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PAPER

# Synthesis and application of poly-SNS-anchored carboxylic acid: a novel functional matrix for biomolecule conjugation†

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Here we report the synthesis of a novel conducting polymer and its properties as an immobilization platform for biosensor application. The conducting polymer has functional groups used for the formation of amide bonding with the enzyme immobilized on the polymer surface. After covalent immobilization of glucose oxidase (GOx) on the polymeric matrix, its application for glucose biosensing was investigated in detail. Scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and contact angle measurements were used to monitor the surface properties of the polymer before and after biomolecule conjugation. The optimized biosensor showed a very good linearity between 0.01 mM and 1.2 mM, a 13 s response time and a detection limit (LOD) of 0.004 mM to glucose. Also, kinetic parameters, operational and storage stabilities were determined. Apparent Michaelis constant ($K_m^{app}$) and $I_{max}$ values of 1.17 mM and 11.28 μA, respectively, were obtained.

## Introduction

Conducting polymers as immobilization matrices appeared as an attractive field of research for the improvement of biosensor design.[1–4] They enable the electric charge produced by biochemical reactions to be delivered to an electronic circuit. Their great electrical, electronic and optical properties make conducting polymers promising materials for biosensor applications.[5,6] Moreover, functionalized conducting polymers are expected to be excellent supports for biomolecules.

When enzyme-based electrochemical biosensors are considered, the enzyme immobilization step can be regarded as the most fundamentally crucial issue for the successful construction of the biosensor. Therefore, the choice of a suitable matrix and the rigidity of the matrix are also important for the successful immobilization of enzymes for the preparation of durable and functioning biosensors.[7,8] In this manner, adapting the electrode surface to construct such a functional biosensor remains a challenge.[9] Among all the immobilization methods, to immobilize enzymes on electrodes *via* robust covalent bonds is preferred in order to improve the durability of enzyme electrodes.[5,10] The basic limitations of conventional immobilization methods, such as adsorption, include entrapment which occurs during continuous operation of the biosensors due to the instability of the immobilized proteins. In conventional methods, the biomolecule is situated in the bulk and obstructs the accessibility of the substrate; covalent immobilization can be generated on the top layers. The related studies are directed to fulfilling the requirements for support materials for enzyme immobilization providing excessive enzyme loading and an accordant microenvironment. The necessity for maximum enzyme loading with excessive activity encourages research into the improvement of matrices with a large surface area and easy bioconjugation. For this purpose, functionalized conducting polymers are promising and can be used to conjugate biomolecules on electrode surfaces through functional groups.[11–16] Electrogeneration of a functionalized polymer on the electrode constitutes the first step. The second step is the covalent attachment of biomolecules to the polymer coated surface.[17] This approach allows the optimization of each step and provides less denaturation and preselected positions for binding. Owing to the well-organized molecular structure of the conducting polymers on the substrates, they constitute a wonderful three dimensional matrix for the immobilization of biomolecules maintaining their biological activity for a long period of time even under adverse conditions.[18] Carboxylic acid group functionalized conducting polymers bring many advantages for the immobilization of biomolecules and the method offers stable, rapid and sensitive substrate detection.[19–22]

In current years, great attention has been paid to the development of reliable glucose biosensors.[23–26] On account of their

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† Electronic supplementary information (ESI) available: Details of syntheses and characterizations; $^1$H-NMR, $^{13}$ C-NMR, MS, HRMS spectra of the products; preparation and measurement details of *G. oxydans* culture mediums. See DOI: 10.1039/c1jm12048d

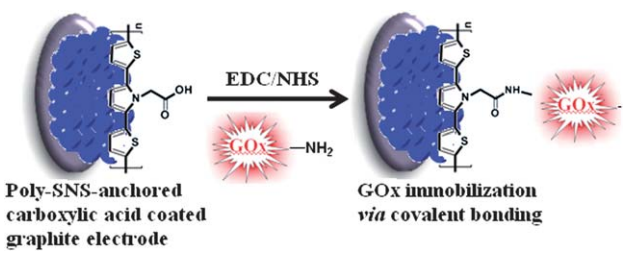
simplicity, low cost, fast detection, high sensitivity and selectivity, glucose oxidase-based electrochemical biosensors provide the possibility of carrying out diagnostic procedures simply and hence, they became the main focus of biosensor research.[27,28,29]

