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PAPER

Functionalization of poly-SNS-anchored carboxylic acid with Lys and PAMAM: surface modifications for biomolecule immobilization/stabilization and bio-sensing applications

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Poly(2-(2,5-di(thiophen-2-yl)-1*H*-pyrrol-1-yl) (SNS) acetic acid) was electrochemically deposited on graphite electrodes and functionalized with lysine (Lys) amino acid and poly(amidoamine) derivatives (PAMAM G2 and PAMAM G4) to investigate their matrix properties for biosensor applications. Glucose oxidase (GOx) was immobilized onto the modified surface as the model enzyme. X-Ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) were used to report the surface properties of the matrices in each step of the biosensor construction. The biosensors were characterized in terms of their operational and storage stabilities and the kinetic parameters (K_m^{app} and I_{max}). Three new glucose biosensors revealed good stability, featuring low detection limits (19.0 μM, 3.47 μM and 2.93 μM for lysine-, PAMAM G2- and PAMAM G4-functionalized electrodes, respectively) and prolonged the shelf lives (4, 5, and 6 weeks for Lys-, PAMAM G2- and PAMAM G4-modified electrodes, respectively). The proposed biosensors were tested for glucose detection on real human blood serum samples.

Introduction

Conducting polymers are exciting materials having delocalized electronic structures which support the conductivity along the conjugated organic backbone.[1] The conducting and semiconducting properties of these polymers provide wide range of electronic, optoelectronic and biotechnological applications.[2–5] The use of conducting polymers as appropriate immobilization matrices for biomolecules leads to the improvement of biosensors as faster and more economical tools for clinical and pharmaceutical analyses.

In that manner, electrically conducting polymers can be deposited on an electrode surface to enhance stability, sensitivity and efficient electron transfer ability for the biosensors.[6–9] Also, electropolymerization enables easy control over several properties such as morphology and thickness. Furthermore, their chemical functionalizations offer a better microenvironment for biomolecules and electrochemical transduction of biological events.

The isolation of the enzymes from their natural microenvironment results in a decrease in their operational stability and sensitivity. Due to these limitations, immobilization of enzymes is the most noteworthy step to be able to use them in the most efficient way. For this reason, there is always an urgent need for new immobilization matrices and this brings up the quest for enhancement of the immobilization of biomolecules. The functionalization of the immobilization matrix plays a significant role, since the placement and orientation of the bioreceptor molecules (enzymes, antibodies, DNA, *etc.*) affect the overall performance of a biosensor. Ideally, a biosensor matrix should be a well-organized surface that provides a high ligand density having stable attachment of biomolecules throughout the immobilization step, biocompatibility and an easy accessibility. In this regard, two-dimensional matrices and three-dimensional supramolecular architectures have been developed to define the optimum sensor platforms in the past decades.[10]

Dendrimers are highly branched and nanospherical macromolecules with their three-dimensional structures.[11,12] They have attracted great interest in many research areas such as diagnostics, chemical and biochemical sensing, coating and nanotechnology improvements.[13–17] In particular, poly(amidoamine)

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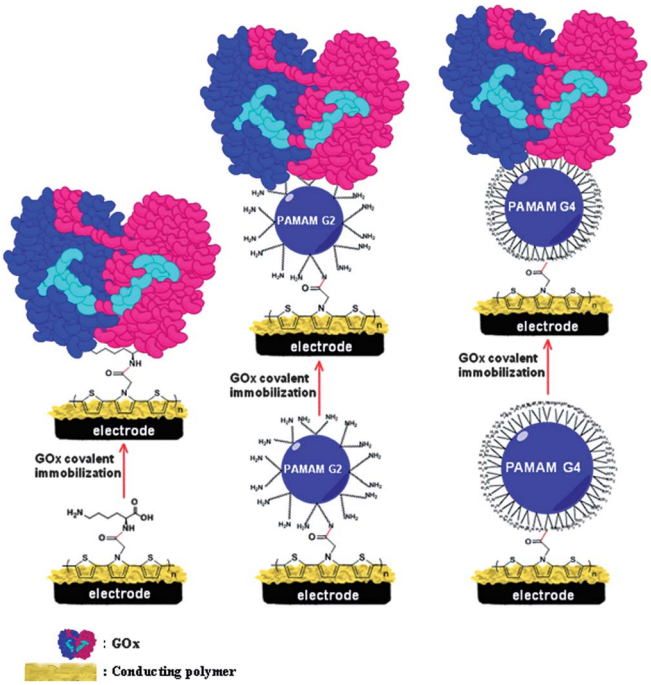
(PAMAM) dendrimers contain a number of terminal amino groups which are promising moieties for the attachment of biomolecules such as antibodies, DNA and enzymes.[18–20] Moreover, PAMAM dendrimers can also be used to modify an electrode surface owing to their good biocompatibility and functional groups for chemical attachment.[21–23] As the density of the terminal amino groups on the surface increases, the generation of PAMAM dendrimer grows and this enables the attachment of biomolecules (enzymes, antibodies and nucleic acids) quite easily.[24,25] Due to these advantages, dendrimers are effectively used in biosensor applications.[26–29]

