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Application of an Efficient Amperometric Glucose Sensing Electrode Based on a Bilayer Polymer Film Platform

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A new approach was developed using a combination of a conducting polymer; poly(3,4-ethylenedioxythiophene) (PEDOT) with the electrochemically produced polymer of N-ferrocenyl-3-(1*H*-pyrrol-1-yl)aniline, (PFcPyBz) layer for the enzyme scaffolding resulting in excellent analytical parameters. To organize such a surface, graphite electrode was coated with a PEDOT layer and it was used as a transducer for electrochemical deposition of the polymer of a newly synthesized FcPyBz monomer. Using a PEDOT layer as the working electrode improved localization of the PFcPyBz on the transducer surface while enhancing the biosensor performance. A simple binding of glucose oxidase (GOx) as a test enzyme on this new polymeric platform was achieved using glutaraldehyde (GA) as the cross linker. The low limit of detection and high sensing sensitivity on glucose for the biosensor are estimated as 54 μM and 112.2 μA/mMcm^2, respectively. The surface characterizations of the modified electrodes were investigated by cyclic voltammetry (CV), attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy and scanning electron microscopy (SEM) techniques. Finally, different kinds of beverages were tested for sensor reliability with high accuracy.
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A well-known disorder, diabetes mellitus occurs via an increase in glucose concentration in human blood. Since it is spreading worldwide, its careful and precise detection gained importance to reduce the threat.[1,2] Mirroring the importance of the disease is the worldwide interest leading to publishing a number of articles per year. These developments have opened ways to the wide array of emerging applications in glucose biosensors. Development of the glucose oxidase based enzyme biosensors by different immobilization techniques on various kind of electrodes had been studied for several decades.[3,4] Conjugated polymers (CPs) promise to advance a number of real-world technologies. Of these applications, they are particularly attractive for uses in enzyme biosensors for environmental and health monitoring. Their unique properties such as high electrical conductivity, ease of preparation and good environmental and chemical stability have motivated the use of CPs in the form of thin films for biosensors.[5,6] CP based biosensors bring simple, accurate, reliable and low-cost determination of various analytes and act as a very effective analytical tool in the multiple areas. This description is also supported by a number of researchers using the CPs as an immobilization architecture.[7–10]

PEDOT has been reported to exhibit superior stability in conductivity compared to other available polymers and this property prompted us to use the PEDOT film as a transducer in order to obtain a more stable scaffolding for the glucose sensor. Brett and co-workers developed a PEDOT/poly (methylene blue) (PMB) modified glassy carbon electrode (GCE) for a GOx-based biosensor.[11] PEDOT films generated on top of PMB modified bare electrode was used to enhance the stability of PMB modified electrode. The proposed sensing architecture (GOx/PEDOT/PMB/GCE) showed better biosensor performance than the ones for GOx/GCE and GOx/PEDOT/GCE biosensors. In another work, Si reported a simple synthesis of a hybrid film by electropolymerizing 3,4-ethylenedioxythiophene (EDOT) on nanoporous gold (NPG) for applications in amperometric glucose biosensors.[12] They finally concluded that the NPG/PEDOT/GOx biosensor prepared by optimum film thickness is appropriate for effective substrate diffusing. PEDOT is also used as the working electrode for glucose sensing by Ho and co-workers.[13] In this work, PEDOT and ferrocene (Fc) containing polymer were used for not only reducing the working potential but also for improving the stability of the sensor. Moreover, after the invention of the Fc molecule in 1951, scientist paid attention to this sandwich like molecule in many other research areas.[14,15] A huge number of molecules containing Fc moiety were designed and assessed for their possible use in glucose sensing applications.[16,17] Electrodeposited copolymer of pyrrole and ferrocene carboxylate modified pyrrole p(Py-FcPy),[18] co-deposition of 4-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl) aniline (SNS-NH$_2$) and 4-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl) amidoferrocenyldithiophosphonate (SNS-NH2-Fc)[19] and copolymer of O-4-(1H-pyrrol-1-yl)-ferrocenyldithiophosphonate (TPFc) with 4-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl)butane-1-amine (TPA)[20] are the examples of the conjugated polymer based electron-mediating support materials for developing GOx immobilized electrodes. Under the light of this, this work will show a significant way to create CPs-based glucose biosensors exhibiting selective responses to a target analyte. Such approach predominantly includes functionalization of PEDOT bearing surface with newly synthesized Fc moiety containing polymer.

In this report, for the first time a PEDOT transducer was designed and a new one-pot synthesized ferrocene containing monomer was electrochemically deposited on a well-known polymer (PEDOT) surface for glucose sensing. Without the PEDOT layer it was difficult to coat PFcPyBz layer on the bare graphite surface. For this reason, the electrode surface is first modified with PEDOT and then coated with PFcPyBz. We described the importance and effect of CPs in biosensor construction that was highlighted in our previous works.[21,22] However, need for a promising analytical devices for glucose sensing with high accuracy and sensitivity has motivated us to design a new sensing system. Designing of the new surface in this study, a highly sensitive and reliable glucose sensor was developed with the help of glucose oxidase (GOx), as a model enzyme. Detailed optimization studies, surface characterization of the polymer layers and the amperometric characterization were performed. Additionally, the testing of the biosensor was conducted using different kinds of commercial beverages.

