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PAPER

Potentiometric glucose biosensing using camphor sulfonic acid doped polyaniline

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In this work, we explored the possibility of developing an enzymatic potentiometric glucose biosensor using polyaniline–camphorsulfonic acid–glucose oxidase (PANI–CSA–GOD) composite films. Glucose oxidase was covalently immobilized on the polyaniline–camphorsulfonic acid modified electrode that was used as a potentiometric sensor matrix. The PANI–CSA–GOD transducer responded linearly to glucose molecules in the concentration range of 1 to 50 mM and the sensitivity was found to be 0.09 mV mM^{-1}. The modified electrode showed excellent sensitivity and better selectivity in the presence of some common interfering molecules.

1. Introduction

Diabetes mellitus, a metabolic disorder resulting from insulin deficiency that causes blood glucose concentrations higher or lower than the normal range of 80–120 mg dL^{-1} (4.4–6.6 mM). The difficulties of battling diabetes are numerous, including higher risks of heart disease, kidney failure and blindness. These complications can be greatly reduced through stringent personal control of blood glucose concentration that requires periodic and constant monitoring.[1] Right from Clark and Lyons'[2] concept of glucose enzyme electrodes, different routes have been suggested for developing reliable devices for blood glucose monitoring.

Electrochemical detection of glucose concentrations has become popular because of its affordable cost, fast response and patient friendliness. It can be achieved by two major methods namely, enzymatic[3–6] and non-enzymatic[7–10] methods. The enzymatic method involves the enzyme, glucose oxidase (GOD) that oxidizes glucose into gluconic acid and produces hydrogen peroxide as a byproduct. The amount of hydrogen peroxide produced is usually determined by amperometric oxidation at the working electrode. Since it is an unstable molecule, it is quantitatively measured by means of indirect mediated electrochemical determination. The non-enzymatic glucose sensor involves direct amperometric oxidation of glucose at an electrode surface and it requires a large overpotential.[11] Tremendous progress is reported in the development of electrochemical glucose biosensors for direct (mediator-less) electron transfer and enzyme catalysis by GOD.[12] However, these methodologies are more tedious and some of the designed sensors show inaccuracy and ambiguity in measurements.

In contrast, potentiometric detection is a simple approach of developing a glucose biosensor, leading to direct and reagent-less measurement. The enzyme activity is determined based on the measurement of pH changes in the enzymatic layer of the sensor matrix.[13] The advantages of PANI as an advanced potentiometric pH transducer that can be used in biosensors have been reported by Karyakin *et al.*[14] Shoji *et al.* reported a non-enzymatic potentiometric glucose sensor based on the pK_a changes on the poly(aniline boronic acid) that showed improved sensitivity for fructose compared to glucose.[15,16] Polyaniline–polyvinyl sulfonate–glucose oxidase modified electrodes are reported as a potentiometric sensor for glucose estimation.[17]

Based on all these facts, we have explored a potentiometric glucose biosensor based on a GOD immobilized CSA doped polyaniline composite matrix. The matrix was characterized by spectroscopy, electron microscopy and electrochemical techniques. The sensor performance was optimized in neutral phosphate buffer solution, in terms of enzyme loading, glucose concentration and introduction of interfering species.

2. Experimental

2.1 Reagents

Glucose oxidase (Fluka, from *Aspergillus niger*, 21 700 units per g), aniline (Aldrich), ammonium persulfate (Aldrich), and sulfuric acid (Merck) were used without further purification. β D(+)-Glucose (Aldrich) in phosphate buffer of pH about 7.4 was used as the analyte solution. Sodium dihydrogen phosphate (Merck) and disodium hydrogen phosphate-2-hydrate (Merck) were used to prepare phosphate buffer solution (PBS). A Nafion solution perfluorinated ion-exchange resin (5 wt% in lower aliphatic alcohols and water, contains 15–20% water) was

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purchased from Aldrich. All other chemicals were of analytical grade and were used as received. All solutions were prepared using water from a Milli-Q ultra-pure water (18.2 MΩ cm) system. All measurements were carried out at room temperature (25 ± 1 °C).

