increased the interaction with enzyme due to hydrophilic structure of monomer and increased the effective surface area. Apart from the conjugated polymer, a multiwalled CNT was inserted to sensing system since MWCNT provided fast electron transfer and chemical stability in the constructed system.

Amperometric biosensor response was investigated at -0.7 V in a buffer solution (pH 7). As a result of enzymatic reaction between alcohol oxidase and the substrate, changes in current due to change oxygen level were monitored in terms of the signal of biosensor. To obtain the best sensor response, parameters that affect the biosensor response were optimized. After finding the optimum conditions, a calibration curve was constructed for ethanol detection. Then, kinetic, and analytical characterization of the proposed biosensor were completed. The constructed biosensor was found to have linear dynamic range between 0.0085 mM and 5.95 mM with a detection limit of 0.11 mM and sensitivity of 7.99 $\mu A\ mM^{-1}cm^{-2}$. Compared to similar studies reported in the literature, proposed biosensor has good kinetic parameters, a high

dynamic range, and a low limit of detection. Besides, to show the specificity of the proposed biosensor, different interferants were tested, and fabricated biosensor was found to be very specific for alcohol. Apart from the interference effect to investigate the surface morphology, scanning electron microscopy (SEM) was used. Finally, fabricated biosensor was used in the quantification of alcohol in several beverages and revealed high accuracy.

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