Experimental

Chemicals and instrumentation.—All chemicals which were used for monomer synthesis and electrochemical polymerization, were purchased from Aldrich and used without further purification. Materials used for biosensor construction were also obtained from Aldrich. Glucose oxidase enzyme used in this study was (GOx, β-D-glucose: oxygen 1-oxidoreductase, EC 1.1.3.4, 17300 units/g solid) from *A. Niger*. Electrochemical polymerizations of the EDOT and the FcPyBz

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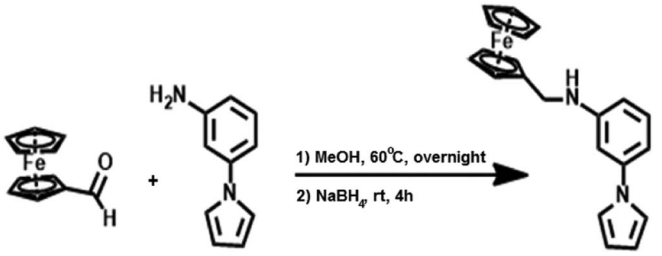

Scheme 1. Synthetic route for the ferrocene containing monomer (FcPyBz).

monomers were performed by GAMRY Reference 600 potentiostat/galvanostat using three electrode system containing platinum counter and silver wire reference electrodes. Amperometric measurements were performed using Palm Instrument (PalmSens, Houten, The Netherlands) with the same electrode configuration. Graphite electrodes (type RW001, 3.05 mm diameter and 13% porosity) were obtained from Ringsdorff Werke GmbH, Bonn, Germany. In amperometric analyses, the data were given as the average of three measurements and standard derivations were recorded as ±SD. JEOL JSM-6400 models SEM was used to investigate the layers of the fabricated biosensor.

Shynthesis of FcPyBz.—Ferrocene containing monomer was synthesized in a single step reaction using a similar procedure in literature[23] (Scheme 1). 676.5 mg (3.16 mmol) ferrocene carboxaldehyde and 500 mg (3.16 mmol) 3-(1*H*-pyrrol-1-yl)aniline were dissolved in 15 mL methanol in a 100 mL round bottom flask fitted with a condenser and stirred overnight at 60°C. After the solution was cooled, 177 mg (4.74 mmol) $NaBH_4$ were added into the solution and stirred for an additional 4 hours at room temperature. Then, the reaction mixture was poured into 100 mL of distilled water and the solution was extracted using ethyl acetate (EtOAc) for several times. The organic layer was dried with Na_2SO_4 and the solvent was evaporated under vacuum. The purification of the residue was performed by column chromatography using hexane:EtOAc (3:1) as the eluent. Yellow crystals were obtained with a yield of 27%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.22 (t, 1H), 7.08 (d, 2H), 6.74 (dd, 1H), 6.65 (s, 1H), 6.54 (dd, 1H), 6.33 (t, 2H), 4.26 (t, 2H), 4.20 (s, 5H), 4.17 (t, 2H), 4.04 (s, 1H), 3.99 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 149.3, 142.0, 130.2, 119.5, 110.4, 110.0, 109.9, 104.9, 85.9, 68.6, 68.2, 68.0, 43.4. HRMS (ES^+) calculated for $C_{21}H_{20}N_2Fe$ 356.0976, found 356.0961.

Deposition of bilayer polymeric platform and amperometric detection.—Sequential electrochemical polymerization of the EDOT and FcPyBz were performed on graphite electrodes by cyclic voltammetry using platinum wire as the counter electrode and the silver wire as the reference electrode. Graphite rods were hand-polished with an emery paper, washed with distilled water and left to dry at ambient conditions right before the deposition. Firstly, pristine PFcPyBz film was tried to electrochemically coat on the graphite surface however it was observed that there was no homogenous PFcPyBz film coverage. For this reason, EDOT electrochemically polymerized on the graphite surface to have a more convenient platform for FcPyBz polymerization. Thanks to its electrical conduction characteristics, PEDOT enabled to obtain a very homogenous PFcPyBz film.

10^{-2} M EDOT solution was prepared in propylene carbonate containing 0.1 M $LiClO_4$, then potentiodynamic coating of PEDOT layer was performed with a repeated scan interval between −600 mV and 1600 mV with a scan speed of 100 mV/s in 10 cycles. Then, PEDOT coated graphite rods were immersed into propylene carbonate solution containing 10^{-2} M FcPyBz and 0.1 M $LiClO_4$ for electrochemical polymerization by potential cycling between −600 mV and 1600 mV for 15 cycles at a scan rate of 100 mV/s. After sequential deposition of PEDOT and PFcPyBz films on graphite electrode, enzyme immobilization was performed by drop casting of 5 μL enzyme solution (1.25 mg of GOx is dissolved in 50 mM phosphate buffer (pH = 7.0)) with the help of 5 μL GA (% 1) and dried thoroughly at room temperature. Dried electrode was rinsed with distilled water just before the measurement to remove the unbounded species. Construction route for the glucose biosensor is illustrated in Figure 1.

