Electrospun poly(vinylidene fluoride)/poly(aminophenylboronic acid) composite nanofibrous membrane as a novel glucose sensor

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Abstract

Electrospinning was used to prepare the nanofibrous membrane (NFM) of the composite comprising poly(vinylidene fluoride) and poly(aminophenylboronic acid) (PVdF/PAPBA-NFM). The PVdF/PAPBA-NFM displayed an excellent linear response to the detection of glucose for the concentration range of 1 to 15 mM with a response time of less than 6 s. Further experiments on amperometric sensing of glucose were performed in the presence of interferents such as uric acid, ascorbic acid, acetaminophen, fructose, mannose, etc. using PVdF/PAPBA-NFM. The interferents did not give significant overlapping current signal during the determination of glucose. Also, PVdF/PAPBA-NFM possesses better reproducibility toward glucose detection and storage stability.

Keywords: Glucose; Amperometric sensor; Fibrous membrane

Intensive research activities have been devoted to the development of amperometric biosensors. Glucose is a universal nutrient preferred by most organisms and serves fundamental roles in energy supply, carbon storage, biosynthesis and carbon skeleton and cell wall formation. The development of amperometric glucose sensors is an intensively investigated research area because of its importance in the treatment of diabetes mellitus [1].

Most of the recent work related to the development of glucose sensors has been based on the immobilization of an enzyme such as glucose oxidase [2] or glucose dehydrogenase [3] which catalyzes the oxidation of glucose to gluconolactone. Enzyme-immobilized electrode surfaces are fabricated to achieve rapid and direct electron transfer [4]. Also, in most cases, the enzyme-based sensors require a redox mediator [5] that is used to increase the selectivity and sensitivity of the sensors. However, the amperometric enzyme electrodes have several inherent problems such as the relatively low output current density and the gradual deterioration of the enzyme activity. The stability and toxicity of some mediators limit their in vivo applications. Further, sensors based on enzyme immobilization are susceptible to denaturing. The hydrogen peroxide produced by the reaction damages the enzymes.

Attempts to develop glucose sensors without the use of enzymes have been made. In one approach, glucose is electrocatalytically oxidized at metal surfaces such as platinum [6], gold [7], copper [8], and nickel [9]. However, the metal electrodes rapidly lose their activity due to the accumulation of chemisorbed intermediates, which block the electrocatalyst surface. Another disadvantage of these metal electrodes is that they lack the essential requirement of the sensor, selectivity for glucose. Few of the structurally similar organic substances are also simultaneously oxidized along with glucose at these electrode surfaces and they give interfering electrochemical signals.

Hence, fabrication of a robust and an enzymeless glucose sensor is an important goal. Boronic acids are known to interact with cyanide [10], fluoride [11], and diols [12] and...
have been used in sensor development. To date, many studies concerning boronic-acid-based enzymeless glucose sensors have been reported. Different sensing approaches including fluorescence [13,14], UV-visible [15], near-infrared [16], surface plasmon resonance spectroscopy [17,18], potentiometry [19,20], and quartz crystal microbalance measurements [17] have been developed.

Recently, the electrospinning method has been used to produce polymeric nanofibers for sensing applications [21]. The nanofibers produced through the electrospinning process possess higher specific area and hence have applications as sensors and catalysts. It is reported that optical sensors based on electrospun nanofibrous membranes (NFM) showed higher sensitivity and selectivity than film sensors for the detection of metal ions and nitro compounds [18,22,23].

In the present investigation, a novel sensor electrode based on the composite electrospun nanofibrous membrane of poly(vinylidene fluoride) (PVdF) and poly(aminophenylboronic acid) (PAPBA) was fabricated on ITO glass plate. The glucose sensing ability of the nanofibrous membrane was assessed.

**Materials and methods**

**Chemicals**

3-Aminophenylboronic acid, PVdF, glucose, acetaminophen, ascorbic acid, uric acid, D-galactose, D-mannose, D-fructose, and D-maltose were of analytical grade and used as received. Double-distilled water was used throughout the experiments. Aqueous solutions of glucose were prepared afresh at the time of performing the electrochemical experiments in phosphate buffer (pH 7.5). An ITO-coated glass plate with a specific surface resistance of about 10 Ω was used for fabricating the sensor electrode. Before performing the experiment, the surface of the ITO-coated plate was degreased with acetone and further rinsed with distilled water.