2.2 Apparatus and measurements

Scanning electron microscopy (SEM) images were obtained by using a HITACHI Model S-3000H. Fourier transform infrared spectra (FTIR) were recorded on a Nexus-670, Thermo Electron Corporation. Pellets of composites in potassium bromide were prepared to register the IR spectra. Potential measurements were done using a Hewlett Packard E2373A digital multimeter. A two electrode cell was employed for potentiometric measurements comprising the modified electrode as a working electrode and Ag/AgCl (saturated KCl) as a reference electrode. The electrochemical impedance spectroscopy (EIS) measurements were performed with an IM-6 impedance analyzer in 0.01 M potassium hexacyanoferrate + 0.1 M potassium nitrate solution by applying an alternating current voltage with 5 mV amplitude in a frequency range from 0.01 Hz to 100 kHz and THALES 4.13 software was used for curve fitting.

2.3 Chemical synthesis of polyaniline

The synthesis was based on the classical chemical oxidative polymerization of aniline. Aniline was introduced in aqueous sulfuric acid (0.5 M) and cooled to 0 °C. 0.54 M solution of ammonium persulfate was added slowly to the aniline solution, with stirring. The reaction mixture was allowed to stir at ice temperature for 4 h, then filtered and washed with aqueous sulfuric acid. The dark green product obtained was allowed to dry at 50 °C for 12 h. The resulted product was de-doped by treating it with ammonium hydroxide solution. It is further treated with 50 wt% of CSA to obtain CSA doped PANI composites.

2.4 Preparation of modifier solution

The modifier solution is prepared by dispersing 5 mg of CSA doped PANI, 5 mg of GOD (corresponding to 21.2 units of GOD) and 1% Nafion in water. 0.5% of glutaraldehyde was used to covalently immobilize the enzyme in the PANI matrix. The mixture was then sonicated for about 30 minutes, stored in a refrigerator for 24 hours and used for electrode modification.

2.5 Preparation of the CSA–PANI–GOD–Nafion modified electrode

Prior to its modification, the glassy carbon electrode (GCE, diameter of 3 mm) was carefully polished with 4 μm alumina slurry, rinsed in water and then subjected to ultrasonic cleaning with Milli-Q water. The electrode surface is then ready for modification. It was prepared by syringing 3 μL of the modifier solution on the electrode surface using a micropipette.

3. Results and discussion

3.1 FTIR characterization

Fig. 1 shows the FTIR spectra of PANI–CSA, PANI–CSA–GOD and PANI–CSA–GOD–Nafion composite materials. In all the spectra, the band at around 2940 cm^{-1} could be assigned to aromatic C–H stretching modes. The characteristic absorbance at around 1680 cm^{-1} indicated the signature of the PANI backbone due to the stretching modes of the protonated quinoid and the benzenoid rings. The two stretching vibrations might be overlapped giving one unresolved intense peak. The strong aromatic C–N stretching modes in secondary aromatic amines were responsible for the peaks at around 1360 cm^{-1}. In the lower frequency region, the peaks at around 1160 cm^{-1} were due to the aromatic C–H in-plane deformation, which was used to evaluate the electron delocalization in polymers. The strong band in this region was described as the "electronic-like band" and is considered a measure of the degree of delocalization of electrons and thus it is a characteristic peak of PANI conductivity.

The absorptions at around 1060 and 660 cm^{-1} by the CSA doped PANI samples were the best confirmations for the presence of sulfonate functional groups attached to the aromatic rings and the groups exhibited S═O stretching mode. This indicated the doping of the sulfonic acid group in the PANI backbone. The peaks at around 660 cm^{-1} were also attributed to the C–H bending in CH═CH in all the samples. The absorption at 1064 cm^{-1} by PANI should be caused by the doping due to SO_4^{2-}.