In this work, a novel target monomer; 2-(2,5-di(thiophen-2-yl)-1*H*-pyrrol-1-yl) (SNS) acetic acid was synthesized through pyrrolysis and subsequent hydrolysis processes following 1,4-di(thiophen-2-yl)butane-1,4-dione synthesis. The corresponding monomer design was really attractive due to its carboxylic acid group which is open to amide bonding. Also, the thiophene-pyrrole-thiophene unit has the great advantage of its ability to polymerize easily. The conducting polymer of 2-(2,5-di(thiophen-2-yl)-1*H*-pyrrol-1-yl) (SNS) acetic acid was electrochemically synthesized onto a graphite electrode by cyclic voltammetry technique *via* repeated electrochemical cycling at the oxidation potential of the monomers using a scan rate of 0.1 V $s^{-1}$. The electropolymerization of the monomer comprises an E(CE)n (electrochemical, chemical, electrochemical) mechanism consisting, as the first step, the formation of the radical cation.[30a,b] Glucose oxidase (GOx), which catalyzes glucose oxidation in the presence of molecular oxygen, was used as the model enzyme. Immobilization of GOx onto the polymer surface was performed through covalent binding using the well- established two-step carbodiimide coupling method.[31,32] Poly-SNS-anchored carboxylic acid served as an excellent immobilization matrix forming a robust electrode configuration for glucose sensing. An efficient matrix for enzyme immobilization was obtained which was biocompatible while maintaining its protein structure and thereby its biological activity. Scheme 1 displays the procedure for construction of the proposed amperometric glucose biosensor. Finally, the enzyme immobilized matrix was characterized and favorably applied for the estimation of glucose contents in *Gluconobacter oxydans* culture medium.

## Results and discussion

### Synthesis of methyl 2-(2,5-di(thiophen-2-yl)-1*H*-pyrrol-1-yl) acetate

1,4-Di(thiophen-2-yl)butane-1,4-dione was synthesized according to the literature.[33] 1,4-Di(thiophen-2-yl)butane-1,4-dione ($4 \times 10^{-3}$ mol, 1.0 g), glycine methyl ester ($5.6 \times 10^{-3}$ mol, 702 mg), propionic acid ($4.32 \times 10^{-3}$ mol, 320 mg, 0.323 mL) were dissolved in 20 mL toluene. The mixture was stirred and refluxed for 16 h under argon atmosphere. Toluene was evaporated through vacuum. Product was isolated by flash column chromatography (eluent: 1 : 5 EtOAc/Hexane) (0.752 g, 62%). $^1$H-NMR ($CDCl_3$ + $CCl_4$): $\delta$ 7.22 (dd, $J = 1.0$ Hz, $J = 5.1$ Hz, 2H), 6.97 (dd, $J = 3.5$ Hz, $J = 5.2$ Hz, 2H), 6.89 (dd, $J = 1.0$ Hz, $J = 3.5$ Hz, 2H), 6.30 (s, 2H), 4.66 (s, 2H), 3.69 (s, 3H). $^{13}$C-NMR ($CDCl_3$ + $CCl_4$): 168.2, 132.6, 127.5, 126.0, 124.8, 124.4, 109.6, 51.0, 45.8. MS (EI) *m*/*z* (relative intensity): 303.0 (100), 304.0 (18), 305.0 (10), 244.0 (37), 230.0 (22).