In order to enhance the stability, enzyme molecules can be assembled to dendrimers. PAMAM G2 and G4 moieties possess 16 and 64 primary amine groups on their surface, respectively. These multiple surface groups of hyperbranched polymeric materials enable the multiple interactions and conjugations with the biomolecules.[30] This promotes the stability of the enzyme. Hence, the shelf life of the biosensor increases. Furthermore, amino acids are rarely used in surface modifications.[31,32] Bearing different functional groups, amino acids are appropriate for modification of the desired surfaces. Lysine (Lys) bears two amino groups in its structure which have the potential to be utilized in covalent attachments. Therefore, Lys was chosen to enhance the immobilization of the enzyme onto the carboxylic acid group-functionalized conducting polymer.

Glucose oxidase (GOx) is widely used as a model enzyme in analytical chemistry, biochemistry, food and clinical chemistry.[33–35] With the help of molecular oxygen GOx catalyzes the oxidation of β-D-glucose to δ-gluconolactone which is subsequently hydrolyzed into gluconic acid.[36] The chemical resistance to acid media and denaturing agents such as urea and sodium dodecyl sulfate and commercial availability at a low price allow this enzyme to be used in many applications as a glucose sensor in clinical analysis, food and pharmaceutical industries.[37] Considering its advantages, glucose oxidase was used as the model enzyme.

In this study, poly(2-(2,5-di(thiophen-2-yl)-1*H*-pyrrol-1-yl) acetic acid) (SNS-acetic acid) was electrochemically deposited onto a graphite electrode *via* cyclic voltammetry using a scan rate of 0.1 V s^{-1}. It was reported that the poly-SNS-anchored carboxylic acid structure was a convenient matrix with the carboxylic acid moiety for glucose sensing in our previous work.[38] Herein, we aimed to functionalize the polymer surface using lysine, PAMAM G2 and G4 to get more reactive spots to attach GOx enzymes for more efficient glucose sensing. These three kinds of modifications can lead to many functionalizations and make a great contribution to the literature as regards to surface modification of the conducting polymers. For this purpose, amino acid and PAMAM derivatives were covalently attached to the carboxylic acid groups of the conducting polymer using EDC (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride) and NHS (*N*-hydroxysuccinimide) coupling reagents.[39,40] GOx was immobilized on the modified electrode surfaces using glutaraldehyde (GA) as the cross-linker. The proposed systems were tested by amperometric detection technique. In these amperometric studies, the decrease in oxygen level as a result of enzymatic reaction is monitored at −0.7 V *versus* Ag/AgCl and correlated with the substrate concentration. Scheme 1 depicts the surface modifications. The biosensors were used for glucose detection in human blood serum samples and their accuracies were tested.



Scheme 1 Schematic representation of the surface modifications in enzyme immobilizations.

Results and discussion

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

2-(2,5-Di(thiophen-2-yl)-1*H*-pyrrol-1-yl) acetic acid was synthesized as reported in the literature.[35] Methyl 2-(2,5-di(thiophen-2-yl)-1*H*-pyrrol-1-yl)acetate (0.7 mmol, 212.38 mg) was dissolved in 15 mL methanol at room temperature. After the addition of KOH (1.9 mmol, 106.6 mg) to the mixture, the reaction was allowed to proceed under reflux conditions until all esters were consumed. The crude product was concentrated and extracted with water and diethyl ether. 6.0 N HCl was added to the aqueous phase to acidify it to pH 1.0 and then extracted with diethyl ether. The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure (0.171 g, 85%).

Preparation of poly(2-(2,5-di(thiophen-2-yl)-1*H*-pyrrol-1-yl) acetic acid) substrates

Prior to electrochemical polymerization, spectroscopic grade graphite rods were polished on emery paper and washed thoroughly with distilled water. Cyclic voltammograms were obtained with a three electrode configuration. Electrochemical polymerization of monomer was potentiodynamically carried out between the potential range −0.3 V and 1.2 V in 0.1 M ACN/$NaClO_4$/$LiClO_4$ medium at a scan rate of 0.1 V s^{-1} on the graphite electrode (Fig. 1).