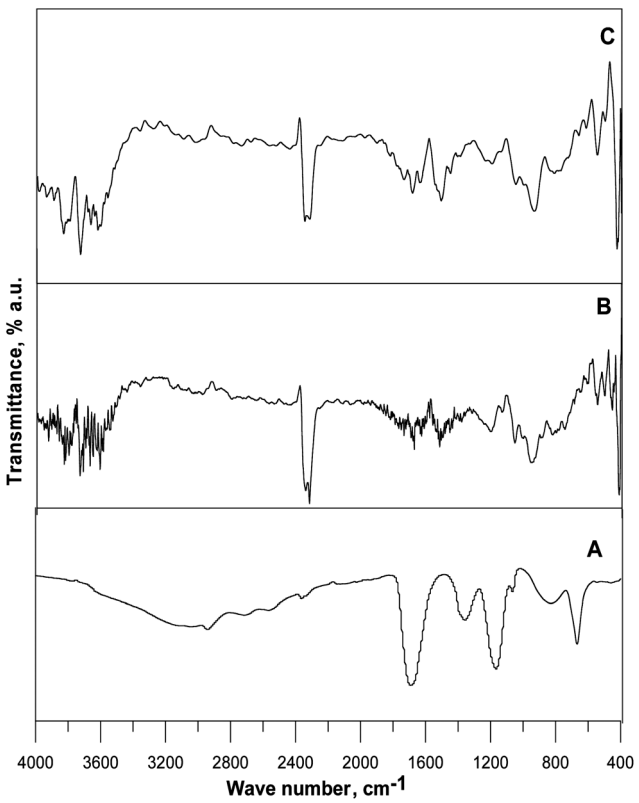

Fig. 1 FTIR spectra of (A) PANI–CSA; (B) PANI–CSA–GOD; and (C) PANI–CSA–GOD–Nafion.

GOD incorporation in a matrix is widely confirmed by the amide I and II IR bands that are used for monitoring conformational changes in proteins. As shown in Fig. 1C, two amide bands, within ranges of 1700–1600 cm^{-1} and 1600–1500 cm^{-1}, were observed at both PANI–CSA–GOD and PANI–CSA–GOD–Nafion. The former (1652 cm^{-1}, amide I band) is caused by C═O stretching vibrations of peptide linkages in the GOD backbone, whereas the latter (1574 cm^{-1}, amide II band) resulted from a combination of N–H in-plane bending and C–N stretching of the peptide groups.[18] The PANI–CSA–GOD–Nafion showed a peak at 1063 cm^{-1} corresponds to the $-SO_3$ group and the peaks at 1066 and 876 cm^{-1} correspond to the presence of a vinyl fluoride group confirming the presence of Nafion in the polymer matrix.

3.2 SEM studies of CSA–PANI–GOD composite

Fig. 2 shows the surface morphology of PANI–CSA, PANI–CSA–GOD and PANI–CSA–GOD–Nafion modified composite films. The CSA doped PANI showed a micro-porous structured morphology and it is found to be uniform throughout the sample. The micro-porous structure does not get affected (Fig. 2B and C) by the introduction of modifiers such as GOD and Nafion. The micro-dimension pores were readily accommodating these materials that results a smooth morphology. Thus, the PANI exhibited a uniform homogeneous composite matrix, consistent with enzyme loading and offering a better conductive platform for electron transfer.

3.3 Electrochemical impedance measurement

Fig. 3 shows the EIS of CSA–PANI, CSA–PANI–GOD and CSA–PANI–GOD–Nafion modified GCE electrodes. In general, all impedance curves exhibited a semicircle region lying on the axis followed by a straight line as the frequency approaches lower values. The semicircle portion observed in the higher frequency region corresponds to the charge-transfer process and the straight line part in the lower frequency region represents the diffusion-limited process. It is interesting to note that the R_{ct} value of the CSA doped PANI is found to be 1.2 kΩ and introduction of the GOD enzyme increased the film resistance to a value of 12 kΩ. This increased interfacial resistance indicated the successful formation of the CSA–PANI–GOD composite film. This value reduced to 4 kΩ by the addition of Nafion, which is known to be an ionic conducting membrane. Thus, the resistance is brought down to a concession value for the CSA–PANI–GOD–Nafion film. This film is used as the sensor matrix, which portrays the better conductivity suitable for analytical purposes.