**Scheme 1** A schematic representation of the construction of the proposed glucose biosensor.

### Synthesis of 2-(2,5-di(thiophen-2-yl)-1*H*-pyrrol-1-yl)acetic acid

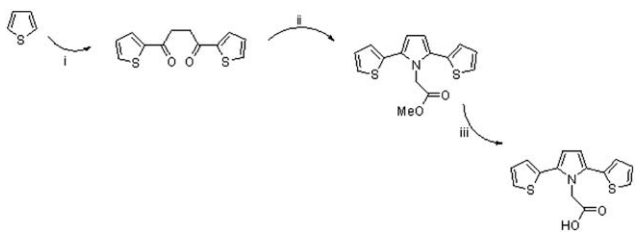
KOH (1.6 mmol, 89 mg) was added to methyl 2-(2,5-di(thiophen-2-yl)-1*H*-pyrrol-1-yl)acetate (0.59 mmol, 180 mg) dissolved in methanol. The mixture was allowed to reflux until all the esters had disappeared and the methanol had evaporated. The residue was dissolved in water and then extracted with ether. The aqueous phase was acidified to pH 1.0 with 6.0 N HCl and then extracted with ether. Latter ethereal extracts were washed with water and brine, and dried over $MgSO_4$. The solvent was evaporated under vacuum (0.145 g, 85%). $^1$ H-NMR ($CDCl_3$): $\delta$ 9.10 (brs, 1H), 7.26 (dd, $J = 1.0$ Hz, $J = 5.2$ Hz, 2H), 7.00 (dd, $J = 3.6$ Hz, $J = 5.1$ Hz, 2H), 6.94 (dd, $J = 1.0$ Hz, $J = 3.5$ Hz, 2H), 6.34 (s, 2H), 4.73 (s, 2H). $^{13}$C-NMR ($CDCl_3$): 175.4, 134.0, 129.3, 127.8, 126.8, 126.4, 111.6, 47.2. MS (EI) *m*/*z* (relative intensity): 289.0. HRMS: Calculated $[M]^+$ 289.0231, Measured $[M]^+$ 289.0229. Scheme 2 shows the reagents and conditions.

### Preparation of poly-SNS-anchored carboxylic acid-coated substrates

Spectroscopic grade graphite rods were polished on emery paper and washed thoroughly with distilled water prior to electropolymerization. Electrochemical polymerization of SNS anchored carboxylic acid was potentiodynamically carried out between −0.3 V and 1.2 V (*versus* Ag/AgCl) in 0.1 M ACN/$NaClO_4$/$LiClO_4$ solvent/electrolyte system at a scan rate of 100 mV $s^{-1}$ on graphite (Fig. 1). After the polymerization, the surface of the electrode was rinsed with acetonitrile and then with distilled water to remove organic impurities.

### Optimization of biosensor fabrication

In this study, GOx is immobilized onto the –COOH functional conducting polymer using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) chemistry. The presence of the free carboxylic acid groups on the conducting polymer backbone can be utilized for the




**Scheme 2** Reagents and conditions: (i) thiophene, aluminum chloride, succinyl dichloride, DCM, 150 °C, 5h, 75%, (ii) glycine methyl ester, propionic acid, toluene, reflux, 16 h, 62%, (iii) KOH, methanol, $H_2O$, reflux, 3 h, 85%.

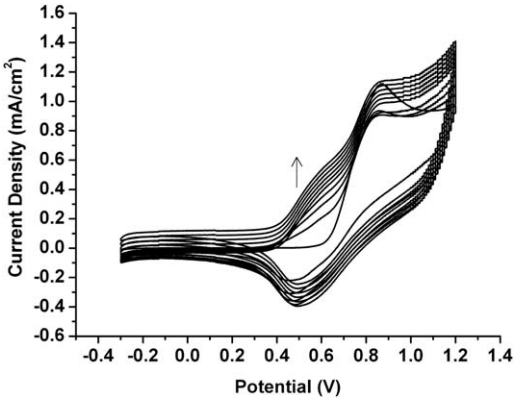



**Fig. 1** Repeated potential-scan electropolymerization of SNS-anchored carboxylic acid in 0.1 M ACN/$NaClO_4$/$LiClO_4$ solvent/electrolyte system at a scan rate of 100 mV $s^{-1}$ on graphite (up to 10 cycles).



covalent attachment of enzymes *via* formation of amide bonds. In this case, the free carboxylic acid groups on the surface are converted to reactive intermediates using the linking reagents EDC and NHS *via* carbodiimide activation.[31,32] By the help of conducting properties of the polymer, it was used as not only a substrate, suitable matrix for the enzyme immobilization but also as a redox mediator of the enzyme reaction.

