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Pectin coated polyaniline nanoparticles for amperometric glucose biosensor

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Abstract

An amperometric glucose sensor based on nanoparticulate form of polyaniline (PAni) is reported herein. The PAni nanoparticles were synthesized using pectin (PAni- Pec NPs) and functionalized with glucose oxidase (GOx- PAni- Pec NPs) to create highly specific biosensor. Biopolymer pectin stabilizes the colloidal nanoparticles of PAni while also allowing homogenous and high degree functionalization of the nanoparticles with glucose oxidase via covalent coupling. This helped to arrest enzyme leaching and enhance biosensor stability. The biosensor showed high sensitivity (79.49 μA $mM^{-1}cm^{-2}$), wide linear range (0.06- 4 mM) and a low detection limit (43.5 μM) at a working potential of 0.6V. The sensitivity exhibited by GOx-PAni- Pec NPs reported herein was three times higher than that of conventional polyaniline for the same amount of GOx immobilized by physical adsorption method. Efficient loading and organization of GOx on the nanoparticles could afford adequate availability of the substrate to the enzyme sites leading to better sensitivity. The biosensor did not respond to presence of electroactive interferents (ascorbic acid, urea, and uric acid) coexisting with glucose in physiological fluids, thereby revealing its specificity. The analytical performance of the biosensor was evaluated for estimation of glucose in blood serum samples. The reliability and

stability of the glucose biosensor indicate its potential for application in routine analysis of glucose in physiological fluids.

Keywords**:** Polyaniline nanoparticles, glucose sensor, pectin, glucose oxidase, amperometric biosensor, conducting polymers

1. Introduction

Glucose monitoring is an integral part of medical diagnostics, especially for Type 1 diabetes mellitus management. In a healthy adult human being, the normal value of blood glucose concentration ranges from 4.4–6.6 mM. There are numerous reports on development of glucose sensors based on different techniques like fluorescence[1], electrochemiluminescene[2], colorimetry[3], electrochemistry[4], surface plasmon resonance[5], etc. However, electrochemical technique like amperometry comes with many advantages such as miniaturization, low cost instrumentation, rapid response, intrinsic selectivity and good sensitivity. Despite impressive progress in the development of electrochemical glucose biosensors, there are still many challenges and obstacles related to the achievement of a highly stable and reliable continuous glycemic monitoring [6].

Generally, carbon nanotubes (CNT) [7], graphene[8], metal/ metal oxide nanoparticles[9, 10], metal hexacynoferrates[11], redox polymers[12], conducting polymers[13], hydrogels[14], and their combinations are used for the development of electrochemical glucose biosensors. Amongst these, conducting polymers (CPs) like PAni have attracted lot of attention as they can perform dual task, as a physicochemical transducers as well as an immobilization matrix for biomolecules. PAni modified electrodes are used in environmental monitoring of toxins (like

pesticides[15], harmful gases [16, 17]) and common analytes (like glucose, urea, uric acid, creatinine, dopamine, ascorbic acid, cholesterol, biomarkers, etc. [18-24]) with glucose taking the topmost position in the hierarchy. Polyaniline in nanostructured morphology can provide high interfacial area for efficient immobilization of enzyme and analyte interaction, resulting in improved sensitivity of the biosensor. The nanostructured polymer is also known to display exclusive properties such as enhanced electrical conductivity and fast electrochemical switching speeds [25]. Various approaches have been reported for synthesis of polyaniline nanostructures either in the form of nanowires (using hard/soft template, interfacial polymerization) or nanoparticles (using special type of dopant, polymeric stabilizer, etc) [26]. Each of these methods has its own advantages and drawback, which are summarised in ***Supplementary Information***. We have earlier reported synthesis of mesoporous PAni (using soft template method) which displayed excellent electrocatalytic response for the detection of H_2O_2 and glucose compared to conventional polyaniline [27]. Generally, the nanowire morphology helps to expedite electron-transfer efficiency in addition to the enhanced surface area. On the other hand, nanoparticulate morphology provides a 3D surface for enzyme immobilization and can also help to solve the processibility problem associated with polyaniline as PAni NPs are readily dispersible in water.

Enzyme loading is considered as one of the crucial step in the biosensor design. The performance of an enzyme electrode is largely governed by the materials used for immobilization and the mechanism of its assembly on the surface of the electrode [28]. Techniques like dropcasting, covalent coupling, encapsulation and physical adsorption are commonly employed to immobilize enzyme on the sensor surface. While some of these methods face problem of enzyme leaching and an eventual lowering of sensor activity with time, it becomes vital to work in this direction to prolong biosensor's life. Covalent coupling of enzymes to nanostructured PAni during biosensor

construction can solve these issues to a great extent. The biocompatible nature of PAni can help to preserve the active sites of enzymes while allowing permeation of analytes to catalytic sites of the enzymes [29, 30].

Herein we present an amperometric glucose biosensor based on biopolymer pectin coated PAni nanoparticles (PAni-Pec-NPs). Glucose oxidase (GOx) enzyme is efficiently loaded over polyaniline nanoparticle via glutaraldehyde coupling in facile two-step process. The PAni-Pec-NPs were synthesized by a simple method without the use of any organic solvents to obtain highly water dispersible NPs. Pectin acts as a stabilizer for PAni nanoparticles and also permits entrapment of covalently bound GOx. Moreover the gelatinous structure of pectin around polyaniline nanoparticles behaves as a porous network allowing appropriate transport of analytes leading towards development of a wholesome biosensor.