3.4 Optimization of enzyme electrode performance

The fabrication of the GOD immobilized PANI in a GCE electrode is illustrated in Fig. 4. It is well known that the reaction of GOD with glucose produces hydrogen peroxide that will interact with the polyaniline, which is highly sensitive in shifting the free energy change. This could be observed in terms of potential variation at the interface, which corresponds to the glucose concentrations. To optimize the enzyme loading in the sensor matrix, the amount of GOD loaded on the surface was varied under fixed experimental conditions. The change in free energy increased with enzyme loading up to 100 units and beyond that the response decreases. Hence, the 100 unit loading was chosen as the optimum GOD content for the preparation of the glucose biosensor. This could be explained in terms of the decrease in conductivity of the composite film. This saturation kinetics in addition may lead to the increased protein concentration in the bio-composite film which might be due to the fact that the increased protein content decreased the conductivity of the composite film.[19]

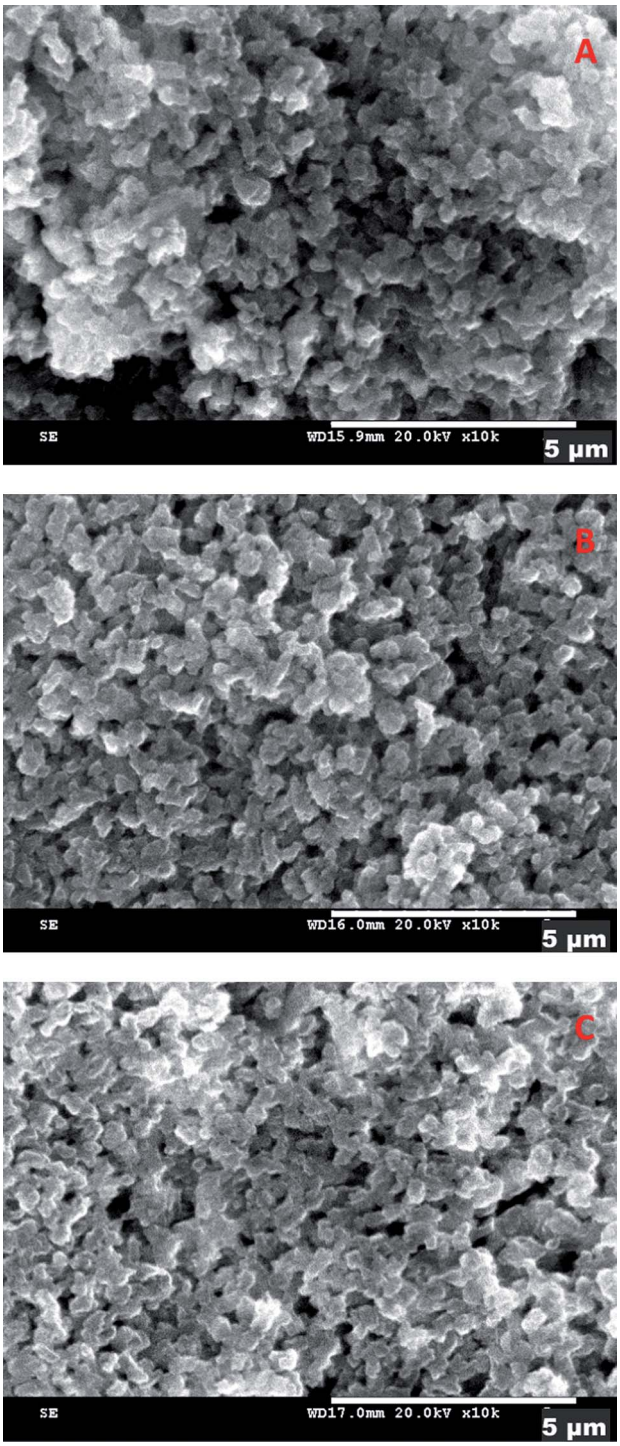

Fig. 2 SEM images of (A) PANI–CSA; (B) PANI–CSA–GOD; and (C) PANI–CSA–GOD–Nafion (scale bar 5 µm).