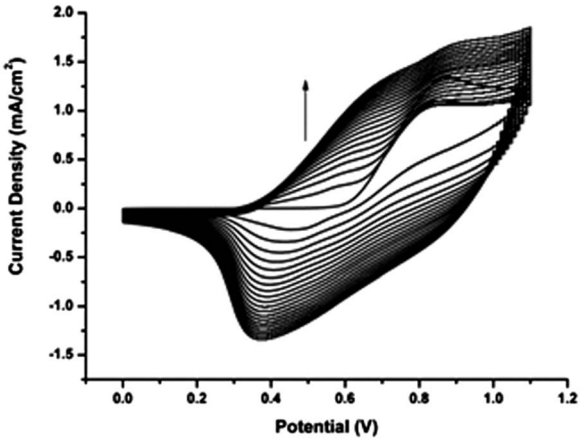


Fig. 1 Repeated potential-scan electropolymerization of SNS-anchored carboxylic acid in 0.1 M ACN/$NaClO_4$/$LiClO_4$ solvent/electrolyte system at 0.1 V s^{-1} on graphite electrode (up to 20 cycles).

Construction of biosensors

In present study, three novel glucose biosensors were developed *via* surface modification of the conducting polymer with Lys, PAMAM G2 and PAMAM G4. To prepare the Lys-modified biosensor, the polymer-coated graphite electrode was immersed in 1.5 mL EDC : NHS (4 : 1) in 50 mM sodium phosphate buffer (pH 7.0) for 3 h. After washing with distilled water, the electrode was put into 1.5 mL Lys solution (4.5 mg in 50 mM sodium phosphate buffer, pH 7.0) for 3 h. During this procedure, amide bond formation was achieved between the carboxylic acid groups of the polymer and the amine groups of Lys. For the immobilization of enzyme, a proper amount of GOx solution (2.5 mg in 5.0 μL, 50 mM sodium phosphate buffer, pH 7.0) and glutaraldehyde (5.0 μL, 1.0% in 50 mM sodium phosphate buffer, pH 7.0) were spread over the modified electrodes and treated for 2 h at ambient conditions prior to use.

The PAMAM dendrimer-modified electrodes were prepared as follows: in this process, the polymer-coated graphite electrode was immersed in EDC : NHS (4 : 1) solution (5.0 μL) in 50 mM sodium phosphate buffer, pH 7.0 for 30 min. Then, the electrode was left in PAMAM solution (1.0% PAMAM in 50 mM sodium phosphate buffer, pH 7.0) for 1 h. Afterwards, the electrode was placed in $NaBH_4$ solution (5.0 mM) to reduce the Schiff base formation.[41] GOx (1.0 mg in 2.5 μL 50 mM sodium phosphate buffer, pH 7.0) and GA (2.5 μL, 1.0% in 50 mM sodium phosphate buffer, pH 7.0) solutions were spread over the modified electrode and it was allowed to stand at room temperature for 2 h. After immobilization, the non-bound enzyme molecules were removed by rinsing the electrode surface with phosphate buffer solution and distilled water.

Amperometric measurements

Amperometric studies were carried out in sodium acetate buffer *via* application of −0.7 V *versus* Ag/AgCl electrode while mildly stirring at room temperature. In all experiments, current change due to the consumption of the molecular oxygen was detected; therefore, the difference between the baseline current and the steady state current after the addition of substrate was monitored. All measurements were performed by addition of glucose to the buffer solution (10 mL) in an electrochemical cell at steady state. After each measurement, the buffer solution in the cell was refreshed and the electrodes were washed with distilled water. The kinetic parameters were determined by testing the biosensors on various glucose concentrations; on the other hand, the same amounts of substrate were used for each biosensor.

Optimization studies of designed biosensors

The thickness of the conducting polymer affects both the quality of the film and the biosensor response. There should be adequate thickness of polymer on the bare electrode to functionalize with amino acid, dendrimers and biomolecule. The increase in the thickness of the polymer film may hinder the electron transfer resulting in a longer response time. When the film is thin on an electrode surface, there exists a larger interaction area with the analyte molecules. By contrast, the thin film refers to the small amount of functional groups for the covalent attachment which may cause little attachment of the biomolecule revealing a low response. Hence, to determine the optimum film thickness, polymer was electrodeposited on the electrode surface in different scan numbers varying between 10 and 40 cycles. Then, the certain amounts of amino acid and dendrimers were covalently attached to the polymer surface. GOx and GA were immobilized on the surface in certain amounts for each biosensor. According to the results, 20 cycles of polymer production was determined as the optimum thickness for the Lys- and PAMAM G4-modified electrodes. On the other hand a 10 scan polymerization was found to be the optimum for the PAMAM G2-modified biosensor (Fig. 2). The charge and film thickness in the optimum conditions were also calculated as 60 mC (1.33 μm) and 120 mC (2.67 μm) for 10 and 20 cycles of polymer, respectively.