**Fabrication of sensor electrode**

PAPBA was prepared by performing the oxidative polymerization of 3-aminophenylboronic acid (50 mM in 1 M HCl) using ammonium persulfate (0.1 M in 1 M HCl) as oxidant at 5°C. The green-colored precipitate, PAPBA, was filtered, washed with 1 M HCl, and dried in a vacuum oven. Adequate amounts of PVdF and PAPBA were dissolved in N,N-dimethylformamide/acetone mixture (7:3 v/v) to obtain solution of composites. Electrospinning of the composite solution was performed at a flow rate of 10 mL/h with a potential difference of 25 kV. A distance of 15 cm between the syringe tip and the collector was maintained. The composite membrane was collected on the ITO glass plate (Scheme 1).

**Characterization**

The morphology of the sensor electrode (PVdF/PAPBA-NFM) was examined by field emission scanning electron microscope (FESEM) Hitachi S-4300 with a field emission gun operated at 200 kV. The Fourier transform infrared spectra were recorded using Bruker IFS 66v FT-IR spectrophotometer.

**Instrumentation**

All electrochemical measurements were carried out using an EG&G PAR 283 Electrochemical Analyzer. The amperometric response of the sensor electrode was recorded under steady state conditions in the phosphate buffer (pH 7.5) by applying a constant potential of 0.04 V to the working electrode. The amperometric experiment was performed in a standard single-compartment electrochemical cell that contained the sensor electrode, a saturated calomel reference electrode (SCE), and a platinum wire auxiliary electrode. The background response of the sensor electrode was allowed to decay to a steady state with stirring. When the background current became stable, a solution of glucose was injected into the electrolytic cell, and its response was measured. In the case of flow analysis, current vs time for variable glucose concentration injections were recorded for injection of a series of concentrated solutions of glucose to the phosphate buffer solution (pH 7.5).

**Results and discussion**

Sensitivity, selectivity, and mechanical stability are the essential requirements for a sensor. We have fabricated a nanofibrous PVdF/PAPBA membrane (PVdF/PAPBA-NFM) as glucose sensor. The boronate groups in PAPBA preferentially sense the glucose. PAPBA is anchored into the mechanically robust matrix of PVdF through an electrospinning process. Scheme 1 describes the fabrication process involved in the PVdF/PAPBA-NFM electrode through electrospinning and the microstructural features that provide characteristics for sensing glucose.

**Morphology and microstructure of PVdF/PAPBA-NFM**

Fig. 1 presents the FESEM image of the electrospun, nonwoven membrane of the PVdF/PAPBA composite deposited onto the ITO-coated glass plate (Scheme 1). Few interesting features can be seen in the morphology of the electrospun PVdF/PAPBA composite membrane. The FESEM image of the PVdF/PAPBA composite reveals the nanofibrous morphology. The fibers are nearly uniform with an average diameter of ~150 nm. The nanofibers are

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1 **Abbreviations used**: NFM, nanofibrous membrane; PVdF, poly(vinylidene fluoride); PAPBA, poly(aminophenylboronic acid); ITO, indium tin oxide; FT-IR, Fourier transform infrared; SCE, saturated calomel electrode; FESEM, field emission scanning electron microscope; AA, ascorbic acid; AP, acetaminophen; UA, uric acid.
interconnected to each other and form an interconnecting network between the components. The surface of the composite nanofiber is smooth. The morphological features of the PVdF/PAPBA-NFM are distinctly different from those of the electrospun PVdF membrane prepared under similar conditions. Fibers of PVdF are rigid and straight [24] with an average diameter of ~450 nm. The average diameter of PVdF membrane is much higher than the average diameter of PVdF/PAPBA composite membrane (~150 nm). The smaller size of the nanofibers of PVdF/
PAPBA provides higher surface area for sensing action and the boronic acid groups in PAPBA are the sources for the preferential selectivity and sensing of glucose.

The interconnected network morphology of the PVdF/PAPBA composite arises from the intermolecular interactions between the NH2 groups in PAPBA and the C–F group in PVdF. The FT-IR spectrum of PVdF/PAPBA-NFM (Fig. 2a) provides evidence for the intermolecular interactions between PVdF and PAPBA. The FT-IR spectrum of PVdF/PAPBA-NFM shows characteristic bands corresponding to PVdF and PAPBA. The composite membrane has spectral bands corresponding to CF2 vibration (1410 cm⁻¹), CF2 wagging (480 cm⁻¹), B–O stretching (1350 cm⁻¹), B–C stretching (1090 cm⁻¹), and C–N stretching of aromatic quinoid imine (1600 cm⁻¹). The molecular interactions between the PVdF and the PAPBA are evident from the shifts in the positions of CF2 stretching and CF2 wagging bands with respect to the bands in simple PVdF (Fig. 2b). The presence of a band corresponding to quinoid imine stretching (~1600 cm⁻¹) in the FT-IR spectrum of PVdF/PAPBA-NFM indicates that PAPBA exists in the self-doped state [12].