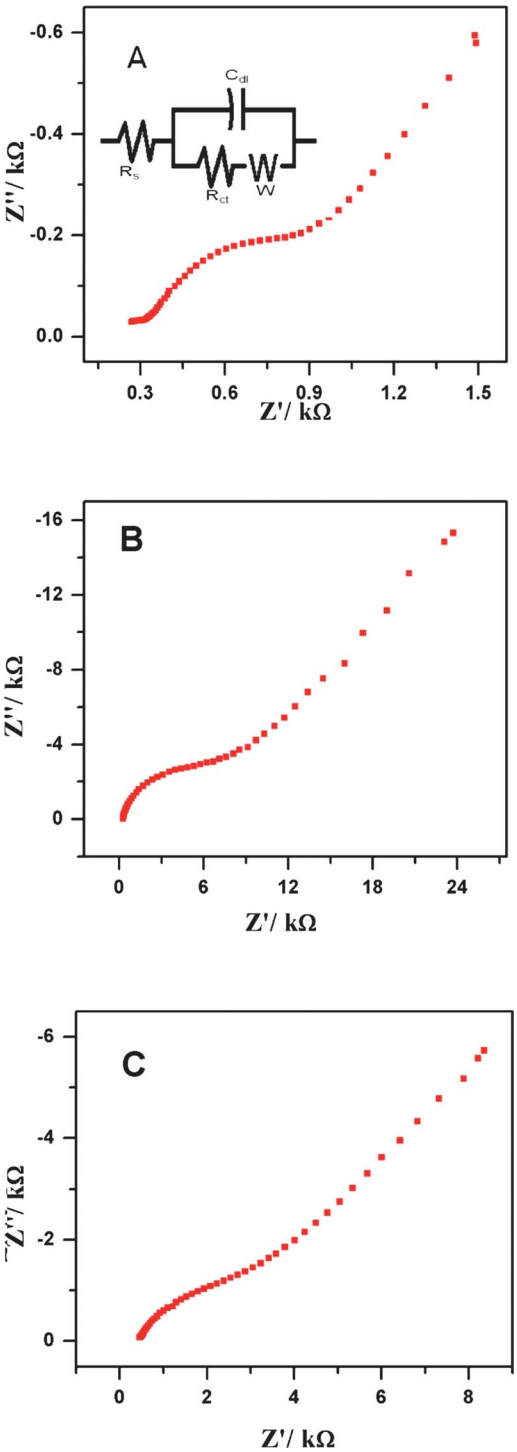

Fig. 3 Nyquist plots of (A) PANI–CSA; (B) CSA–PANI–GOD; and (C) CSA–PANI–GOD–Nafion modified electrodes. Frequency range: 0.01 Hz to 100 kHz; bias potential: 0.2 V; amplitude: 5 mV; electrolyte: 0.01 M potassium hexacyanoferrate in 0.1 M potassium nitrate; inset: equivalent circuit model.

3.5 Potentiometric glucose sensing

The modified electrodes were activated by immersing in phosphate buffer solution for a period of 30 minutes. Measurements were carried out by introducing the electrodes in phosphate buffer solution of pH 7.4 containing glucose of varying concentrations (1–50 mM). With increasing glucose concentrations, the potential response also increases and finally reached a steady state value.[20] The resulting potential perturbations (ΔE_p) for a response time of 1 minute were measured against a Ag/AgCl reference electrode. To ensure the sensing characteristics, the experiment was carried out in buffer solution without glucose addition and no change in potential in the system for an hour was observed, which confirms that the potential changes can be accounted for by the addition of glucose sensing. Fig. 5 shows the calibration curve for a potentiometric glucose biosensor, where the potential signal increases linearly with the increase in glucose concentration with the correlation coefficient of 0.9807 ($N = 10$) and sensitivity of 0.09 mV mM^{-1}, which is the minimum detectable concentration using these modified electrodes. The reproducibility and storage stability of the biosensor have also been studied. The relative standard deviation (RSD) of the biosensor response to 5.0 mM glucose was 2.4% for 13 successive measurements. The RSD for six sensors prepared under same conditions in response to 5.0 mM

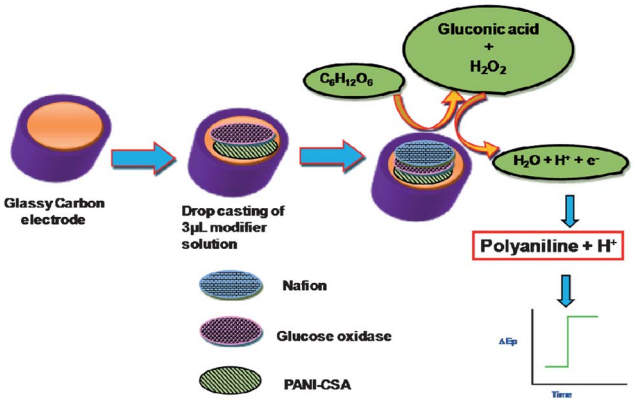

Fig. 4 Schematic representation of PANI–CSA–GOD–Nafion modification in a glassy carbon electrode and sensing mechanism.

