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Electrochemical Polymerization of 2-Thiophen-3-yl-malonic Acid for Biosensor Application

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ABSTRACT: Poly(2-thiophene-3-yl-malonic acid) (P3TMA) was synthesized electrochemically using tetrabutylammonium perchlorate ($TBAClO_4$) as electrolyte into acetonitrile (ACN) for glucose biosensor application. The properties of P3TMA were investigated using FTIR, SEM, and electrochemical measurements. The glucose oxidase enzyme (GOD) was chemically immobilized onto P3TMA modified electrode using glutaraldehyde as crosslinking. It was observed that response current of P3TMA/GOD enzyme electrode increased linearly with loading glucose concentration. Michaelis Menten constant (K_m) of P3TMA/GOD enzyme electrode was calculated as 0.03517 m*M*. FTIR and SEM analyses were used to confirm immobilization of GOD onto surface of P3TMA-modified electrode.
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INTRODUCTION

Conducting polymers are an important and interesting class of organic conductors because of their mechanical and electronic properties such as inexpensive, flexibility and facile processability and so on. These properties have made them extremely useful for practical applications including biological sensor, molecular electronic devices, solar cell, and chemical sensitive devices, etc. Moreover, conducting polymers are suitable materials for electromagnetic interference (EMI) shielding applications. The principal sensing mechanism of conducting polymer is generally based on the modification of doping level due to redox interaction of analyte and resulting in change in conductivity.[1–3]

Electropolymerization and chemical polymerization of aromatic and heteroaromatic compounds such as aniline, pyrrole, thiophene have been extensively studied. Among the conducting polymers, the polythiophene and its derivatives have been synthesized and characterized in the last 15 years.[4,5] Among which, polythiophene is regarded as one of the most technologically promising conducting polymer because of its easy of preparation, low cost, high environment and thermal stability, relatively stable electrical conductivity and its special properties.[6–8] As an illustration, polythiophene and its derivatives do not easily suffer ring opening reactions when compared to polyfurans and polypyrrols.[9] Highly π-conjugated polythiophene has been composed by the electrochemical method. For that reason electrochemical polymerization may facilitate preparation of conductive organic polymers. Moreover, polythiophene derivatives are particularly important since they can solve serious problems of solubility and processability of polythiophene.[10–12] As a result polythiophene family has been used in a lot of applications including light emitting diodes (LED), thin-film transistors (TFT), solar cells, and sensors, etc.[8] Especially, the position-3 in the thiophene group can modify polymer properties such as thermal stability, high electrical conductivity, biosensor and optoelectronic properties.[13,14] In recent years, it has been particularly focused in the development of poly(thiophene)s with electron-withdrawing carboxylic acid groups in the 3-position of the thiophene ring.[15,16] Because they are able to form covalent bonds with both biologically active materials and inorganic nanoparticles.[17] Yoon et al. synthesized polymers including –COOH, -NH_2, -CN groups into 3-position of the terthiophene ring and compared solar cell performance of conducting polymers. A carboxylic acid group was more strongly bound to the TiO_2 layer in comparison with other groups. Although the band gap energies of polymers were similar, the carboxyl-functionalized poly(terthiophene) derivative showed a higher energy conversion efficiency when compared with other derivatives. The poly(terthiophene) including carboxylic acid groups has indicated the best cell efficiency.[18] Bertran et al. prepared poly (2-thiophen-3-yl-malonic acid) and obtained important improvements such as thermal stability and electrical conductivity.[19]