All amperometric measurements were carried out at room temperature in a reaction cell filled with 10 mL PBS (phosphate buffered saline, pH 7.0) under mild stirring by applying −0.7 V constant potential. For consecutive measurements, the electrodes were first washed with distilled water and then the buffer solution was refreshed. Amperometric signals were followed after initial equilibration. The proper amount of substrate was added to the reaction cell. As a result of the enzymatic reaction between GOx and the substrate, the oxygen consumption level associated with the substrate concentration was examined under a constant potential. The difference (under the obtained equivalence signal) between before and after the addition of glucose called as the biosensor response and reported as current (μA). The proposed sensing system was also tested by determining the glucose content in several beverages. The samples were injected into the cell instead of the glucose substrate without any pretreatment. The responses of the biosensor for each sample were recorded and the values were estimated from the calibration curve.

Results and Discussion

Electrochemical characterization.—Electroactivities of the PEDOT, PFcPyBz and PEDOT/PFcPyBz films were investigated in 0.1 M $LiCO_4$ solution in propylene carbonate in the range between −600 mV and 1600 mV at a scan rate of 100 mV/s. The related films were obtained on ITO (indium tin oxide) substrates via cycling the potential for optimum numbers which were determined to obtain the highest current response for the biosensor using same electrolytic medium and 10^{-2} M of corresponding monomer. As it is shown in the CV spectra of the films in Figure 2, a broad redox couple was obtained for the PEDOT film in a monomer free environment. On the other hand, irreversible oxidation and reduction peaks were obtained for the PFcPvBz film on ITO surface which are located at 1240 mV and −268 mV, respectively. These peaks were also very low in intensity, which shows that it is not possible to obtain a homogenous polymer film on the electrode surface. However, when it was deposited on a PEDOT coated surface (blue curve in Figure 2), a reversible redox couple was obtained for the same monomer concentration and electrolytic medium at 723 mV/177 mV for oxidation/reduction. Notably, the trend of the cyclic voltammograms are similar with that trend in surface morphology quality.

Another electrochemical study was carried out in 5.0 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl to investigate the electroactive surface area after depositing the polymer layers and the immobilization of the GOx (Figure 3). Cyclic voltammograms were obtained at a scan rate of 100 mV/s versus silver wire electrode using platinum wire as the counter. The average values were calculated according to the Randles-Sevcik equation[24] and found to be 0.313 cm^2 for the bare graphite electrode, 0.405 cm^2 for the graphite/PEDOT/PFcPyBz and the 0.256 cm^2 for the graphite/PEDOT/PFcPyBz/GOx. As it is also discussed in the surface characterization, the porous surface of the deposited polymer layers increases the electrode surface and enhances the electron transfer reactions. The immobilization of an insulating biomolecule on the conducting polymer surface decreases the electroactivity of the corresponding electrode which also indicates an effective attachment of the enzyme to the electrode surface.

Surface characterization.—ATR-FTIR measurements were conducted for PEDOT and PEDOT/PFcPyBz surfaces of the films (Figure 4). Electrochemical depositions of the films were performed by cyclic voltammetry on ITO coated glasses as the working electrodes instead of graphite rods for an easy handling of the samples for the structure characterization. The characteristic peaks of the PEDOT film were observed at 890 cm^{-1}, 1027 cm^{-1} and 1160 cm^{-1}, for the

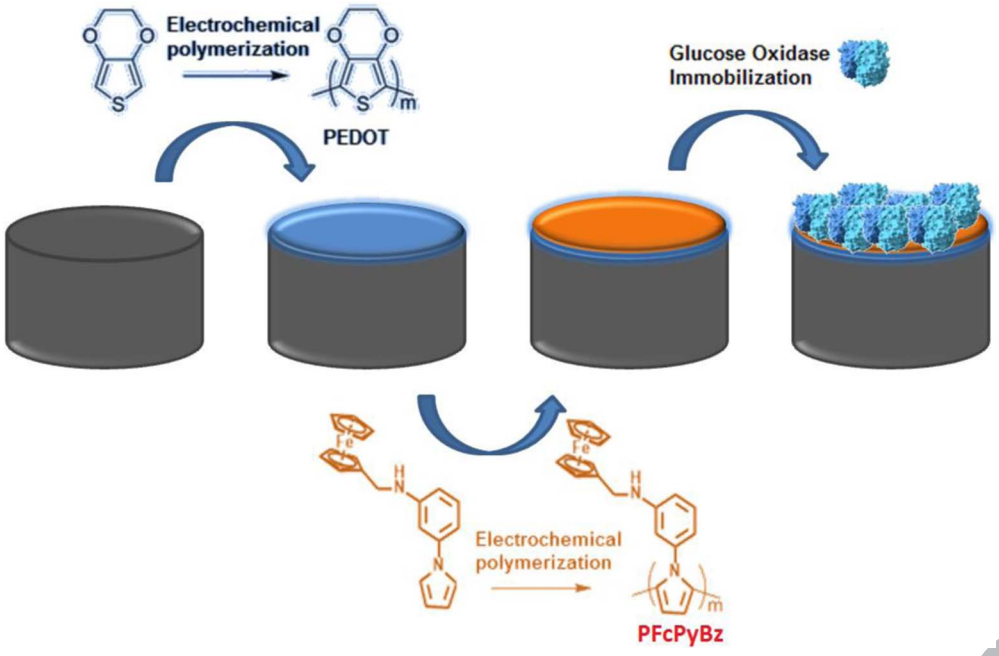

Figure 1. Fabrication of the glucose biosensor used in this work.