The optimum Lys, PAMAM G2 and PAMAM G4 amounts were determined using the optimum thickness of the polymer, and a certain amount of enzyme (GOx) and GA. In accordance

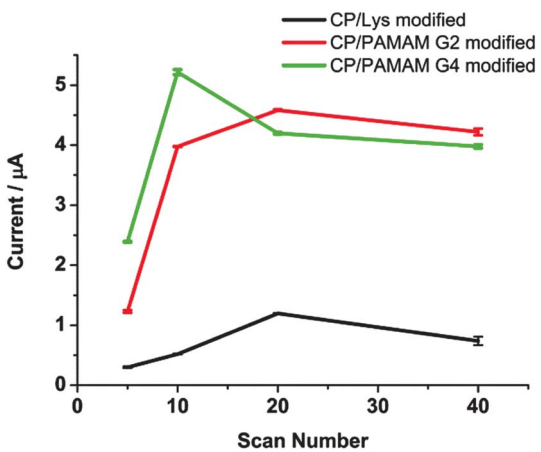


Fig. 2 The effect of conducting polymer (CP) film thickness on biosensor response (in 50 mM sodium acetate buffer, pH 5.5, 25 °C). The corresponding measurements were performed with 0.75 mM glucose. Error bars show the standard deviation (SD) of three measurements.

with the responses, 2.5 mg Lys in 5.0 μL 50 mM phosphate buffer, pH 7.0, 2.5 μL 0.5% PAMAM G2 and 2.5 μL 0.5% PAMAM G4 amounts were determined as the optimum results.

The amount of immobilized enzyme directly affects the sensitivity of the biosensor. The effect of enzyme loading on the responses was determined with the different electrodes. Different amounts of enzyme between 0.5 mg (10.40 Units) and 6.0 mg (125 Units) were immobilized on the modified electrode surfaces coated with the optimum amounts of polymer, amino acid and the dendrimer. The enzyme was stabilized using GA (1.0%). The highest signals were obtained with the biosensors prepared with 4.5 mg (93.75 Units) GOx for Lys-modified and 1.0 mg (20.80 Units) GOx for PAMAM G2 and G4-modified biosensors. Glutaraldehyde chemistry is a versatile technique as regards the stabilization of enzymes on electrode surfaces.[42,43] GA enables cross-linking between the amine groups of Lys, dendrimers and the amine groups of enzyme molecules. In this study, different amounts (0.01%, 0.05%, 1.0%, 2.5%) of GA were used in the preparation of each electrode. A 1.0% GA amount was determined as the optimum GA for each biosensor. Since the bioactivity of the enzyme depends on the pH of the solution, and extreme pH conditions cause enzyme denaturation, the optimum pH values should be determined. The experiments were performed with the prepared optimum enzyme electrodes and the effect of pH on the responses is given in Fig. 3. The biosensors showed the maximum response at 50 mM pH 5.5 sodium acetate buffer.

Surface characterization

The characterization of the modified layers was performed *via* X-ray photoelectron spectroscopy (XPS). It is possible to determine the amide bond formation between carboxylic acid groups of the polymer and amine groups of Lys, PAMAM G2 and G4 molecules. X-Ray photoelectron spectra of the polymer-, Lys-, PAMAM G2- and PAMAM G4-modified electrodes and enzyme-coated surfaces are indicated in Fig. 4. The C1s and N1s spectra were well fitted by a fitting program. The Lys immobilized surface (Fig. 4c) exhibited a signal at 287.841 eV (–N–(C═O)) which was assigned to the presence of an amide bond between the polymer and Lys molecules, as expected. In addition to the C1s data, there

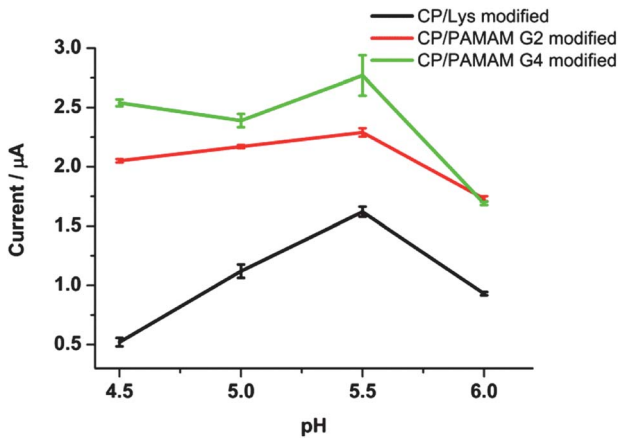


Fig. 3 The effect of pH on GOx immobilized electrodes, 25 °C, –0.7 V. The measurements were performed with 0.40 mM glucose. Error bars show the standard deviation (SD) of three measurements.