2. Experimental

2.1. Chemicals

Aniline (Sigma Aldrich) was distilled under vacuum before use. Glucose oxidase (EC 1.1.3.4, 17.3 units/mg, from Aspergillus niger), horse radish peroxidase (HRP), N, N- diethyl aniline, fluorescein isothiocyanate (FITC), and 4-Amino antipyrine were obtained from Sigma Aldrich, and d-(+)-glucose (99.5%) from Loba Chemie, India.Pectin was purchased from Sisco Research Laboratory (SRL), India. Na_2HPO_4, KH_2PO_4, NaCl and KCl (for making phosphate buffer solution), glutaraldehyde solution (25 %) and ammonium persulphate (APS) were procured from SRL, India. Aqueous solutions were prepared using deionized water from a Milli-Q purification system.

2.2. Instruments

FTIR measurements of the samples were carried out using Bomen Hartman and Braun, MB Series FTIR instrument. SEM images were recorded using Carlzeiss EVO40 SEM instrument. Fluorescence microscopy was performed using Olympus (U- CMADC, Tokyo, Japan) microscope. DLS measurements were performed using a Malvern 4800 Autosizer employing 7132 digital correlator. The light source was He-Ne laser operated at 633 nm with a maximum output power of 15 mW. The average decay rate was obtained by analyzing the electric field autocorrelation function vs. time data recorded at 90° using method of CONTIN [31] Autolab Potentiostat - Galvanostat, model PG STAT 20 (Eco Chemie, Netherlands) was used for cyclic voltammetry and amperometry measurements. All the experiments were carried out using a three-electrode system with Ag/AgCl (3mM KCl) as reference electrode and platinum (Pt) counter electrode. The working electrode was Pt (Biologic A- 002422 with 3mm diameter) which was modified with PAni- Pec NPs and GOx- PAni- Pec NPs for further studies.

2.3. Preparation of Glucose biosensor

The various steps involved in fabrication of glucose biosensor are shown in **Fig. 1** and the procedure is described below in brief.

2.3.1. Synthesis of PAni- Pec nanoparticles

The polyaniline – pectin nanoparticles (PAni- Pec NPs) were synthesized as per the procedure reported earlier by our group [32]. 25 ml of aqueous solution of ammonium persulfate (2.28 g) was added drop-wise to a 60 ml solution containing 1.862 g pectin (Pec), 9 ml HCl (11 N) and 0.9313 g of aniline (Aniline: Pec = 1:2). The reaction was carried out at room temperature under constant stirring for 4 h to yield homogeneous dispersion of PAni-Pec. The PAni-Pec was

precipitated in 1:1 mixture of ethanol-water followed by filtration with Whatmann 41 filter paper and washing with 1:1 mixture of ethanol-water to get pure PAni-Pec precipitate. The precipitated PAni-Pec NPs were re-dispersed in nanopure water by ultrasonication where its final concentration was estimated to be 28.2 mg/ ml.