C-S, alkylenedioxy and C-O-C stretchings, respectively.[25] The spectrum of PEDOT/PFcPyBz film clearly exhibits the decreased peak intensities of the PEDOT and formation of vibrational bands of pyrrole and ferrocene rings. The conjugated C-N peak was observed at 1174 cm^{-1} also the C-H stretching vibration and C-H deformation peaks are located at 967 cm^{-1} and 907 cm^{-1}, respectively.[26,27] Additionally, the band observed at 1402 cm^{-1} was for the asymmetric stretching vibration of C-C bonds of cyclopentadienyl rings of ferrocene.[28]

To correlate the morphology of the modified electrodes with the electrochemistry and the FTIR data, top-view SEM images of the bare graphite electrode, PEDOT coated graphite, PEDOT/PFcPyBz coated graphite and PEDOT/PFcPyBz/GOx immobilized graphite were collected as indicated in Figure 5. Additionally, the SEM image of the PFcPyBz coated graphite surface without PEDOT layer is also given in Figure 6.

As confirmed by SEM image in Figure 6, a total coating of the bare graphite surface by electrochemical deposition of the PFcPyBz could not be achieved (whereas some partial deposition was observed). On the other hand, a cauliflower like structure with high porosity was monitored from the surface of the PEDOT on graphite (Figure 5b), which is a typical network for conducting polymers. High porosity also means a larger surface area, which was stated in the electrochemical study in Figure 3. More importantly, an obvious difference was observed between the PEDOT and the PEDOT/PFcPyBz surfaces (Figure 5c), which proves the deposition of PFcPyBz onto PEDOT surface. This result is also in accordance with the results of the CV and ATR-FTIR. From the SEM image of PEDOT/PFcPyBz/GOx surface in Figure 5d, it can be deduced that a successful GOx immobilization was performed with a smooth surface that is related to good anchoring of the biomolecules on the electrode surface.

Optimization studies of the biosensor.—Optimization studies were carried out by following four steps; PEDOT layer thickness optimization on graphite electrode, PFcPyBz layer thickness optimization on PEDOT transducer, GOx amount and the pH value of

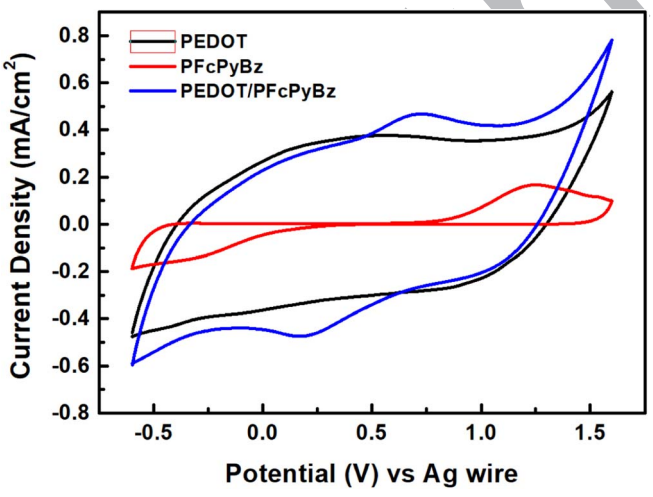

Figure 2. Cyclic voltammograms of the relating polymer surfaces on ITO electrodes obtained in a monomer free environment.

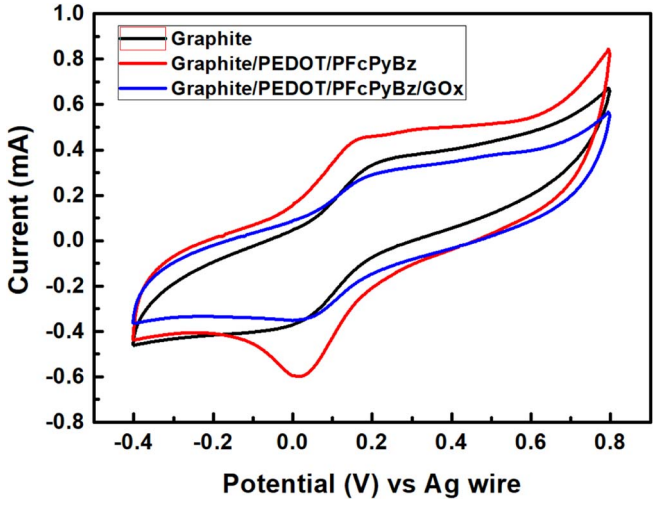

Figure 3. Cyclic voltammograms of the relating surfaces on graphite electrodes in 5.0 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl.