The relationship between the response of the biosensor and the enzyme amount is exhibited in Fig. 2. Four different electrodes were prepared with 0.80 mg (17 Unit), 1.20 mg (25 Unit), 1.60 mg (34 Unit) and 3.20 mg (68 Unit) of GOx while the amount of the other components was kept constant. The highest signals were obtained with the biosensor prepared with 1.60 mg (34 Unit) GOx. To check the response of the nonfunctionalized electrode (with no conducting polymer (CP)), 1.60 mg (34 Unit) GOx was directly immobilized onto the graphite electrode and much lower responses were registered. For further experiments, that optimum amount of enzyme was used in the construction of the electrode.

The effect of the pH of the medium on the biosensor performance for 0.75 mM glucose was determined using buffer solutions with different pH values between 3.5 and 6.5 (sodium citrate buffer at pH 3.5, sodium acetate buffer at pH 4.0; 4.5; 5.0; 5.5 and sodium phosphate buffer at pH 6.0; 6.5, 25 °C). The biosensor shows a maximum response at a wide range of pH. The optimum pH lies between pH 4.5 and 5.5. Therefore, since the response at pH 5.5 was slightly higher, in all of the consecutive experiments, this was used as the optimum pH.

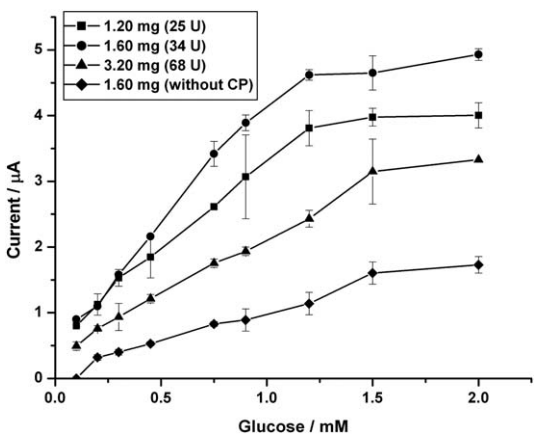



**Fig. 2** Effect of loaded enzyme amount (in sodium acetate buffer, 50 mM, pH 5.5; 25 °C). Error bars show standard deviation (SD) of three measurements for each.

## Surface characterization of designed biosensor

Surface morphologies before and after biomolecule immobilization were examined by scanning electron microscopy (SEM).

Fig. 3a shows the SEM image of the conducting polymer grown on the graphite electrode. In contrast, the surface morphology of the enzyme modified polymer film (Fig. 3b) depicts a rough and nonuniform coating on the surface. This clearly shows that the enzyme is well-immobilized onto the polymer film. On account of the size of the enzyme molecule and the morphology of the polymer film, the penetration of the enzyme into the polymer film seems infeasible and that is why the enzyme molecules were entirely bound at the outer part. This increases the possibility of access of the substrate to the biorecognition part.

In order to gain information on the changes of hydrophilicity of the surfaces before and after conjugation with the enzyme, contact angle measurements were performed. A more hydrophobic nature of the biomolecule conjugated surface due to the presence of different hydrophobic amino acids[34] is evident through an increase in advancing angle from 76.27° (±0.40) for the initial –COOH functional polymer surface to 101.2° (±0.61) for GOx immobilized polymer.

Correspondingly, due to the defined location of the enzyme, the response time of the enzyme electrodes is controlled by the thickness of the polymer layer. The increase in the thickness of the polymer layer makes the diffusion distance longer for the electroactive groups causing lower charge transfer rates. In order to examine the relationship between biosensor response and polymer layer thickness, electrodes were prepared with different electropolymerization cycles, and corresponding biosensor responses were recorded (Fig. 4). The sensitivity and performance of the biosensor are influenced by the polymer film thickness. The decrease in responses could be related to the thickness after 10 scans of electropolymerization. The polymer film obtained after 10 scans may not be thick enough to stabilize

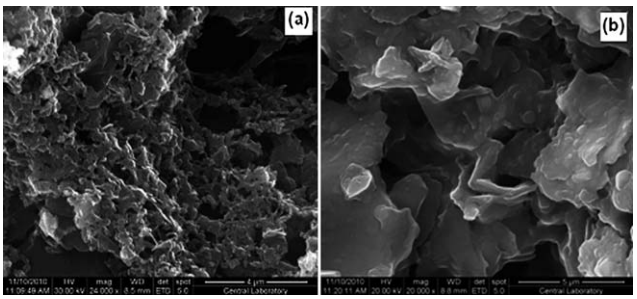



**Fig. 3** SEM images of polymer before (a) and after biomolecule immobilization (b) under optimized conditions. Magnification (a) 24000; (b) 20000.