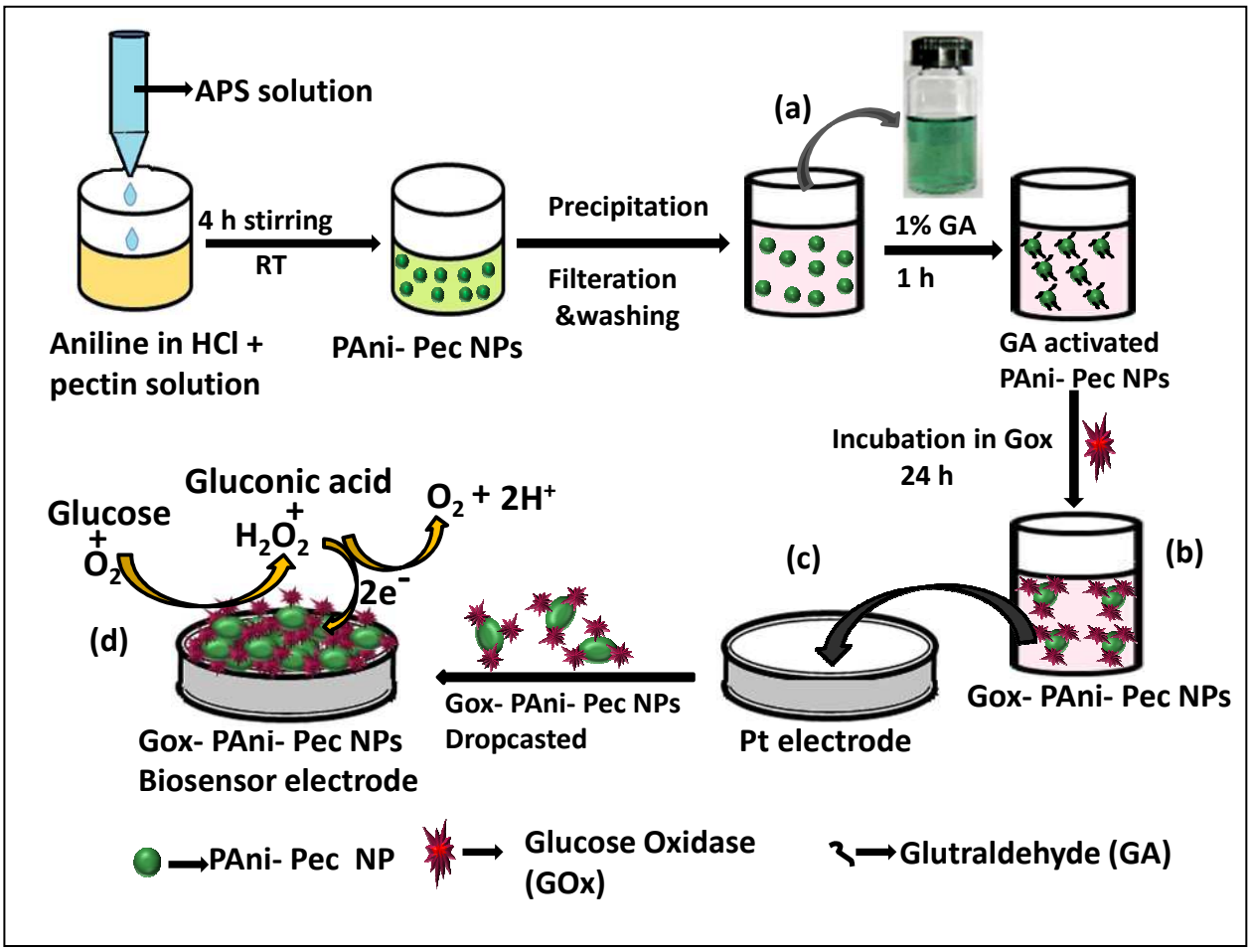

Fig. 1: Scheme for fabrication of GOx- PAni- Pec glucose biosensor depicting (a) synthesis of PAni-Pec NPs, (b) covalent immobilization of GOx on the NPs, (c) casting of the sensor material on platinum electrode and (d) the biochemical reactions involved in glucose sensing.

*APS = Ammonium persulphate, RT= Room temperature, Pt= Platinum.

2.3.1. Covalent coupling of GOx on NPs

0.5ml of above PAni-Pec NPs were dispersed in 1M HCl and then centrifuged. The NPs were then dispersed in 1 ml of 1% glutaraldehyde (GA) solution and kept on a shaker for 1h. The NP solution was centrifuged and washed with PBS (7.4 pH) to remove excess GA. The NPs were dispersed in 100 µl of PBS having GOx (1mg, 3.5mg and 5 mg) and incubated for 24 h at 4°C to allow crosslinking. The resulting enzyme loaded PAni-Pec NPs were again washed with PBS to remove unbound GOx and finally re-dispersed in 100ul PBS to obtain GOx- PAni- Pec NPs.

2.3.2. Electrode modification

The Pt electrode was cleaned using HNO_3 and polished with alumina powder. The polished electrode was cleaned electrochemically in 0.5M H_2SO_4 by cycling the potential between -0.25 and +1.1V at a scan rate of 50 mVs^{-1} until the characteristic cyclic voltammogram for a clean Pt electrode was obtained. 10 µl of PAni-Pec-GOx solution was dropcasted on clean Pt electrode surface and allowed to dry in air for 30 min. Before each electrochemical measurement, the modified electrode was allowed to equilibrate in PBS (pH7.4) till a stable CV was obtained.This is especially important as electrochemical behaviour of PAni is highly sensitive to pH.

The details of colorimetric assay for estimation of enzyme loading, FITC tagging of nanoparticles and biocompatibility studies are given in ***Supplementary Information.***

2.4. Electrochemical measurements

The cyclic voltammetry (CV) measurements were carried out in PBS (pH7.4) in a potential window of -0.2 V to 1 V (scan rate = 50 mV s^{-1}). For studying the interference effect, chronoamperometry was performed at a potential of 0.6 V under constant stirring. Glucose solution (1M) was injected at regular time intervals to the PBS solution to obtain a desired

concentration. Effects of major interferents such as uric acid (0.2 mM), ascorbic acid (0.2 mM) and urea (0.2 mM), on the performance of biosensor was studied. The performance of biosensor for estimation of glucose in human blood serum samples was evaluated using CV. All the experiments were carried out at room temperature. When not in use, the biosensor electrode was stored in an airtight container at 4°C.

3. Results and discussions

3.1. Characterization of the modified Pt electrode

3.1.1. FT- IR studies

Fig. S1 in ***Supplementary Information*** shows the FT-IR spectra of PAni - Pec NPs and GOx-PAni- Pec NPs. Both the spectra show characteristics of PAni, namely, bands at 1496 and 1574 cm^{-1}, attributed to the stretching frequencies of the benzenoid and quinoid rings of PAni, and the adsorption band at 1294 cm^{-1} corresponds to the C-N stretching [33]. The in-plane bending of C–H gives the band at 1143 cm^{-1} and the band at 802 cm^{-1} arises due to the out-of-plane bending of C–H of aromatic benzene ring [34]. In addition to this, the absorption band assignable to C=O is observed at 1646 cm^{-1}, indicating the presence of pectin in both the samples. The peaks at 1652 and 1590 cm^{-1} observable for GOx- PAni- Pec NPs correspond to the stretching of C=O bond and the in-plane vibration of N-H bonds in peptide linkage of GOx, respectively. Also, the bands at 1379 and 1100 cm^{-1} corresponding to CH_2 and C-O bending vibrations of GOx were observed[35]. This confirmed the binding of GOx to PAni- Pec NPs.

3.1.2 Fluorescent microscopy

Fluorescence microscopy images were recorded for fluorescein isothiocyanate (FITC) tagged PAni- Pec NPs and GOx- PAni- Pec NPs (FITC tagging procedure given in ***Supplementary Information***). FITC is known to have strong binding affinity for amino group of proteins. A less intense fluorescence signal was obtained for PAni- Pec NPs (**Fig. 2(a)**) due to the binding of FITC to free amino group of PAni. In case of GOx- PAni- Pec NPs (**Fig. 2(b)**), highly intense green fluorescence could be seen due to binding of FITC to amino groups of GOx. The fluorescence images confirmed efficient immobilization of GOx on PAni- Pec NPs.