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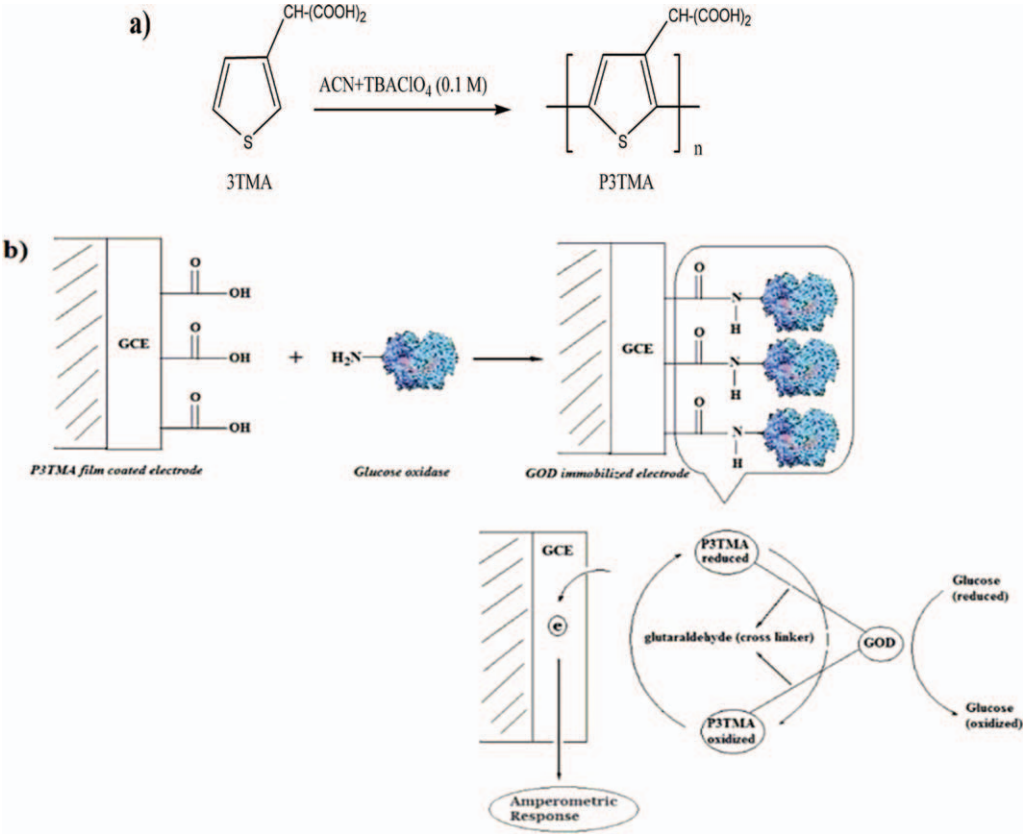

Scheme 1. (a) Electrochemical polymerization of 3TMA monomer (b) Schematic illustration of sensing mechanism for electrocatalytic glucose and immobilization of GOD on the P3TMA-modified glassy carbon surface. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Enzyme electrodes carrying electron transfer mediators have been prepared with conducting polymers. The immobilization of enzymes on conducting polymer films have become area of scientists to fabricate enzyme electrodes for biosensors and biofuel cells.[20] Because of reusability, enhanced stability, continuous operational mode, easy separation from reaction mixture, cheap, immobilized enzymes have more advantageous than free enzymes.[21]

The published methods for enzyme—immobilization contain adsorption, covalent attachment, cross-linking, and entrapment.[22] Enzyme entrapment was firstly made by Foulds et al. into the conducting polymers using electrochemical polymerization.[22] Entrapped procedure contains the electrochemical polymerization of monomers from an enzyme-containing solution.[20] Some widely conductive polymers used for enzyme immobilization are polyaniline, polythiophene and polypyrrole.[22–25] If conducting polymer has functional groups available as covalent bonding sides, enzyme, and mediators can be immobilized firmly on the surface of its film.

This article shows the procedure electrochemical polymerization of 2-thiophene-3-yl-malonic acid (3TMA) and the specificity of ability of P3TMA as the glucose oxidase enzyme bioelectrode, P3TMA/GOD. The characteristics of P3TMA/GOD film have been investigated using cyclic voltammetry (CV), FTIR spectroscopy, and scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

2-thiophene-3-yl-malonic acid (3TMA), glucose oxidase (GOD), acetonitrile (99.8%, anhydrous), and glutaraldehyde (GA) were obtained from Aldrich. The electrochemical grade tetrabutylammonium perchlorate ($TBACIO_4$) and D-(+)-Glucose anhydrous were purchased from Fluka. The buffer solution was prepared using $NaH_2PO_4.2H_2O$ (Merck) and NaOH (Merck).

Characterization Methods

Electrochemical experiments were carried out using Gamry PCl4/300 model potentiostat. Conventional three-electrode cell, modified glassy carbon working electrode, a saturated calomel electrode (SCE) as a reference electrode and a Pt electrode as the auxiliary electrode were utilized. Fourier transform infrared (FTIR) spectra were recorded between 400 and 4000 cm^{-1} with KBr pellets on a Perkin Elmer Spectrum BX FTIR system (Beaconsfield, Buckinghamshire, HP91QA, England). SEM images were taken on a Scanning electron microscope model Tescan Vega II LSU.