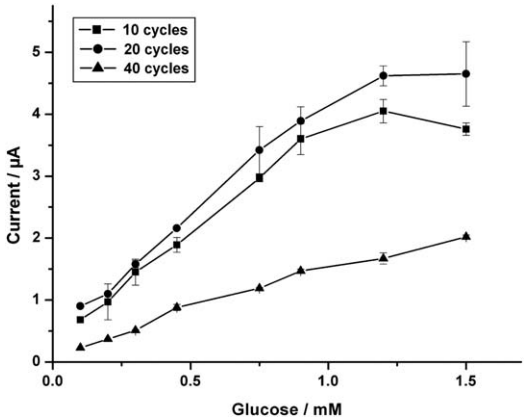



**Fig. 4** Effect of polymer layer thickness (in sodium acetate buffer, 50 mM, pH 5.5; 25 °C). Error bars show standard deviation (SD) of three measurements for each.

a high amount of GOx. Moreover, lack of enough functional groups of the conducting polymer on the bare graphite electrode may cause leaching of the enzyme from the surface. This leads to lower responses. According to the results, the electrode prepared with a 20 cycle electropolymerization process gave the highest biosensor response, hence this is chosen as the optimum polymerization cycle.

X-ray photoelectron spectroscopy (XPS) experiments were carried out to characterize the modified layers. XPS was used to understand the interactions between the carboxyl groups on the polymer chain and the functional amine groups on the enzyme and to investigate the formation of amide bonds. The carbon and nitrogen signals were resolved by a fitting program as depicted in Fig. 5a-d. Specific chemical bonds of the protein or those formed during the protein immobilization were readily detected through XPS analysis. The protein-immobilized surface (Fig. 5b) exhibited two signals at 287.0 eV and 288.1 eV (C═O and (C═O)–N respectively), indicating the presence of an amide bond as expected, in addition to signals representing aromatic bonds (Cα, Cβ of pyrrole ring and C–S at 283.8 eV and 284.9 eV, respectively), C–N, C═N (286.1 eV) groups and characteristic carboxyl group (290.6 eV). No such signal for an amide bond was detected on the untreated polymer coated electrode (Fig. 5a) confirming the successful deposition of GOx molecules on the surface of the SNS-anchored carboxylic acid polymer.

Information regarding the successful attachment of GOx to the polymer surface can also be obtained from the N1s peak. The nitrogen envelope can be fitted into four peak components in addition to the previously obtained spectrum representing only the polymer (Fig. 5c). The peak at 401.2 eV is attributed to the amide nitrogens (Fig. 5d).