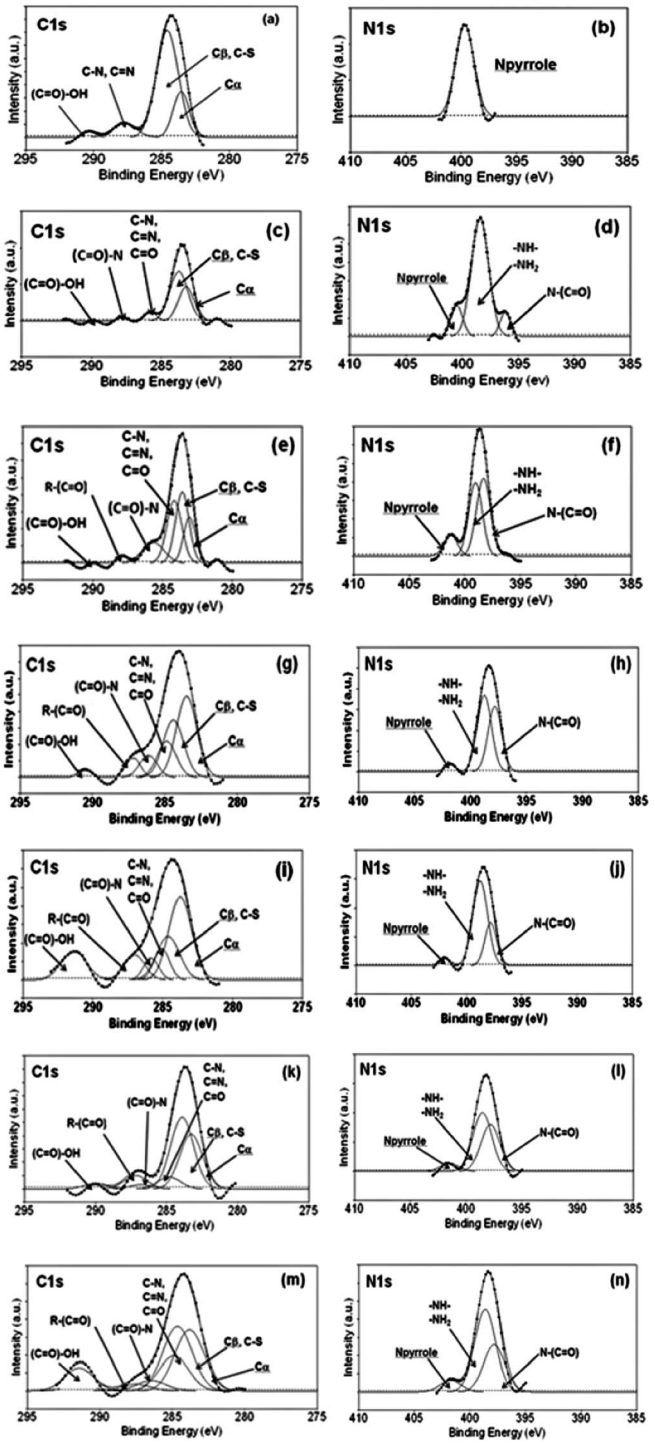


Fig. 4 C1s and N1s XPS spectra of polymer-coated electrode (a,b), Lys, PAMAM G2, PAMAM G4-modified polymer-coated surfaces (c,d; g,h; k,l) and GOx immobilized surfaces on polymer-coated modified surfaces (e,f; i,j; m,n), respectively.

are two new signals representing $–NH_2$ (398.433 eV) and –N–(C═O)– (400.499 eV) in the N1s spectrum of the Lys-modified surface (Fig. 4d).[44] The increase in the intensity of –C═N–, –C═O (284.167 eV), and (C═O)–N (285.675 eV) peaks in the C1s spectrum (Fig. 4e) and the –N–(C═O)– (398.35 eV) peak in the N1s spectrum (Fig. 4f) were observed due to protein

immobilization on the surface. Fig. 4g–j represent the C1s and N1s spectra of the PAMAM G2-modified polymer-coated electrode and the protein immobilized surfaces on these layers, respectively. As illustrated in these spectra, the signals of $-NH_2$ (398.67 eV) and –N–(C═O) (397.67 eV) in N1s (Fig. 4h) show the increase in their intensity depending on the increasing terminal amine groups of PAMAM G2 dendrimers on the surface.[45] These expected results prove that the attachment of PAMAM G2 dendrimer was successfully carried out. The protein immobilized surface exhibited a characteristic signal at 291.36 eV representing the (C═O)–OH group of GOx enzymes on the modified surface (Fig. 4i). Similar results for the modification of the polymer-coated electrode with PAMAM G4 and the immobilization of the biomolecules were reported in Fig. 4k–n.