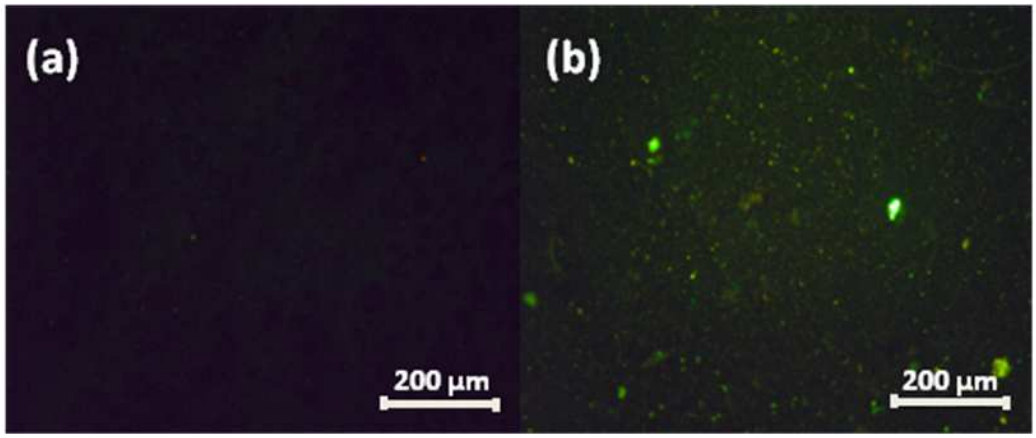

Fig. 2: Fluorescence microscopy images of (a) PAni- Pec NPs and (b) GOx- PAni- Pec NPs

3.1.3. SEM analysis

SEM studies showed flattened oval shaped morphology for PAni- Pec NPs (**Fig. 3**) with average length of ~160nm ± 33nm and width ~94 nm ± 21nm (N=50). GOx- PAni- Pec NPs were roughly spherical with average diameter of ~80 nm ± 21nm (N=50). The size of GOx- PAni- Pec NPs was found to be less than that of PAni- Pec probably due to partial elimination of pectin layer during the 24 h incubation of NPs in GOx solution. The enzyme loaded particles are highly water dispersible indicating that enough pectin is still present on the GOx- PAni- Pec NPs.

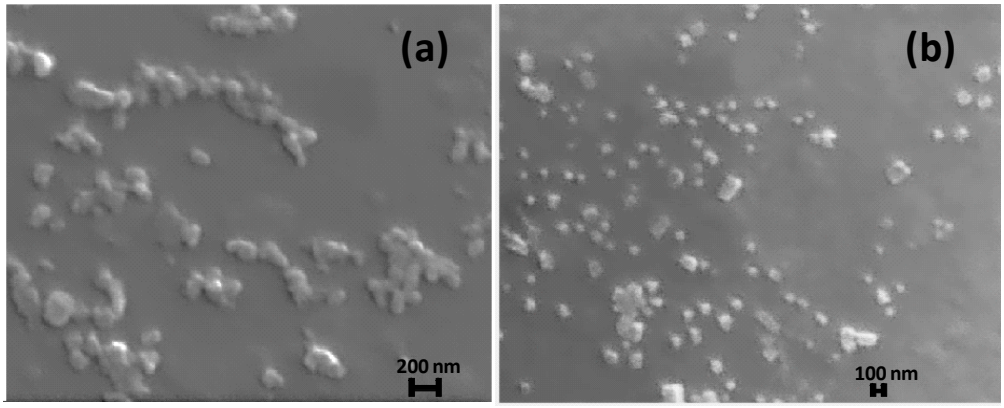

Fig. 3: SEM images of a) PAni- Pec NPs and b) GOx- PAni- Pec NPs

3.1.4. DLS studies

DLS measurements revealed average radius of 275±12.5 nm and 237± 6 nm for PAni- Pec and GOx- PAni- Pec NPs respectively (**Fig. S2** in ***Supplementary Information***). The size of particles obtained by DLS studies is much higher than that obtained from SEM studies. This is because DLS gives the average hydrodynamic radius of diffusing particles. Pectin is known to swell to a large extent in aqueous solutions which leads to a high hydrodynamic radius for PAni- Pec and GOx- PAni- Pec NPs in dispersion. Here too, the size of GOx- PAni- Pec NPs was found to be smaller that PAni- Pec NPs (due to partial removal of pectin layer during the enzyme immobilization step), similar to the observations of SEM studies.

3.2. Electrochemical performance of biosensor

3.2.1. Cyclic voltammetry studies

Cyclic voltammogram of PAni –Pec NPs coated on Pt electrode in 1M HCl solution affirmed that presence of pectin had almost no effect on electrochemical properties of PAni as the CV clearly shows two oxidation peaks at 0.2V and 0.8V and the corresponding reduction peaks at

0.06V and 0.75V respectively, similar to that observed for conventional PAni. The enzyme loading did not have any pronounced effect on electrochemical properties of PAni as evident from the CV of GOx-PAni-Pec in 1M HCl solution. (**Fig. S3** in ***Supplementary Information***). The overall current in CV of GOx-PAni-Pec NPs was found to be higher as compared to that of PAni- Pec NPs, probably due to partial elimination of pectin (an insulating material) during processing of NPs for GOx coupling, as observed by SEM and DLS studies.