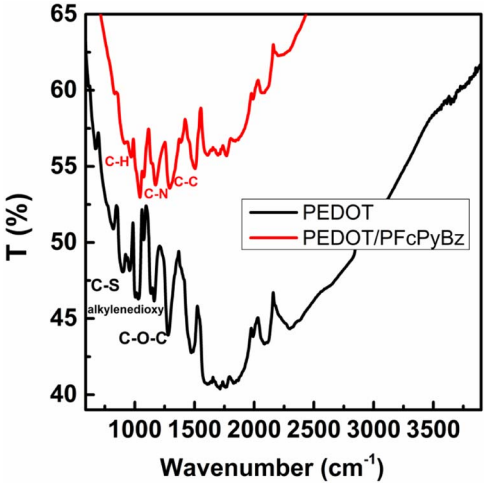

Figure 4. ATR-FTIR spectrum of PEDOT and PEDOT/PFcPyBz before (below) and after (above) PFcPyBz modification.

the buffer solution to obtain the best biosensor performance. Firstly, sequential coatings of the polymers (PEDOT and PFcPyBz) on the graphite electrode were optimized to have a smooth and electronically active surface for the enzyme immobilization. The thickness of the polymer films on the graphite surface is very crucial in terms of an efficient biosensor response. The ferrocene containing polymer (PFcPyBz) serves as an electron transfer mediator between the redox center of the GOx and the electrode surface. Therefore, the number of the ferrocene units is yet another deciding factor in the amperometric response of the corresponding sensor. For an efficient biosensor, polymer layer thicknesses were optimized accordingly. Firstly, EDOT monomer (10^{-2} M) was dissolved in propylene carbonate (PC) containing 0.1 M $LiClO_4$ and deposited on different graphite working electrodes with varying scan numbers between 5 and 20 (5, 10, 15 and 20). The scan number of the second polymeric layer was kept constant as 10 cycles. Comparing the current responses with the scan numbers of the PEDOT layers, the highest response was obtained from the 10 cycles of PEDOT layer deposition and it was decided as the optimum number for the following optimization steps (Figure 7a). The second

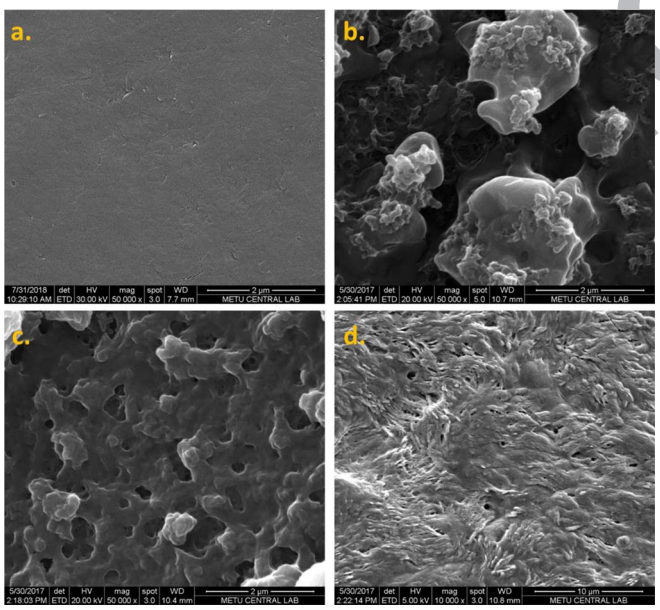

Figure 5. SEM images of (a) bare graphite electrode, (b) PEDOT layer, (c) PEDOT/PFcPyBz layers and (d) PEDOT/PFcPyBz/GOx on graphite surface.

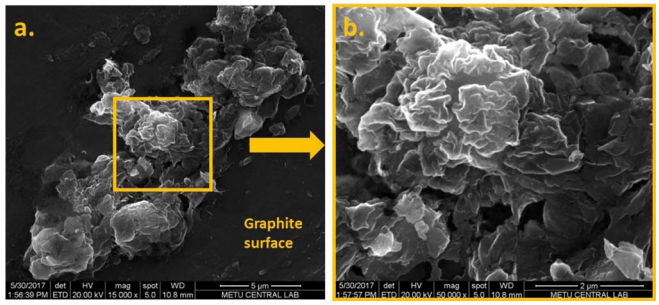

Figure 6. SEM images of PFcPyBz on bare graphite surface with different magnifications a) 15000x and b) 50000x.

optimization was performed for the PFcPyBz using the same electrolytic medium used for the EDOT polymerization. The 10^{-2} mmol of FcPyBz monomer was dissolved in a 1 mL of PC solution containing 0.1 M $LiClO_4$ and the electrochemical deposition was performed onto four different PEDOT coated graphite electrodes using 5, 10, 15 and 20 scan numbers. The highest response was obtained from the 15 cycles of polymerization of the FcPyBz and chosen as the optimum condition (Figure 7b). Without optimizing the thickness range for the sensor system, diffusion problems between the polymer coated electrode and the biomolecule, or denaturation of biomolecules may arise. As shown in Figures 7a and 7b, the highest response for biosensor application was recorded with 10 cycles of PEDOT film and 15 cycles of PEDOT/PFcPyBz film deposition, which corresponds to 120 nm (the equivalent of a 54 mC charge).

For the third optimization step, five different amounts of GOx (GOx; 0.5 mg, 0.75 mg, 1.0 mg, 1.25 mg and 1.50 mg) were dissolved in the same buffer solution and immobilized on the optimized polymer surface with glutaraldehyde (1%). The biosensor response increased with increasing enzyme amount and reached a maximum for 1.25 mg of GOx for the same amount of substrate. However, the current value decreases when the enzyme amount increased to 1.50 mg, which means that the higher enzyme concentration hinders the electron transfer. Additionally, excess loading of enzyme resulted in leaching from the surface since the enzyme molecules on the surface were not sufficiently stable (Figure 7c). On the contrary, inadequate enzyme loading caused low sensitivity of the biosensor. As the final step, the optimization of the pH value of the buffer solution was performed preparing different buffer solutions having the pH values as 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. It is known that the GOx is an active enzyme in the range of pH 3.0 and pH 8.0.[29] According to the current response of the sensors in different pH mediums, pH 7.0 PBS buffer was determined as the optimum solution medium and used for the further analyses (Figure 7d).