Sensor characteristics of PVdF/PAPBA-NFM

The sensitivity, selectivity, and stability of PVdF/PAPBA-NFM as glucose sensor were established through a series of electrochemical measurements.
Selectivity of PVdF/PAPBA-NFM

Selectivity of PVdF/PAPBA-NFM toward glucose was tested by performing experiments in the presence of generally known interfering substances such as ascorbic acid (AA), acetaminophen (AP), and uric acid (UA). UA and AA have been reported to give interferences in the electrochemical response for the detection of glucose with enzyme-based sensors and amperometric sensors [31]. Amperometric current response at the PVdF/PAPBA-NFM (at 0.04 V) for the addition of physiological levels of AA, AP, and UA was determined. The concentration of glucose was kept at 5 mM and the concentration of UA, AA, and AP were maintained at 1 µM each in the studies made for testing the influence of interfering species on electrochemical response for glucose detection. Negligible interference in the current signal upon the addition of the solution of UA, AA, or AP to glucose solution was noticed.

Complexation of boric and boronic acid derivatives with saccharides has been well documented [19,20]. Boronic functional groups participate in complexation with compounds containing vicinal diols through reversible ester formation (Scheme 1). In the present study, the boronic acid groups in PVdF/PAPBA-NFM are expected to bind the vicinal diol groups in glucose or any of the carbohydrates. There can be differences in the binding ability between the various carbohydrates having vicinal diols and boronic acid groups. It is therefore anticipated that other carbohydrates having diols would also influence the determination of glucose. In the present study, we wanted to ensure the extent of current signal from the

Fig. 3a displays the current–time profile obtained with the PVdF/PAPBA-NFM for the successive addition of 1 mM glucose in phosphate buffer (pH 7.5) for an operating potential of 0.04 V vs SCE. When the background current became stable, glucose was added into the electrolyte (phosphate buffer) with stirring. For an initial addition of 1 mM glucose to the electrolyte, a high peak current density (2 A/cm²) was noticed. The current response for the successive addition of glucose was recorded. Upon each addition of glucose, the current at the fibrous membrane electrode increased abruptly and reached a stable value. A stable and fast amperometric response was observed for successive injections of glucose (Fig. 3a). The time required to reach the stable response was less than 6 s, which is much lower than those for the other types of glucose sensors [25,26]. Fig. 3b shows the calibration plot (response current vs concentration of glucose) for the glucose detection. Thus, the fibrous membrane shows a high sensitivity to glucose in comparison with other glucose sensors [27–30].
individual diols during the electrochemical detection of glucose. Hence, amperometric measurements were performed at PVdF/PAPBA-NFM with other carbohydrates to elucidate the influence of the other carbohydrates on the electrochemical determination of glucose. We have done experiments to augment preferential selectivity at PVdF/PAPBA-NFM for glucose in the presence of other diols. Alexeev et al. [32] reported that boronic acid in the presence of crown ether consisting of a 4-acryloylamidobenzo-15-crown 5 functional group selectively binds glucose over the other carbohydrates, galactose, mannose, and fructose. Hence, in the present study, amperometric measurements were performed at PVdF/PAPBA-NFM containing crown ether having the functional group 4-acryloylamidobenzo-15-crown 5 for the detection of glucose in the presence of galactose, mannose, fructose, and maltose. The current responses to the other carbohydrates are normalized with regard to the current response to glucose and expressed as percentage response with respect to glucose (Table 1). Table 1 shows that galactose, mannose, and fructose have 0.56, 1.22, and 2.89% current response with respect to glucose. In the case of maltose, the influence of current response was judged by considering the ratio of glucose to maltose in real blood samples (305.6 \( \mu \)g/mL of glucose and 9.42 \( \mu \)g/mL of maltose). For a mixture of glucose and maltose having milli mole concentration each, maltose shows a 72.35% current response to glucose in comparison to the current response of glucose. Hence, the PVdF/PAPBA-NFM has adequate inter- and intra-electrode reproducibility for the sensing of glucose shows a proportional increase with increasing concentration of glucose. It is also evident that the PVdF/PAPBA-NFM provides good reproducibility for repeated detection of glucose. Interestingly, there is no memory effect in the response on increasing the concentration of glucose. Hence, the PVdF/PAPBA-NFM has adequate inter- and intra-electrode reproducibility for the current signals.