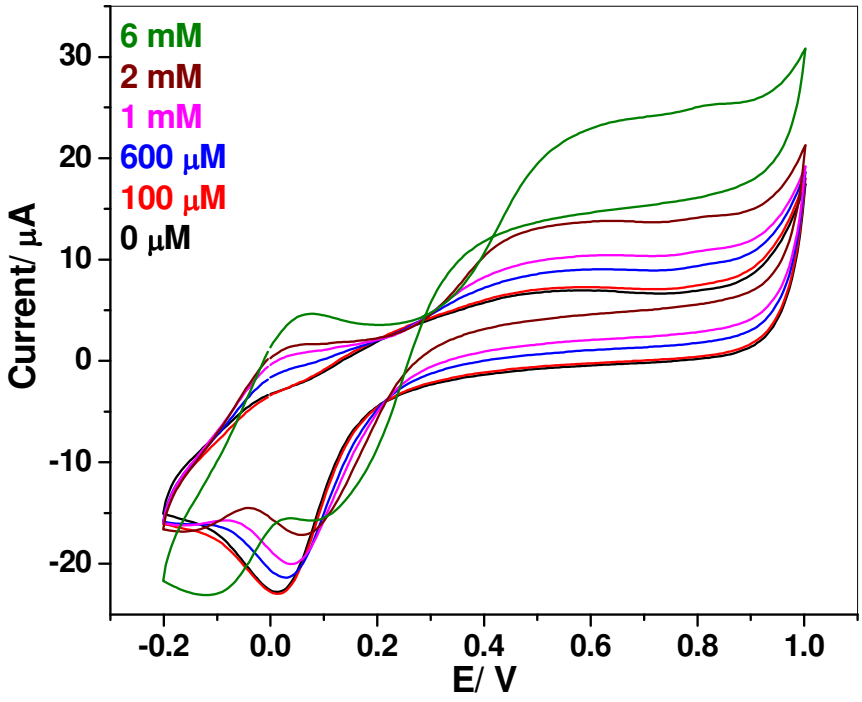

Fig. 4: CV of GOx- PAni- Pec NPs biosensor in PBS with increasing glucose concentration (pH 7.4). Scan rate: 50 mVs^{-1}.

Cyclic voltammetry studies of GOx- PAni- Pec NPs modified electrode were carried out in PBS (pH= 7.4) by increasing the glucose concentration. With addition of each aliquot of glucose, an

increase in oxidation current was observed from 0.4 V to 1V (**Fig. 4**). This increase is attributed to oxidation of H_2O_2, which is released as a result of enzymatic reaction of glucose oxidase with glucose [27]. This was confirmed by carrying out cyclic voltammetry studies on PAni modified electrode with addition of H_2O_2 (**Fig. S4** in ***Supplementary Information***). The nature of CV and peak positions for H_2O_2 oxidation on PAni electrode match well with the CVs in Fig. 4. The effect of scan rate on cyclic voltammogram of GOx- PAni- Pec NPs biosensor was studied at a fixed (2 mM) concentration of glucose (**Fig. S5** in ***Supplementary Information***). The peak current (i_p) was found to be directly proportional to scan rate (ν) indicating that the kinetics is predominantly governed by redox process of surface confined species and not by diffusion of glucose.

3.2.2 Amperometric response of PAni-Pec-GOx biosensor to glucose

Amperometric response of biosensor towards glucose at four different working potentials was studied (**Fig. S6 in *Supplementary Information***). Although the sensor electrode has higher sensitivity at 0.7 V, opting for high working potential increases the chances of plausible interfering effects due to other species present in physiological fluids. At a potential of 0.4 V and 0.5 V, the sensitivity and linear range were found to be low, hence a working potential of 0.6 V was selected for further studies.

As described in the experimental section, the enzyme GOx was covalently immobilized on PAni-Pec NPs by first carrying out glutaraldehyde activation followed by incubation of the NPs in GOx solution for 24 h. The amount of enzyme loaded on the NPs was estimated by colorimetric assay as described in ***Supplementary Information***. When 3.5 mg/ 100 µl GOx was used for coupling to 14 mg NPs, 2.8 mg of enzyme was found to be immobilized on the NPs.

Performance of the biosensor at different loading of GOx on PAni- Pec NPs was studied by carrying out amperometric measurements at the potential of 0.6V (**Fig. 5**). Overall, current response of the biosensor was found to increase with increasing GOx loading; however the increase was not proportional to the amount of enzyme. At higher enzyme loading, the large amount of bulky insulating enzyme molecules on the NPs lead to lowering of the response current. Moreover, it restricts the availability of the substrates and diffusion and products across the biosensor-electrolyte interface eventually lowering the biosensor response [36]. Hence GOx-PAni- Pec NPs prepared using 3.5 mg GOx was considered as optimum loading for further studies.