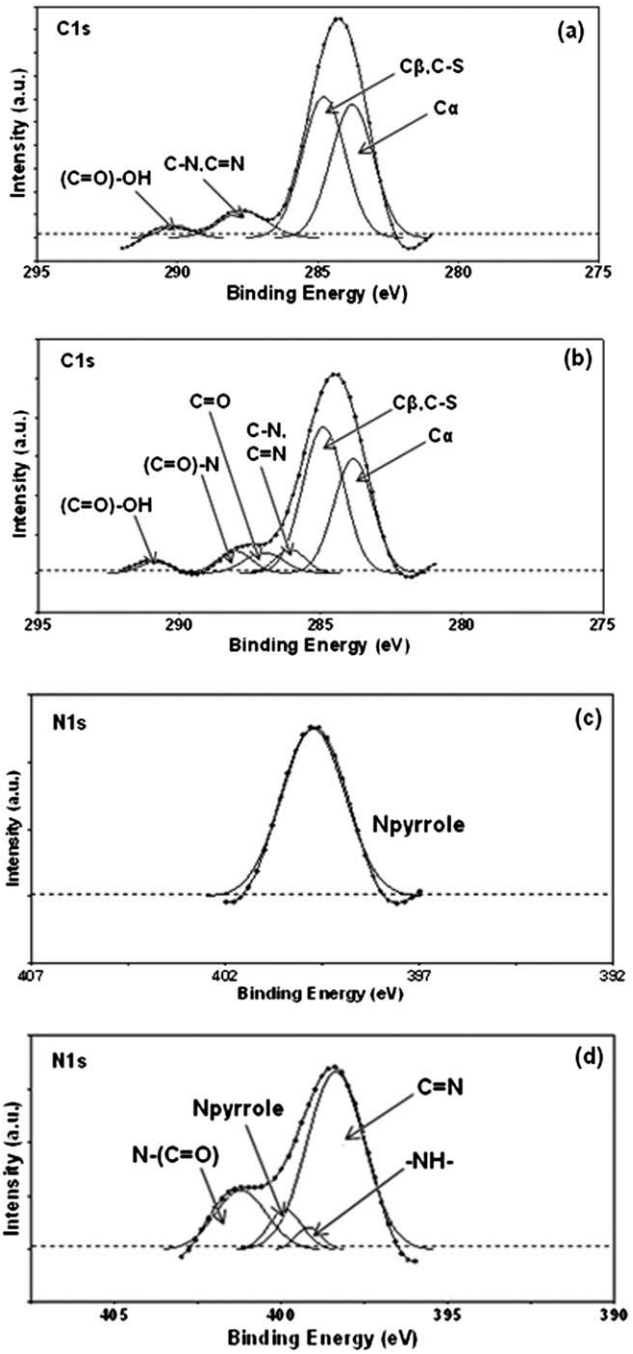



**Fig. 5** C1s and N1s XPS spectra of the polymer deposited surface (a and c) and protein immobilized onto the polymer deposited surface (b and d).

## Biosensing performance

Under the optimized conditions, the analytical characteristics of the fabricated biosensor were explored. The calibration curve for glucose is shown in Fig. 6. A perfect linearity was obtained for 0.01 mM–1.2 mM glucose.

A typical biosensor response was depicted in Fig. 7. The biosensor has a fast and sensitive response to glucose and it reaches a steady-state current after 13 s of glucose addition. Moreover, the limit of detection (LOD) of the fabricated biosensor was calculated as 0.004 mM based on the signal to noise ratio of 3 ($S/N = 3$).

The low Michaelis–Menten constant ($K_m$), as calculated from the Lineweaver–Burk plot, reveals a high affinity of enzyme to its substrate.[35] The other analytical parameters, $K_m^{app}$ and $I_{max}$ values are summarized in Table 1. The apparent $K_m$ is much lower than those reported for the silica gel/MWNT/PAN/GOx (13.9 mM),[36] CHIT/GOx@AgTNPs/Pt (2.35 mM)[37] and ZnO/PVP NF (nanofiber) electrodes (2.19 mM).[38]

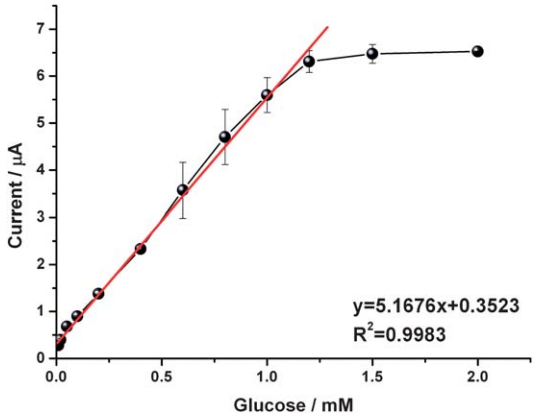



**Fig. 6** Calibration curve for glucose (in 50 mM sodium acetate buffer, pH 5.5; 25 °C; −0.7 V). Error bars show standard deviation (SD) of three measurements for each.