Electrochemical Polymerization of the 3-thiophenemalonic Acid (3TMA)

The conducting polymer film was prepared by the electrochemical polymerization of the 3TMA using the glassy carbon electrode, Pt wire and Ag/AgCl silver electrodes as a working,

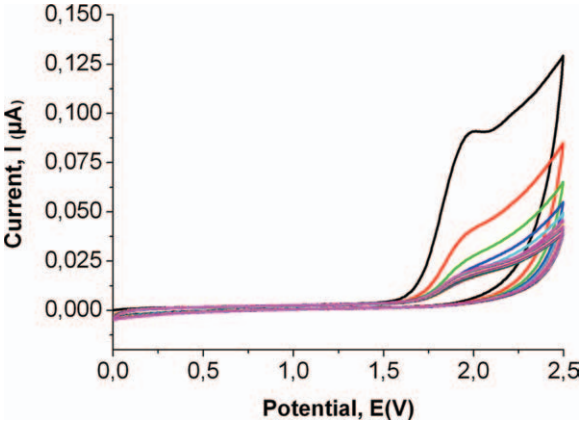

Figure 1. Repeated potential scan of 3TMA between 0 and 2.5 V on the glassy carbon electrode in 0.1*M* $TBAClO_4$/ACN with a scan rate of 50 mV s^{-1}. The voltammograms recorded during the P3TMA/$TBAClO_4$ film growth on glassy carbon electrode. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

counter, and reference electrodes, respectively. Tetrabutylammonium perchlorate ($TBAClO_4$) was used as both dopant anion and supporting electrolyte. 0.1*M* 3TMA + 0.1*M* $TBAClO_4$ solution was prepared into ACN. Then, the electropolymerization

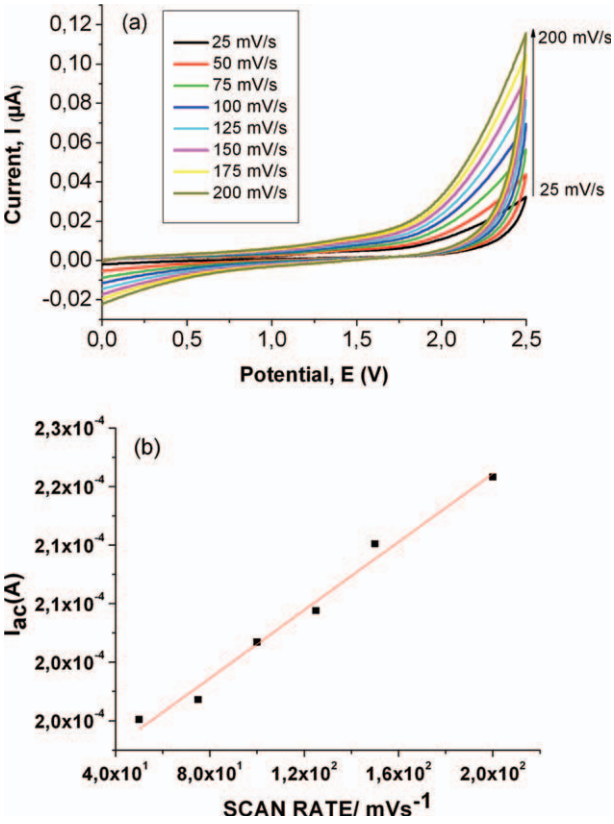

Figure 2. (a) Scan-rate dependence of P3TMA film on a glassy carbon electrode in 0.1*M* $TBAClO_4$/ACN at different scan rates between 25 mV s^{-1} and 200 mV s^{-1}. (b) plot of anodic current vs. scan rate of the P3TMA in 0.1*M* $TBAClO_4$/ACN. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