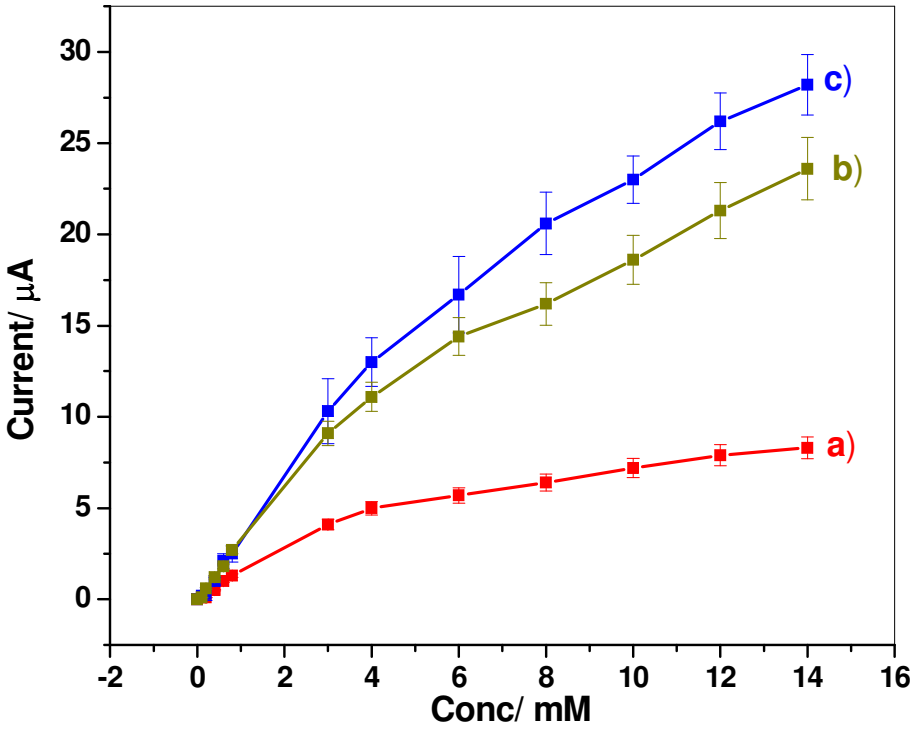

Fig. 5: Amperometric response of the GOx- PAni- Pec NPs biosensor, fabricated using different concentration of GOx during coupling step (*a.* 1mg GOx, *b.* 3.5 mg GOx and *c.* 5 mg GOx), towards glucose addition.

3.3. Michaelis–Menten constant of the composite film based biosensor

Normalized response of the GOx- PAni- Pec NPs biosensor toward glucose addition under the optimized conditions is depicted in **Fig. 6**. The response curve follows a typical Michaelis-Menten behavior showing linearity in initial stages, approaching saturation for higher substrate concentration. A linear relationship was observed between the reciprocal of current response (1/I) and the reciprocal of glucose concentration (1/C) in the concentration range of 0 – 60 mM **(Fig. S7** in ***Supplementary Information***). Based on this data, the slope of the linear fit was used to evaluate Km using the Lineweaver–Burk equation. The value of Michaelis- Menten constant (Km) was found to be 7.65 mM, which is lower than that, reported in literature for some of the glucose biosensors (See **Table 1** in ***Supplementary Information***). Km for the free GOx used in the present study was found to be 62.7 mM, as estimated by colorimetric enzyme assay. A lower value of Km for the GOx- PAni- Pec NP biosensor indicates higher binding affinity of the immobilized GOx for glucose as compared to free enzyme. The biocompatibility of PAni- Pec NPs was assessed from the cell viability of L6 rat myoblast cells (as determined by MTT assay[37]). The percentage of viable cells was 90% for PAni-Pec NPs indicating its biocompatible nature. Thus the improved microenvironment provided by the biocompatible pectin encased PAni NPs lead to lowering of the Km thereby enhancing the sensitivity of biosensor towards glucose.

Under the optimized conditions, the sensor exhibited a linear response from 0.06 mM- 4 mM (correlation coefficient of 0.99) with sensitivity of 79.49 $\mu AmM^{-1}cm^{-2}$ for determination of glucose. The detection limit was found to be 43.5µM at S/ N ratio of 3.

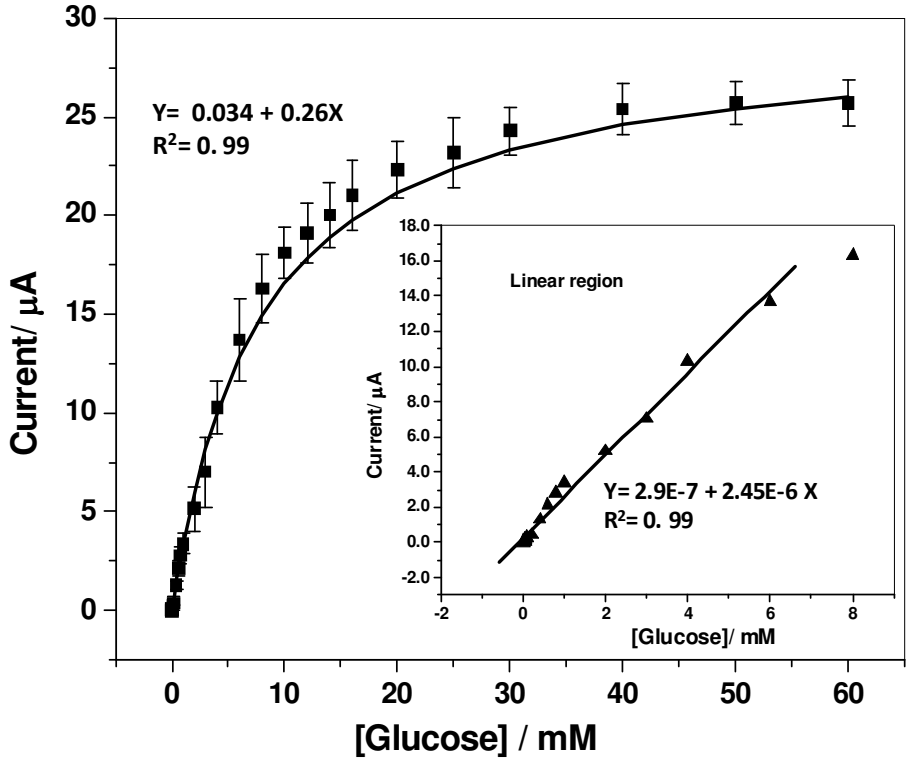

Fig. 6: Normalized response curve (calibration plot) of GOx- PAni- Pec NPs (3.5 mg GOx concentration used for loading the NPs) biosensor toward addition of glucose (n = 3). The solid line depicts the fitted curve based on the Michaelis–Menten equation. Inset shows linear region of the calibration curve.