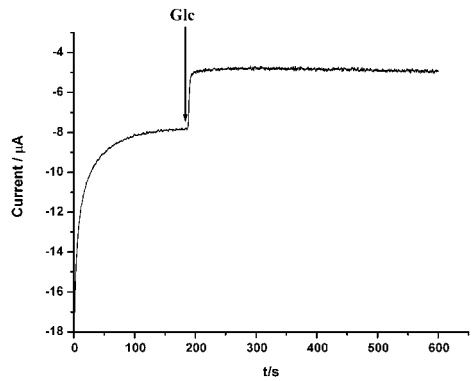



**Fig. 7** A typical biosensor response to glucose (in 50 mM sodium acetate buffer, pH 5.5; 25 °C; −0.7 V, [Glc]; 0.75 mM).

**Table 1** Some characteristics of the proposed biosensor (in sodium acetate buffer 50 mM, pH 5.5; 25 °C, −0.7 V)

| Parameter | |
|---|---|
| $K_m^{app}$ | 1.17 mM |
| $I_{max}$ | 11.28 μA |
| Linear range | 0.01–1.2 mM |
| Sensitivity | 5.59 mM $\mu A^{-1}$ |
| LOD[a] | 0.004 mM |
| Response time | 13 s |
| Operational stability | No decrease for 40 repetitive use |
| Shelf life | No decrease during 30 days |

[a] LOD was calculated according to $S/N = 3$.

On the other hand, the operational stability of the biosensor was analyzed for 0.4 mM glucose with 40 repetitive measurements under optimized conditions and no activity loss was observed. During operation, robust covalent bonding of the enzyme prevents leaching from the electrode surface.

In addition, long-term stability was examined by analyzing the glucose concentration over a month. Measurements were performed everyday over a course of 30 days. The biosensor was stored at 4 °C in contact with sodium acetate buffer (pH 5.5) in between successive measurements. The biosensor maintained its activity and 95% of its initial current response. These results confirm that the enzyme is in a compatible microenvironment.

Moreover, the effect of some possible interfering substances (yeast extract, ascorbic acid, urea, cholesterol between 0.01 M and 1.0 M) was studied. No interfering effects were observed for all concentrations with the addition of yeast extract (between 10 and 1000 μL (5 g $L^{-1}$)), ascorbic acid (1.0 M), urea (1.0 M), cholesterol (1.0 M) during the amperometric measurement of 0.4 mM glucose at the potential used.

### Sample application

Finally, in order to test the biosensor's accuracy and dependability in practical applications, we monitored the concentration of the glucose in fermentation medium. To do this, 1.0 mL samples of cultivation media were taken from the *G. oxydans* cultivation medium hourly for 5 h and suitable amounts of the samples were directly injected through the measurement cell to analyze the glucose concentrations. *G. oxydans* cultivation was carried out according to a previously described procedure.[39] Additionally, HPLC (High Performance Liquid Chromatography) was used to determine the glucose concentrations for the same samples, as a reference method. Both data are shown in Table 2. As depicted in the table, the data agree well with the reference data obtained from HPLC.

## Experimental

### Materials

Glucose oxidase (GOx, β-D-glucose: oxygen 1-oxidoreductase, EC 1.1.3.4, 21 200 Units/g) from *A. Niger* and D-glucose were purchased from Sigma (St. Louis, USA). Acetonitrile, hydrochloric acid, sodium hydroxide were purchased from Merck (Darmstadt, Germany). *N*-Hydroxysuccinimide (NHS) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) were purchased from Fluka (Buchs, Switzerland) and Sigma, respectively. All chemicals used in the synthesis of the monomer (glycine methyl ester hydrochloride, succinyl dichloride, dichloromethane (DCM)) were purchased from Sigma except thiophene which was purchased from Acros Organics (Geel, Belgium). All chemicals used for the electropolymerization were purchased from Aldrich. *G. oxydans* DSMZ 2343 was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). All other chemicals were analytical grade.

**Table 2** Biomonitoring of time dependent glucose consumption in *Gluconobacter oxydans* culture medium

| Sample No. | Given by HPLC (mM) | Measured by biosensor[a] (mM) |
|---|---|---|
| 1 | 0.39 | 0.36 |
| 2 | 0.30 | 0.32 |
| 3 | 0.22 | 0.26 |
| 4 | 0.15 | 0.15 |
| 5 | 0.09 | 0.12 |

[a] Biosensor measurements were performed in sodium acetate buffer (50 mM, pH 5.5, at 25 °C and −0.7 V).