Surface morphology

AFM supplies the morphological information and it was used in intermittent mode. Fig. 5 shows the characteristic AFM images of the surface topography of the modified electrodes at various deposition steps of Lys, PAMAM G2 and G4, respectively. In the vertical direction, darker areas represent the deeper side, contrary to lighter ones. The Lys-modified surface is fairly smooth (Fig. 5a). On the other hand, the GOx immobilized surface is rough and non-uniform (Fig. 5b). Depending on the smaller molecular dimension of Lys, the size of the surface features is relatively smaller on the Lys-modified one compared to PAMAM dendrimer surfaces. Surface roughness reached the maximum value when the GOx was immobilized on the PAMAM G4 layer; this is because the PAMAM G4 matrix is more indented than those of the PAMAM G2 and Lys matrices. Surface roughness (RMS) values were obtained as 0.8 nm and 0.5 nm for the Lys-modified surface. For PAMAM G2-modified one, they were 1.9 nm and 0.8 nm. As for the PAMAM G4-modified surface the values were 2.0 nm and 1.0 nm before and after biomolecule immobilization, respectively. Similar observations were also found a previous work in which the enzyme was immobilized on the gold surface after layer by layer modification with PAMAM G4 structure.[26] It is obvious that the immobilization of the enzyme molecules brings the homogeneity of the formed structure on the surface.

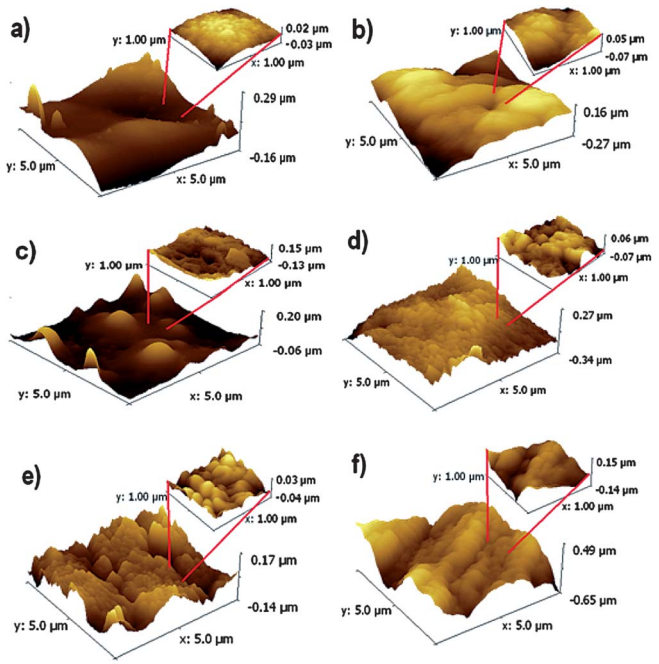


Fig. 5 3-D topographic AFM height images of Lys-, PAMAM G2-, PAMAM G4-modified polymer-coated electrode surface before (a,c,e) and after (b,d,f) biomolecule (GOx) immobilization at the optimized conditions, respectively.

Analytical characterization

The variation of current *versus* different glucose concentrations profile has been shown in Fig. 6. Linearity ranges of each biosensor were determined as 0.01–2.40 mM for the Lys-containing electrode and 0.02–1.20 mM for the PAMAM G2 and G4 ones with the correlation coefficients of 0.999, 0.997, and 0.997, respectively. Kinetic parameters of the optimized enzyme biosensors were found from the Lineweaver–Burk plot at constant temperature and pH 5.5 and are listed in Table 1 comparatively with literature results. Limit of detection (LOD) values for Lys-, PAMAM G2- and G4-containing biosensors were calculated as 19.0, 3.47 and 2.92 µM, respectively when the signal-to-noise characteristics of these data (S/N ratio) is 3. Some electroactive species such as ascorbic acid, urea, oxalic acid, paracetamol *etc.* may affect the biosensor response while working with real samples, especially in physiological fluids. The interference effects of these molecules were investigated between 0.001 M and 0.010 M. No interference effect was observed in all concentrations of ascorbic acid, urea, oxalic acid and paracetamol at the working potential.