Analytical characterization.—The classical amperometric curve of the biosensor is recorded by successively adding different concentrations of glucose at a constant potential. A linearity (inset figure of Figure 8a) was obtained in the range 0.01–0.50 mM of glucose concentration with an equation of $y = 7.5905x + 0.0901$ ($R^2 = 0.997$). Obtained calibration curve was used to calculate the other analytical parameters of the biosensor. The limit of detection (LOD) was calculated as 54 μM according to the concept of signal-to-noise ratio (S/N = 3). The sensitivity of the biosensor and K_M^{app} value (Michaelis-Menten constant) were determined as 112.2 μA/mMcm^2 and 0.05 mM, respectively. Apparent Michealis-Menten constant, K_M^{app} value was obtained from Lineweaver-Burk plot. High sensitivity and such a low K_M^{app} value perform high affinity toward the glucose. It shows that the K_M^{app} is very good compared to that of other studies. For example, a glucose biosensor prepared using a nafion and nano scaled cobalt phthalocyanine (NanoCoPc) film gives a K_M^{app} value of 12.4 mM.[30] In another example, based on a CoPc–CoTPP complexes and Nafion film, it was calculated to be 14.91 mM.[31] Moreover, In 2013, Wang constructed a glucose biosensor containing branched polyethyleneimine

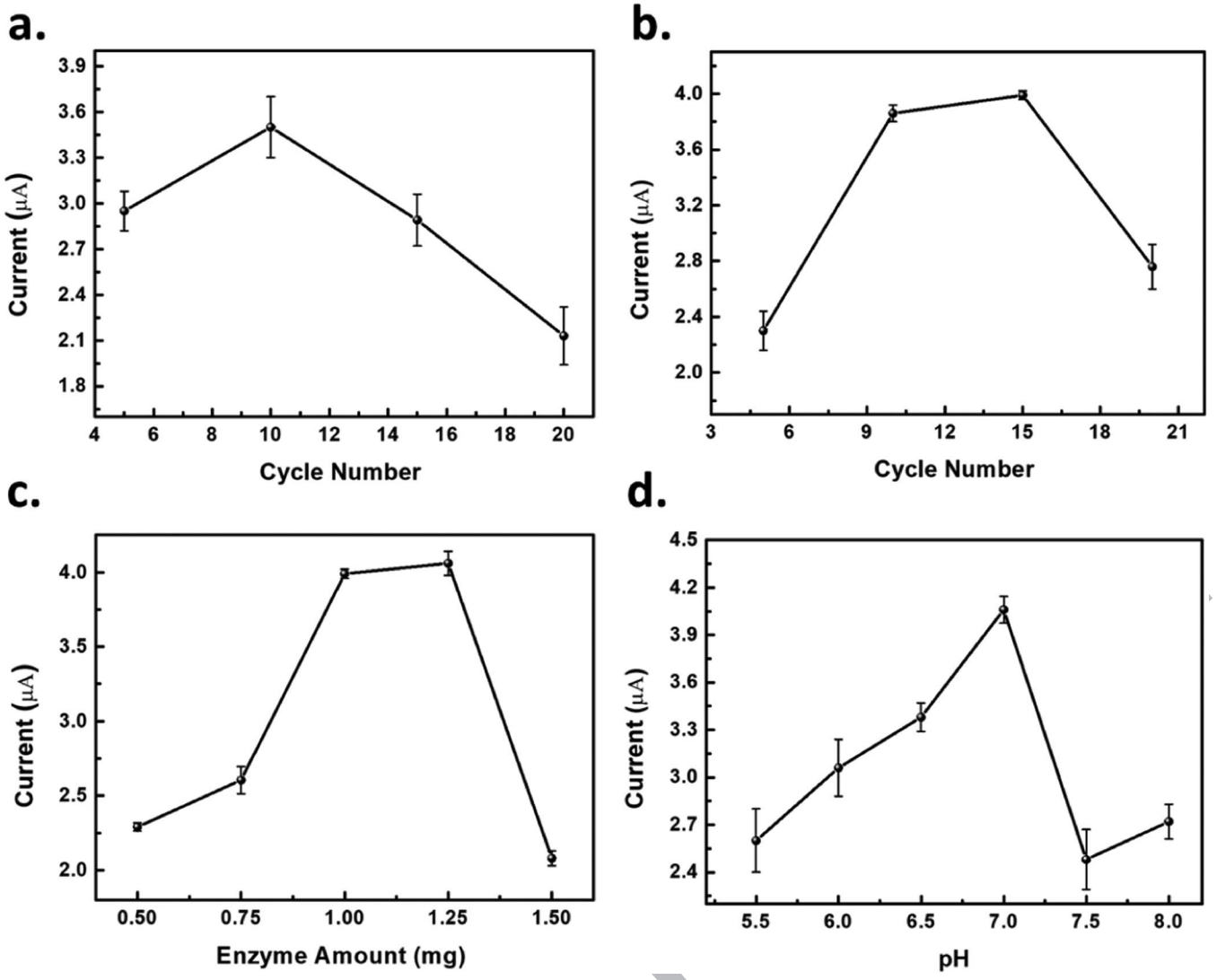

Figure 7. The effect of the cycle number of the PEDOT (a) and PFcPyBz (b), enzyme amount (c) and pH value (d) on the biosensor performance. Error bars show the standard deviation (SD) of three measurements.