Fig. 4. Flow injection amperometric response of the PVdF/PAPBA-NFM to glucose solutions of increasing concentrations (0.1–16 mM); operating potential, 0.04 V; flow rate, 1.0 mL min\(^{-1}\). Inset shows the calibration plot.

Flow injection analysis

Flow injection analysis is widely adopted in analytical chemistry to assess characteristics such as low sample consumption, repeatability of results, high-throughput and versatility. Fig. 4 shows the flow injection amperometric response of the PVdF/PAPBA-NFM at 0.04 V for glucose solutions of increasing concentrations. The current response increases linearly with the concentration of glucose (Fig. 4, inset). The response current corresponding to sensing of glucose shows a proportional increase with increasing concentration of glucose. It is also evident that the PVdF/PAPBA-NFM provides good reproducibility for repeated detection of glucose. Interestingly, there is no memory effect in the response on increasing the concentration of glucose. Hence, the PVdF/PAPBA-NFM has adequate inter- and intra-electrode reproducibility for the current signals.

Nanofibers are expected to have high surface areas. This provides the possibility of large numbers of active sites for sensing glucose. The PVdF/PAPBA-NFM is more suited to the dynamic and continuous monitoring of glucose in comparison to the earlier-reported PAPBA-based glucose sensor [19,20]. For the PAPBA-based glucose sensor reported by Shoji and Freund [20], sensitivity arises from the inclusion of F\(^-\) ions as the dopant into PAPBA. Under the dynamic conditions of glucose sensing, there is a possibility of removal of F\(^-\) ions from the sensor matrix. In the present case, PVdF has C–F groups, and the interconnected morphology in the composite gives close proximity for the fluorine atoms to access the boron atoms which eventually may favor complexation with glucose. Hence, the problem associated with leaching of the fluorine source from the sensor matrix is negligible in the present case. Hydrodynamic experiments with continuous flow of glucose clearly revealed that PVdF/PAPBA-NFM is ideally suited for the detection of glucose in a continuous stream.
Table 2
Results of analysis of glucose in human blood serum samples

<table>
<thead>
<tr>
<th>Glucose found (mol L(^{-1}))</th>
<th>Determined by spectrophotometry (mol L(^{-1}))</th>
<th>Deviation (mol L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.52 (\times) 10(^{-3})</td>
<td>8.13 (\times) 10(^{-3})</td>
<td>0.39 (\times) 10(^{-3})</td>
</tr>
<tr>
<td>7.32 (\times) 10(^{-3})</td>
<td>7.02 (\times) 10(^{-3})</td>
<td>0.30 (\times) 10(^{-3})</td>
</tr>
<tr>
<td>9.56 (\times) 10(^{-3})</td>
<td>9.22 (\times) 10(^{-3})</td>
<td>0.34 (\times) 10(^{-3})</td>
</tr>
</tbody>
</table>

Fig. 5. Effect of storage time of PVdF/PAPBA-NFM stored in phosphate buffer (pH 7.5) at 4 °C.

**Real sample analysis**

To test the reliability of the PVdF/PAPBA-NFM, it was applied to the determination of glucose in human serum samples and the data are presented in Table 2. The values determined were in good agreement with the values obtained by spectrophotometric measurements.

**Stability of PVdF/PAPBA-NFM**

The long-term stability of PVdF/PAPBA-NFM was tested by recording the steady state current response of the sensor in human serum samples (Fig. 5). The long-term storage stabilities of these electrodes were tested every 5 days. After each experiment, the electrodes were washed with a phosphate buffer solution and stored in a phosphate buffer solution (pH 7.5) at 4 °C. The sensor electrode retained 90% of the original activity after 50 days and displayed excellent response to glucose.

**Conclusion**

We have successfully demonstrated that PVdF/PAPBA-NFM prepared through the electrospinning process exhibits sensitive detection of glucose, selectivity in the presence of other carbohydrates, negligible interference, reproducibility, and storage stability. The superior performance for nanofibrous membrane arises from the higher surface area and active sites available for sensing of glucose. The electrospun membrane glucose sensor is ideally suited to the sensing of glucose in a flowing stream. Further, the electrospun technology adopted here can be extended to the fabrication of other sensors through judicious loading of sensing mediators in the fibrous matrix.

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**References**