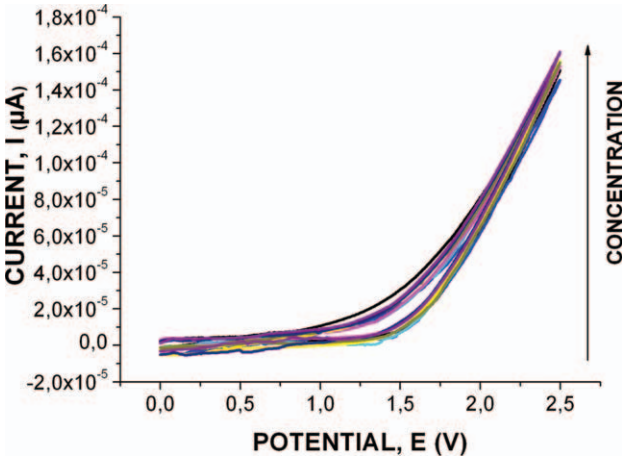

Figure 3. Cyclic voltammograms of P3TMA/glucose bioelectrode with increasing target glucose concentration at 50 mV s^{-1} in pH = 7.4 phosphate buffer solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

was carried out by cyclic voltammetry method by scanning between 0 and 2.5 V potential range at a scan rate 50 mV/s. The working electrode was coated with P3TMA polymer film using 30 cycles according to following electrochemical polymerization reaction (Scheme 1a). The P3TMA film was prepared with the passed charge of 28 mC. For the FTIR and SEM characterizations, the polymer was electrochemically deposited on an indium tin oxide (ITO)-coated glass electrode.

Preparation of Enzyme-Immobilized Electrode

The polymer films of P3TMA were electrochemically formed using the cyclic voltammetry for preparation of enzyme-immobilized electrode. Totally, 5 μL (2.5 mg/500 μL) GOD and 1 μL (5 wt %) glutaraldehyde solutions were successively immobilized on the polymer film, and the electrode was allowed to immobilization for 2 h. After immobilization time, the enzyme electrode was washed two to three times with excess of phosphate buffer (pH = 7.4) to remove nonimmobilizing GOD enzyme (Scheme 1b).

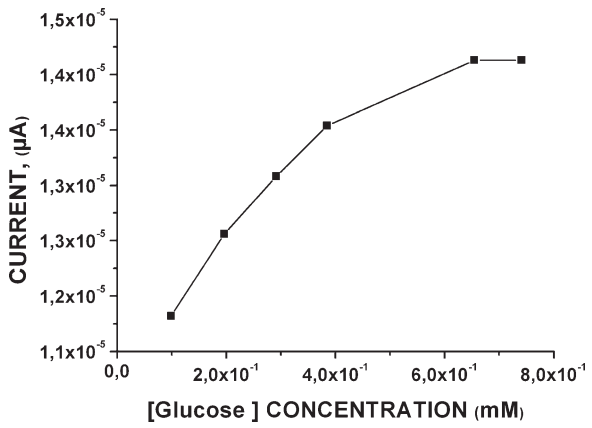

Figure 4. Relationship between the current and glucose concentration in pH = 7.4 phosphate buffer solution.

Table I. Values of the Linear Range, R^2, K_m for P3TMA Electrode

Sample	Linear range (m*M*)	R^2	Equation	K_m (m*M*)	References
3TMA/GOD	0.1–0.8	0.99424	$y = 2608x + 20014$	0. 03517	In this work
PPy/invertase				28.2	[31]
PMTM/PPy/SDS/invertase				30.2	[31]
Th(0.6)/Th-COOH(0.2)/Th-Fc(0.2)				2.98	[32]
PANI/Ch-H_2SO_4/GOD				0.36	[33]
PT2/SiO_2-TTAB				0.025	[34]

Measurement of Response of Enzyme Electrode to Glucose

The enzyme electrode was immersed into a 0.1*M*, pH = 7.4 phosphate buffer solution and used as working electrode. Pt wire and Ag/AgCl electrodes were used as counter and reference electrodes, respectively. Current-voltage changing (CV) was investigated using cyclic voltammetry method on based electrochemical behavior of P3TMA in potential range of 0–2.5 V. Glucose concentration was taken as 10 m*M*. Response current of P3TMA-modified enzyme electrode was measured by adding glucose amount at 1.7 V potential corresponding doping current of P3TMA as schematically showed in Scheme 1b.[26]

RESULTS AND DISCUSSION

Figure 1 indicates cyclic voltammetry curves of 3TMA into ACN/$TBAClO_4$ electrolyte solution during electropolymerization for 30 repeated scans. As seen from Figure 1, the first cycle indicates maximum oxidation peak at 1.7 V showing electrochemical oxidation of 3TMA monomer. Upon the following cycles, the irreversible peak current decreased. This behavior confirms that the insulating polymer film has formed which obstruct the access of the monomer to the electrode surface.[27]