Operational and storage stabilities

The operational stability of each optimized enzyme electrode was obtained by repetitive measurements of 0.4 mM glucose at constant temperature. After 35, 77, and 50 measurements with Lys-, PAMAM G2- and G4-modified electrodes, respectively, no

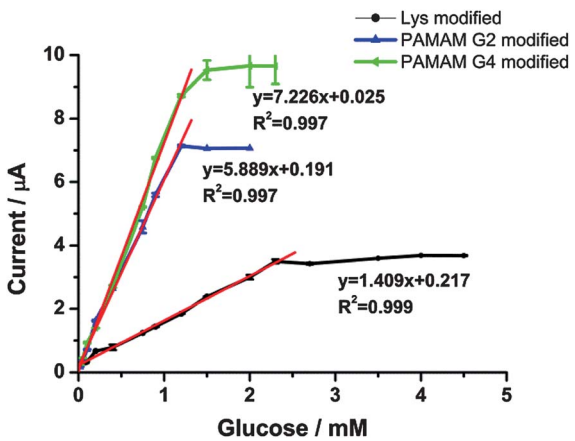


Fig. 6 Calibration curve for glucose (in 50 mM sodium acetate buffer, pH 5.5, 25 °C, –0.7 V). Error bars show standard deviation (SD) of three measurements.

Table 1 Comparison of biosensor examples for GOx (NR: not reported, TW: this work)

Surface modification	Immobilization tech.	K_m/I_{max}	Limit of detection	Shelf life	Ref.
Au/poly(*o*-aminophenol/carbon nanotubes)	Electropolymerization	22.8 mM	0.01 mM	30 days	46
Polypyrrole	Self-encapsulation	25.25 mM/625 nA	9.00 μM	NR	47
Poly[3-(3*N*,*N*-diethylaminopropoxy) thiophene]-SWCNT	Adsorption	3.80 mM	5.00 μM	NR	48
GOx/CNT/Teflon	Adsorption	30 mM	33.00 μM	1 day	49
PANI nanofibers and nanocomposite of Au NPs	Covalent binding	NR	0.500 μM	14 days	50
PAMAM G4	Covalent binding	1.67 mM/4.49 μA	11.84 μM	30 days	51
Poly-SNS-anchored carboxylic acid	Covalent binding	1.17 mM/11.28 μA	4.00 μM	30 days	38
Poly-SNS-anchored carboxylic acid/Lys	Covalent binding	2.25 mM/5.93 μA	19.00 μM	28 days	TW
Poly-SNS-anchored carboxylic acid/ PAMAM G2	Covalent binding	1.19 mM/12.37 μA	3.47 μM	35 days	TW
Poly-SNS-anchored carboxylic acid/ PAMAM G4	Covalent binding	1.59 mM/17.75 μA	2.92 μM	42 days	TW

activity loss was observed during a 22 h operation time. A characteristic biosensor response was illustrated in Fig. 7. The fast response to the substrate can be seen in 5 seconds. It is a representative example of the biosensor responses; all three biosensors have such a quick response to glucose. The shelf life of each biosensor was examined by measuring the amperometric responses every day for 0.4 mM glucose under the optimized conditions. Between each subsequent measurement the biosensors were stored in contact with the working buffer at 4 °C. The shelf lives of the biosensors were determined as 4, 5 and 6 weeks (no activity loss). The aqueous compatibility of the improved immobilization matrices protects the enzyme from the environmental effects.

All analytical parameters, K_m^{app} and I_{max} values are summarized in Table 1. When the immobilization of GOx was achieved with the modifications using carbon nanotubes and gold nanoparticles, much higher K_m^{app} values were recorded.[43,46–48] This may well be not only because of the conducting polymer layer but also the surface modifications. Polymer/Lys or PAMAM matrices exhibit higher affinity toward the glucose substrate; hence, the modifications served our purposes perfectly. Moreover, the response time was also shortened by the help of surface orientation of the enzyme molecules.[38] When the results were compared with a PAMAM-modified glucose biosensor, better analytical parameters and LOD value were recorded.[51] Moreover; the modifications are easier than that of the PAMAM G4-containing biosensor which requires multi-step modification processes. On the other hand, results showed that the hyperbranched structure of PAMAM generations yields more enhanced stability and affinity towards the substrate than for the Lys modification. Furthermore, in the modifications with dendrimers, much smaller enzyme amounts were used in the preparations. This displays that the enzyme molecules were locked more precisely and well-oriented, and hence, the analytical parameters turned out to be better. The polymer/PAMAM G4-modified biosensor showed the best results among the three biosensors. Although it has a higher K_m value than the PAMAM G2-modified biosensor, with the higher number of primary amine groups, the enzyme can be attached more efficiently. Accordingly, the stability and electron transfer and thus the rate of formation of product were improved. Since K_m is the concentration of substrate which allows the enzyme to achieve half I_{max}, it is natural that with the crowded microenvironment around the enzyme molecules, the PAMAM G4-modified biosensor has a higher K_m than that of the G2-modified one to achieve higher I_{max}.