### Apparatus

For the amperometric and cyclic voltammetric measurements, Palm Instrument (PalmSens, Houten, The Netherlands, www.palmsens.com) with a conventional three electrode configuration was used. A graphite electrode (Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 mm diameter and 13% porosity) served as the working electrode, Ag/AgCl (3.0 M KCl saturated with AgCl as an internal solution) and a Pt electrode (Metrohm, Switzerland) were used as reference and counter electrodes, respectively. The electrodes were placed in an electrochemical cell with internal volume of 10 mL. All the measurements were performed at room temperature.

$^{1}$H NMR and $^{13}$C NMR spectra were recorded in $CDCl_3$ on Bruker Spectrospin Avance DPX-400 spectrometer and chemical shifts ($\delta$) were given relative to tetramethylsilane. The mass spectra were recorded on a Thermo Scientific DSQ II Single Quadrupole GC/MS. A Waters Synapt MS System HRMS (High Resolution Mass Spectrometer) was used to confirm the synthesized materials. JEOL JSM-6400 models SEM (Scanning Electron Microscope) was used for surface imaging. XPS (X-ray Photoelectron Spectroscopy) was carried out on a PHI 5000 Versa Probe (Φ ULVAC-PHI, Inc., Japan/USA) model X-ray photoelectron spectrometer instrument with monochromatized Al Kα radiation (1486.6 eV) as an X-ray anode at 24.9 W. Contact angle measurements of a drop of water (2.0 μL) on the polymer surfaces were carried out using the sessile drop method with a CAM 100 KSV (KSV, Finland). Recording the drop profile with a CCD camera allowed to monitor the changes in contact angle. All reported data were given as the average of five measurements ± SD. The experiments were conducted at ambient temperature (25 °C). HPLC (High Performance Liquid Chromatography) with a refractive index detector (RID) controlled by a HP-Chemstation from Agilent (Karlsruhe, Germany) was used as the reference method for independent analysis of the glucose content.

### Covalent immobilization of glucose oxidase (GOx) onto the polymer coated electrode

A graphite electrode coated with the conducting polymer layer was used to immobilize GOx. Suitable amounts of GOx solution (1.6 mg in 3.0 μL, 50 mM sodium phosphate buffer (pH 7.0)), 0.4 M EDC and 0.1 M NHS solutions (3.0 μL for each in 50 mM sodium phosphate buffer (pH 7.0)) were prepared. All three solutions were mixed homogeneously and spread over the polymer coated electrode surface and incubated for three hours at room temperature. The electrodes were kept in a refrigerator overnight. By this step, GOx was immobilized onto the surface *via* amide bonds between the carboxylic acid groups of the conducting polymer and the amine groups of the enzyme. The enzyme electrodes were then rinsed with distilled water to remove unbound enzyme. The solutions were prepared freshly prior to use.

Amperometric determination of glucose was performed at ambient conditions by applying a constant potential at −0.7 V (*versus* Ag/AgCl) and following the oxygen consumption as a result of enzymatic activity in the bioactive surface. When the background current reached a steady state, glucose solution was added with stirring to the electrochemical cell containing working buffer (10 mL) and the steady-state current values were recorded. After each run, buffer solution was refreshed and electrodes were washed with distilled water. The current signal obtained with respect to various standard glucose concentrations were plotted as a calibration curve and glucose concentrations in *G. oxydans* cultures were calculated using the calibration curve.

## Conclusions

The electrochemically polymerized SNS-anchored carboxylic acid performs well as an immobilization matrix for the construction of a glucose oxidase enzyme biosensor. The amperometric biosensor based on conducting polymer was fabricated through the covalent immobilization of the enzyme on a functionalized conducting polymer; poly-SNS-anchored carboxylic acid layer. The presence of the protein and amide bond between the surface and the protein was confirmed by SEM and XPS studies. The fabricated biosensor exhibited excellent kinetic parameters such as $K_m$, $I_{max}$, low LOD and high stability. Moreover, it was successfully applied to *G. oxydans* cultures for the detection of glucose content.

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