The electrochemical behavior of P3TMA was investigated with changing the scan rate. After P3TMA was coated onto the electrode, the electrode was removed from polymerization solution and washed with ACN and dipped into electrolyte solution without monomer. A linear dependence of peak current with scan rates was given in Figure 2(a,b). The anodic peak current responses were determined using CVs and it was found that anodic current responses increase linearly as the scan rate increases [Figure 2(b)]. It was clear that the redox process was nondiffusional and the elecroactive polymer was well adhered to the working electrode surface.[26]

The P3TMA/GOD enzyme electrode was prepared by the immobilizing of the GOD onto P3TMA-coated electrode surface. Figure 3 exhibits the cyclic voltammograms of P3TMA/GOD bioelectrode with increasing target glucose concentration. The maximum enzyme activity and working range of P3TMA/GOD biosensor electrode were determined using the current values versus substrate concentration. It was found that the maximum peak currents increased with adding glucose amount at 1.7 V (Figure 4). This result shows that binding of P3TMA film with the GOD at the P3TMA/GOD electrode surface has occurred and the enzyme electrode has responsed to adding glucose substrate.

According to the Lineweaver-Burk equation, shown in Equation 1, the Michaelis-Menten value was calculated using the linear relation between the mutual of the response current (I^{-1}) and the mutual of glucose concentration (C^{-1}).

$$\frac{1}{I} = \frac{K_m}{I_m} x \frac{1}{C} + \frac{1}{I_m} \quad (1)$$

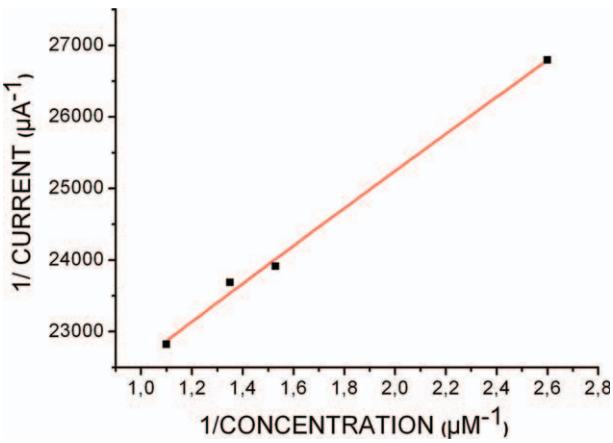

Figure 5. Lineweaver–Burk plot for GOD immobilized in P3TMA film. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

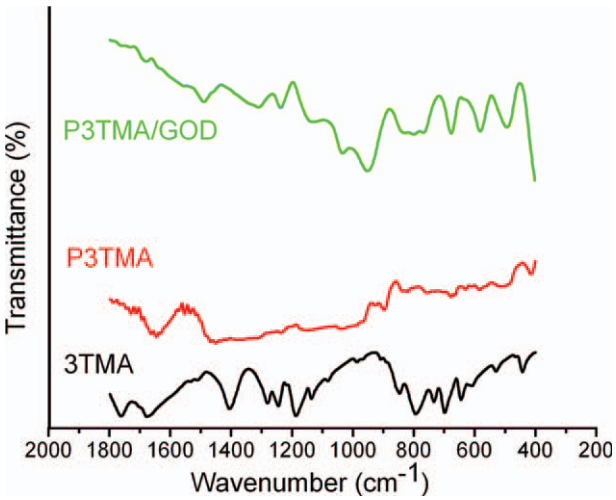

Figure 6. FTIR spectra of monomer (3TMA), polymer film (P3TMA) and P3TMA/GOD film. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