Performance of the GOx- PAni- Pec NP biosensor was compared with GOx- PAni biosensor which was fabricated by physical immobilization of 10 µl of 3.5 mg/ml GOx on conventional polyaniline. As it can be seen from **Fig. 7**, PAni-Pec-GOx biosensor gave three times higher

current response for glucose compared to conventional PAni based biosensor for the same enzyme loading. The detection limit for the GOx- PAni biosensor was found to be 780µM at S/ N ratio of 3 and the linear range was 0.8 - 4 mM. The better sensing performance of the GOx- PAni- Pec NP biosensor can be attributed to nanoparticulate form of PAni-Pec used for loading the enzyme. The increased surface area of PAni-Pec nanoparticles helps to provide an effective exposure of active enzymatic sites resulting in higher output of biosensor.

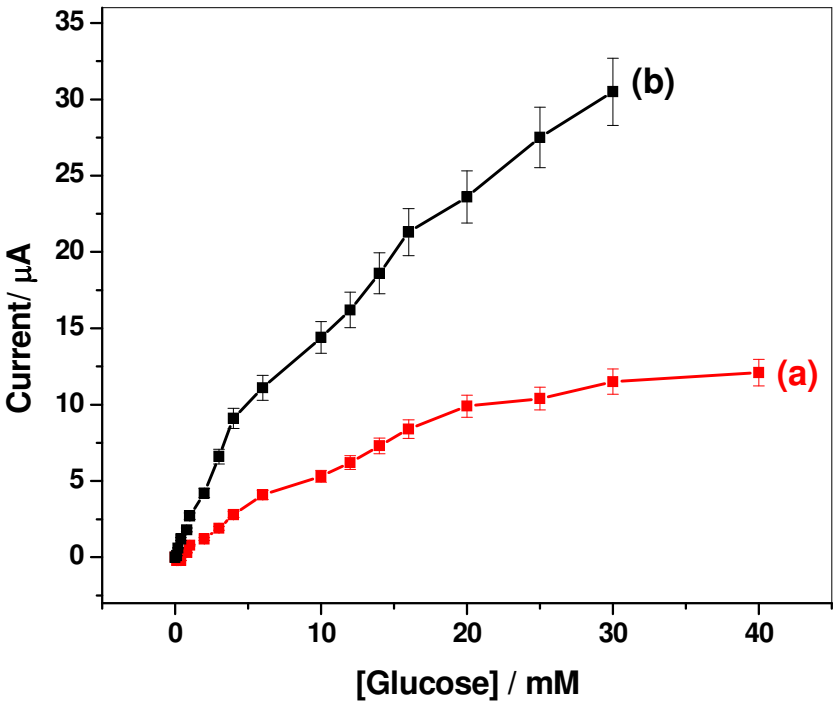

Fig. 7. Amperometric response of a) GOx-PAni and b) GOx- PAni- Pec NPs biosensor towards glucose addition.

Table 1 in ***Supplementary Information*** shows the response of other glucose biosensors reported in literature with respects to some of the important parameters like LOD, sensitivity and interference effects. The sensitivity value obtained in the present study was much higher as

compared to the value reported in literature for the various PAni based glucose biosensors (See **Table1** in ***Supplementary Information***) such as PAni-CNT-platinum nanoparticle composite based biosensor[10]. A simple control over the morphology of PAni could improve the sensitivity to a value much higher than that attained by using CNTs or metal nanoparticles. As far as comparison with reports on nanostructured PAni is concern, the current response and sensitivity obtained in the present study is one order of magnitude higher than that reported for PAni nanowires synthesized by interfacial polymerization[38]. Horng *et al.*[35] have reported a high sensitivity of 2.5 mA mM^{-1} cm^{-2} for PAni nanowires deposited on carbon cloth electrode but their detection limit (0.05mM) is comparable to that achieved in the present study. Very low detection limits (~0.3µM) have been achieved by Wang *et al.* [36] with highly ordered polyaniline nanotubes synthesized using anodic aluminum oxide membrane as a template but their sensitivity is of the same order as that obtained in the present study using PAni-Pec nanoparticles. The linear range for the GOx- PAni- Pec NPs based biosensor (0.06- 4 mM) was much wider as compared to that reported for glucose biosensor based on PAni nanowires [38]. Thus the glucose sensor reported herein exhibited excellent performance, especially in terms of sensitivity and detection limit, in comparison with other several other glucose-sensors reported in literature.

3.4. Reproducibility, selectivity and stability of the enzyme electrode

The reproducibility of biosensor was evaluated by carrying out glucose estimation with three different freshly prepared GOx- PAni- Pec NPs electrodes. A relative standard deviation (RSD) of 4.16 % was obtained for three measurements at glucose concentration of 0.4 mM, thus revealing a reasonably good reproducibility of the biosensor electrode (**Table S2** in

Supplementary Information). The repeatability of the biosensor was studied by carrying out three different measurements using the same GOx- PAni- Pec NPs electrode. A RSD of 4.05% was obtained at glucose concentration of 0.4mM, indicating an acceptable repeatability of measurements (See **Table2** in ***Supplementary Information***).