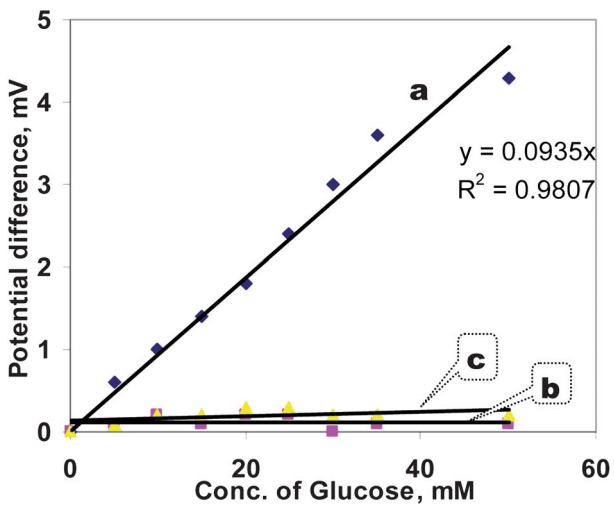

Fig. 5 Calibration curve of potentiometric glucose sensing using a CSA–PANI–GOD–Nafion modified GCE electrode (a) with glucose, (b) buffer alone and (c) CSA–PANI modified GCE electrode with glucose.

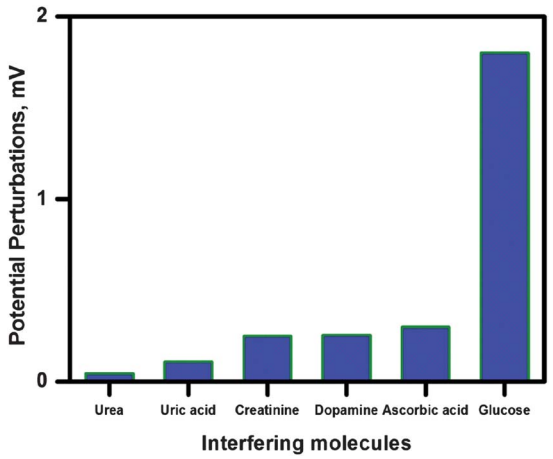

Fig. 6 The response of a CSA–PANI–GOD–Nafion modified GCE electrode with different interfering molecules.

glucose was 3.9%. The biosensor was stored dry at 4 °C and measured at intervals of a week. After one week, the sensor matrix retained 92% of its initial sensitivity.

Experiments with interfering molecules including ascorbic acid (AA), uric acid (UA), urea, dopamine (DA) and creatinine were performed to show the selectivity of the present modified electrode. Potential shifts observed for 20 mM of glucose, 5 mM of AA, UA, DA, urea and creatinine with the CSA–PANI–GOD–Nafion modified GCE electrode are shown in Fig. 6. The high specificity of the present modifier may be accounted for by the fact that perfluorinated Nafion used as a membrane minimizes the contribution of negative interference such as ascorbic acid, uric acid, *etc.* while sensing the glucose molecule.[21] Good selectivity and sensitivity of the CSA–PANI–GOD–Nafion modification for glucose sensing suggested its potential application as an advanced material in biosensors.

4. Conclusions

In this work, we have demonstrated a PANI–CSA–GOD composite film modified glassy carbon electrode as a potentiometric glucose biosensor. The present modifier showed good linear responses to glucose concentrations for a range from 1 to 50 mM, which well encompasses the physiological range for normal and diabetic patients. The biosensing platform offered a sensitivity of 0.09 mV mM^{-1} for glucose detection. The matrix did not experience interferences from AA, DA, UA, urea and creatinine during glucose detection. The current results suggest that the designed matrix shows great potential for the development of a potentiometric glucose biosensor for use in *in vivo* continuous monitoring of glucose.

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