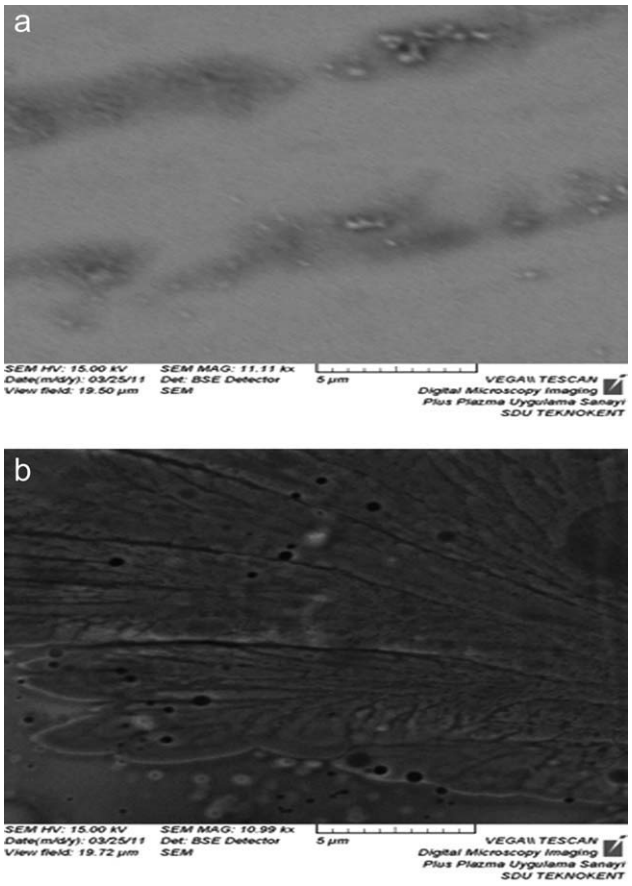

Figure 7. SEM images of (a) P3TMA, (b) P3TMA/GOD onto ITO electrodes.

This is the form of $y = mx + n$, which equation of a linear line with a slope of $m = K_m/I_m$ and intercept of $n = 1/I_m$. K_m and I_m were calculated as 0.03517 m*M*, 1.476 μA, respectively.

Generally, K_m has been used to evaluate enzyme activity. As the enzyme loading increases, a bigger fraction of the GOD locates near to the surface of the electrode, so that the average enzyme-substrate binding possibility increases, with K_m decreasing accordingly.[28–30]

The linear dynamic ranges and equations of the coefficient values of the P3TMA electrode are given in Table I. The response current increases with increasing glucose concentration. For P3TMA/GOD system, a linear calibration graph (Figure 5) was obtained for current density vs. substrate concentration from 0.1 to 0.8 mM and the detection limit was found to be 0.09901 μM. A linear relation was defined by equation: $y = 2608x + 20014$ ($R^2 = 0.99424$). The P3TMA/GOD bioelectrode has a good linear range and high sensitivity for glucose sensor applications.

Figure 6 compares the FTIR spectra of T3MA monomer and PT3MA polymer. The presence of the acid group in both T3MA and PT3MA is clearly detected by the carbonyl absorption region (Figure 6). Vibrational two bands at 1751 cm^{-1} and 1657 cm^{-1} are due to the free C=O stretching and C=O associated with intermolecular hydrogen bonds in the monomer, respectively. But, the intermolecular hydrogen bonds belonging to carboxyl groups are not observed in the polymer. The carbonyl vibrations (1751 cm^{-1}) shift to lower wave number (1705 cm^{-1}) in the polymer because of intramolecular hydrogen bonds between the polymer chains. In spectrum of pure 3TMA, the vibrational band at 795 is assigned to the aromatic C—H out-of-plane deformation. In the polymer, this peak disappeared. Because polymerization forms α-α' position of the thiophene rings.[35]

After immobilization of GOD on the P3TMA surface, the vibrational bands at 1685 cm^{-1} and 1500 cm^{-1} are observed the combination of N—H in-plane bending and C—N starching vibrations of the peptide groups.[36,37] This might confirm that GOD is successfully immobilized on the electrode surface (Figure 6).

SEM images were also taken to be able to confirm immobilization of GOD enzyme onto P3TMA coated electrodes (Figure 7). P3TMA has a smooth surface morphologies [Figure 7(a)]. After immobilization of GOD onto polymer coated electrode, there is a remarkable change of the surface morphology of P3TMA. This result has also confirmed GOD immobilization onto electrode surface.

CONCLUSIONS

P3TMA/GOD biosensor electrode was successfully prepared and its properties was investigated for glucose detection. Michaelis-Menten constant, K_m value was calculated using the Lineweaver-Burk equation and it was found as 0.03517 m*M*. This value indicates that there is no diffusion limitation at the surface of the enzyme electrode.[38,39] Moreover, the K_m value of the P3TMA/GOD electrode is smaller than that of GOD in solution (free enzyme; ~0.500 m*M*), known from and studied in our earlier literatures, because of the stronger affinity between enzyme and electrode.[33,34] All the results show that P3TMA-GOD films could provide useful platform for biosensor applications.

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