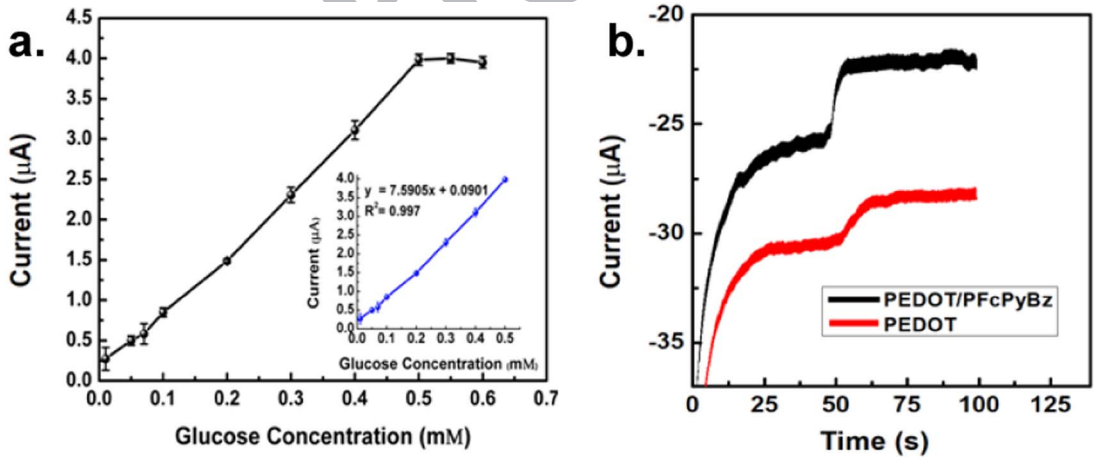

Figure 8. a) Calibration curve for glucose concentration in 50 mM PBS, pH 7.0, −0.7 V). Error bars show the SD of three measurements (Linear range as an inset.) b) Comparison of the current responses of PEDOT/PFcPyBz and PEDOT surfaces for 0.5 mM glucose.

Table I. List of the glucose biosensor based on conjugated polymers.

Electrode matrix	Sensitivity ($\mu A\ cm^{-2}\ mM^{-1}$)	K_M^{app} (mM)	LOD (μM)	Linear Range (mM)	Reference
BPEI-Fc/PEDOT:PSS/GOx	66	2.4	NR	0.5–4.5	32
Poly(BEDOA-6)/AuNPs/MPA	14.97	0.81	25	0.025–1.25	33
Py0.2/PyCO2H0.2/PyFc0.6	1.796	4.73	6.9	1.0–4.0	34
BNNTs-Pani-Pt-GOD	19.02	3.4	0.18	0.01–5.5	35
PDDA/PSS/{PDDA-MWCNTs/GOx}5	5.60	NR	58	Up to 5.0	36
PEDOT/PFcPyBz/GOx	**112.2**	**0.05**	**54**	**0.01–0.5**	**This work**

binding with ferrocene having a sensitivity value of 66 $\mu A\ cm^{-2}\ mM^{-1}$ and 2.4 mM K_M^{app} value.[32] In the same year, a similar type of glucose biosensor showed a very low K_M^{app} value (0.81 mM) by modification of gold nanoparticles of the electrode matrix.[33] Senel and coworkers also developed another glucose biosensor containing pyrrole and ferrocene moieties and sensitivity and K_M^{app} values were estimated as 1.796 $\mu Acm^{-2}mM^{-1}$ and 4.73 mM, respectively.[34] Other references cited in Table I, also describes a similar glucose sensor application with similar sensor performances.

Electroanalytical performance of the corresponding biosensor was tested by addition of required amount of glucose into 10 mL 50 mM PBS buffer medium under applied potential of −0.7 V. Before a detailed analytical characterization of the proposed biosensor (PEDOT/PFcPyBz/GOx), the biosensor without the PFcPyBz layer (PEDOT/GOx) was also constructed and the current responses were compared. As shown in Figure 8b, The PEDOT/PFcPyBz/GOx gives a higher response toward glucose than the PEDOT/GOx modified one. Through the use of PFcPyBz layer, the sensing response of the biosensor was increased by as much as two folds.