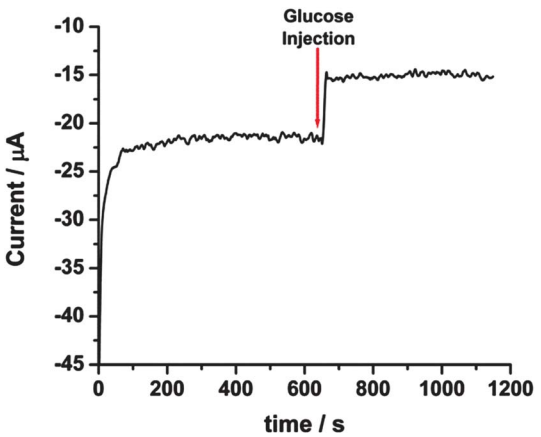


Fig. 7 A characteristic biosensor response of polymer/PAMAM G4-modified biosensor to glucose (in 50 mM sodium acetate buffer, pH 5.5, 25 °C, −0.7 V, [Glc]; 0.75 mM).

Sample application

The biosensors were tested for real human serum samples to determine the concentration of glucose in order to test biosensors' accuracies. All experiments were performed in compliance with relevant laws and ethical committee approved the experiments. These samples were first analyzed in a local hospital and the actual glucose levels were determined with a reference method. During the measurements, the certain amounts of blood serum samples were injected as the substrate in the buffer solution instead of the glucose solution. The experiments were performed at the optimized conditions and a constant temperature of 25 °C. The results were compared with the ones obtained in the hospital results in Table 2. The results confirm that the designed biosensors are suitable for use with real human blood samples.

Table 2 Comparison of the constructed biosensors for glucose analysis in the serum samples

Sample	Hospital data/mM	Lys-modified biosensor/mM	Relative error (%)	PAMAM G2-modified biosensor/mM	Relative error (%)	PAMAM G4-modified biosensor/mM	Relative error (%)
1	0.200	0.195	2.500	0.205	2.500	0.201	0.500
2	0.189	0.184	2.600	0.187	1.050	0.192	1.587
3	0.185	0.186	0.540	0.184	0.540	0.186	0.540
4	0.183	0.181	1.092	0.182	0.546	0.185	1.092
5	0.179	0.180	0.558	0.180	0.558	0.177	1.117
6	0.170	0.166	2.352	0.172	1.176	0.169	0.588

Experimental

Chemicals

Glucose oxidase (GOx, β-D-glucose:oxygen 1-oxidoreductase; EC 1.1.3.4, 21 200 units per g) from *Aspergillus niger*, D-glucose were purchased from Sigma (St. Louis, USA). PAMAM G4 (25% C_{12} dendrimer, 10 wt% solution in methanol), PAMAM G2 (25% C_{12} dendrimer, 20 wt% solution in methanol) and glutaraldehyde (GA; 50 wt% solution in water) were purchased from Sigma-Aldrich. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Sigma and Fluka (Buchs, Switzerland) respectively. Acetonitrile (ACN), hydrochloric acid, sodium hydroxide were purchased from Merck (Darmstadt, Germany). All chemicals used in the synthesis of the monomer (glycine methyl ester hydrochloride, succinyl dichloride, dichloromethane (DCM)) were purchased from Sigma except for thiophene which was purchased from Acros Organics (Geel, Belgium). All chemicals used for electropolymerizations were purchased from Aldrich. All other chemicals were analytical grade. Real human serum samples were obtained from the Middle East Technical University (METU) Medical Center from patients volunteered for that matter.

Apparatus

Amperometric and cyclic voltammetric measurements were carried out using a Palm Instrument (PalmSens, Houten, The Netherlands, www.palmsens.com) with a conventional three electrode configuration. As the working electrode (placed 1 cm from the reference electrode) graphite electrodes (Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 mm diameter and 13% porosity) were used. Ag/AgCl (3 M KCl saturated with AgCl) and a Pt electrode (Metrohm, Switzerland, www.metrohm.com) were used as reference and counter electrodes, respectively. During all electrochemical measurements, the electrodes were placed in an electrochemical cell with an internal volume of 10 mL. All measurements were carried out at room temperature. Veeco MultiMode V AS-12 ("E") model AFM (Atomic Force Microscope) was used for surface characterization. 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. The pressure inside the analyzer was maintained at 10^{-7} Pa. The binding energy scale was referenced by setting the C–H peak maximum in the C1s spectrum to 285.0 eV and the atomic composition estimated using Multipak software.

Conclusions

In this study, three new conducting polymer-based glucose biosensors were presented. The surface modifications of the polymer with Lys and PAMAM dendrimers provide excellent immobilization matrices for the glucose oxidase enzyme. The constructed enzyme electrodes have good K_m^{app}, I_{max} and very low LOD values with substantial shelf lives. Finally, the biosensor systems were successfully applied to human blood serum samples to detect the glucose concentration.

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