To examine the repeatability of the biosensor responses, signals corresponding to 0.5 mM glucose standard solutions were measured for seven times using the same sensor. The standard deviation (S.D) and relative standard deviation (RSD) values were calculated as 0.051 and 1.29%, respectively. The signals were recorded under the same experimental conditions. According to the data, it can be said that PEDOT/PFcPyBz/GOx based glucose biosensor is capable to generate a reproducible output while performing series of measurements. Moreover, as for reproducibility of the devices, three independent sensors were prepared by measuring of the current responses to 0.5 mM glucose at the optimized working conditions. The results showed that all devices showed almost the same properties after each preparation and 1.13% relative standard derivation (RSD) was obtained. Precision of the PEDOT/PFcPyBz/GOx electrode was also evaluated by assaying one enzyme electrode for three replicated determinations in 0.5 mM glucose and its responses were compared with the ones for beverages. As shown in Table II, results are very close to the product label values proving that the fabricated biosensor is feasible for practical sample testing with reliable accuracy and precision.

Interference study was also performed for the proposed biosensor (Figure 9A). Ascorbic acid and urea were used as the interfering agents to observe the affinity of the PEDOT/PFcPyBz/GOx sensor to these materials. Ascorbic acid and urea solutions were tested repeatedly in the same system. Only a slight negative biosensor signal has been observed after the injection of ascorbic acid while the injection of the same concentration of urea into the reaction cell did not alter the electrochemical response of the biosensor at all. When a slight negative biosensor signal of the ascorbic acid was compared with the huge signal of the proposed sensor, it is negligible. Moreover, the selectivity of the biosensor toward the substrate was tested by injecting different sugars. As seen in Figure 9B, no signal was detected after the injection of 0.5 mM of galactose, mannose, fructose, sucrose and Xylose. This shows that the biosensor exhibits excellent selectivity and performs well in the presence of different compounds.

Sample application.—Various beverages were used to evaluate the reliability of the corresponding biosensor under optimized conditions. PEDOT/PFcPyBz/GOx biosensor was used to determine the glucose level of an ice tea, lemonade and milk samples without further dilution. Amperometric responses were calculated using the linearity equation and compared with the reference values. As seen from Table II, it

Table II. Amperometric results of glucose levels using PEDOT/PFcPyBz/GOx biosensor in various beverages.

Sample	Product Label (mM)	Biosensor Response (mM)	Relative Error (%)
L ice tea peach	0.383	0.373	2.61
U lemonade	0.389	0.374	3.95
S milk	0.125	0.121	3.20

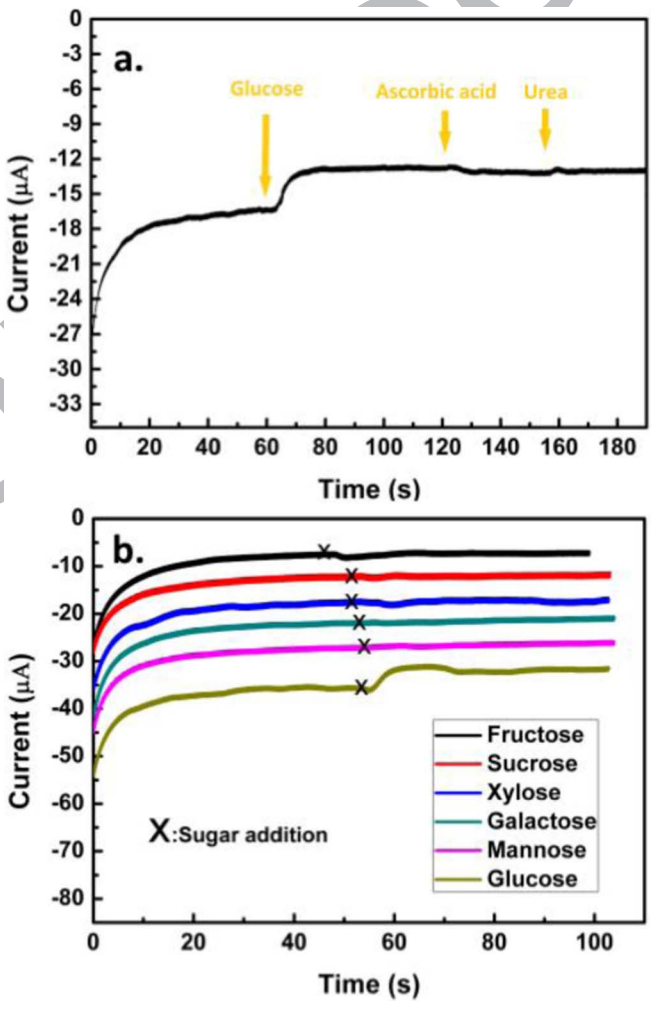

Figure 9. a) Amperometric responses of PEDOT/PFcPyBz/GOx biosensor to glucose and interference studies with ascorbic acid and urea, b) the selectivity of the biosensor toward the different sugars.

is possible to detect the glucose level of commercial products even having different ingredients with a very low error.

Conclusions

In this study, electrochemically produced PFcPyBz and PEDOT combination was used for an effective glucose detection. By this way, the PEDOT coated layer was used as a transducer surface for coating of FcPyBz monomer. With this aspect, a novel polymeric platform was designed and successfully constructed for glucose biosensor application. From the electrochemical analyses it was observed that PEDOT modification of an electrode surface improves the electrochemical deposition of a molecule having steric hindrance. According to the analytical characterization, preparation of the polymeric platform has the main effect on biosensor performance. High sensitivity and low K_M^{app} values show that constructed biosensor has a high affinity to the glucose substrate selectively and it is possible to detect the glucose amount in commercial beverages with very small relative error values.

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