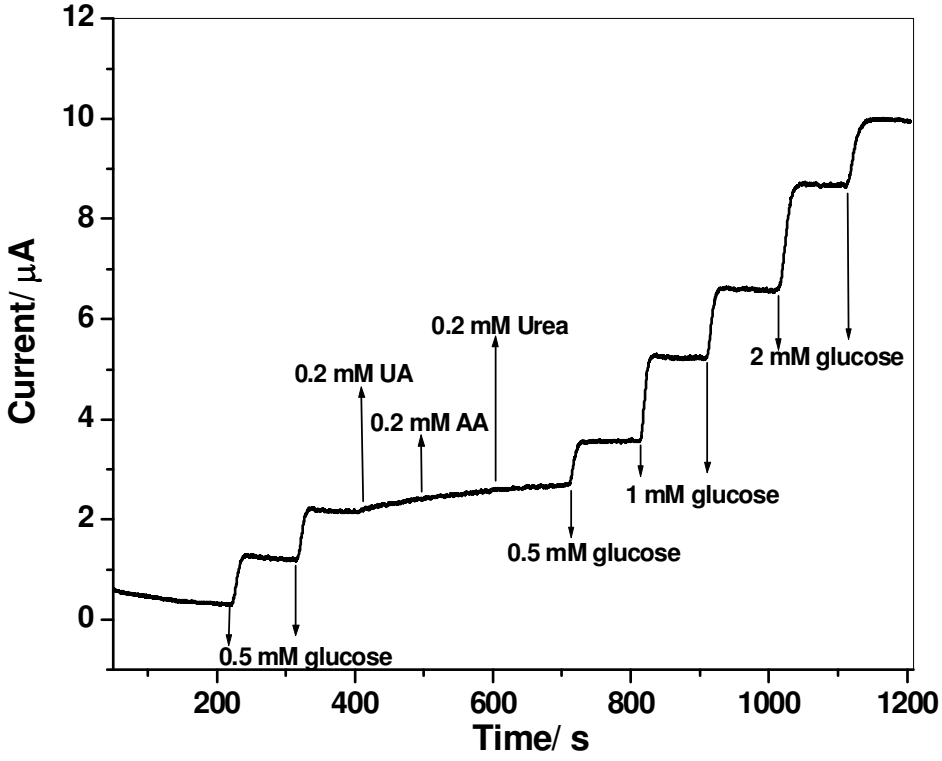

Fig. 8: Chronoamperometric response of GOx- PAni- Pec NPs biosensor to successive addition of glucose, (UA) uric acid (0.2 mM), (AA) ascorbic acid (0.2 mM) and 0.2 mM urea in PBS (pH= 7.4) at an applied voltage of 0.6 V.

Performance of the biosensor was evaluated in presence of ascorbic acid (0.2mM), urea (0.2mM) and uric acid (0.2mM) which can serve as potential interferents during glucose monitoring. The chronoamperometric response (**Fig. 8**) clearly shows that even in the absence of a perm-selective

or substrate selective membrane, the biosensor displayed good selectivity and its performance at this operating potential was not influenced by the presence of other electroactive species in the test sample.

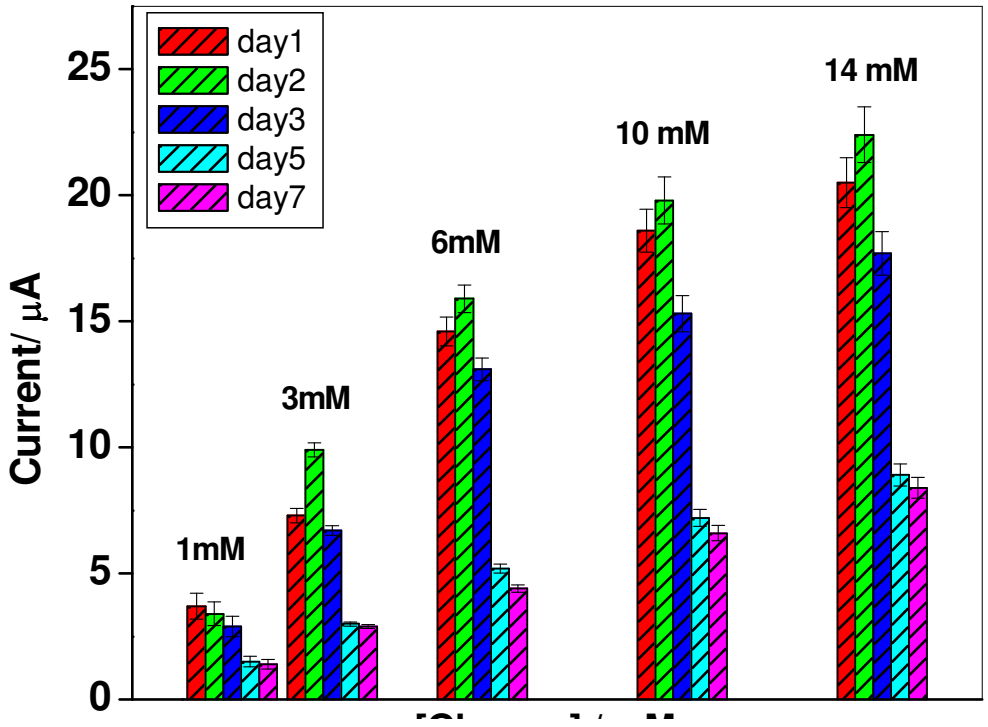

Fig. 9: Stability of the GOx- PAni- Pec NPs biosensor as depicted from the current response for estimation of glucose over a period of 7 days.

Stability of the biosensor was monitored for a period of seven days. **Fig. 9** depicts current response of biosensor to various concentration of glucose for measurements carried out on different days. Activity of biosensor was found to increase on the second day which may be due to increased porosity of the PAni-Pec NP matrix allowing efficient diffusion of substrate. On the

third and seventh day, 83% and 55% of the initial activity was retained respectively. Though the biosensor exhibited an excellent response for initial two days, the electrode activity was found to decreases to a large extent on repeated use for seven days. This could be due to leaching of the Gox-PAni- Pec NPs from the electrode surface on repeated use. The leaching can be reduced by using binders (such as nafion) for entrapment of the enzyme loaded nanoparticles, which provide good entrapment of NPs while allowing diffusion of the analytes.

3.5. Glucose determination in serum samples

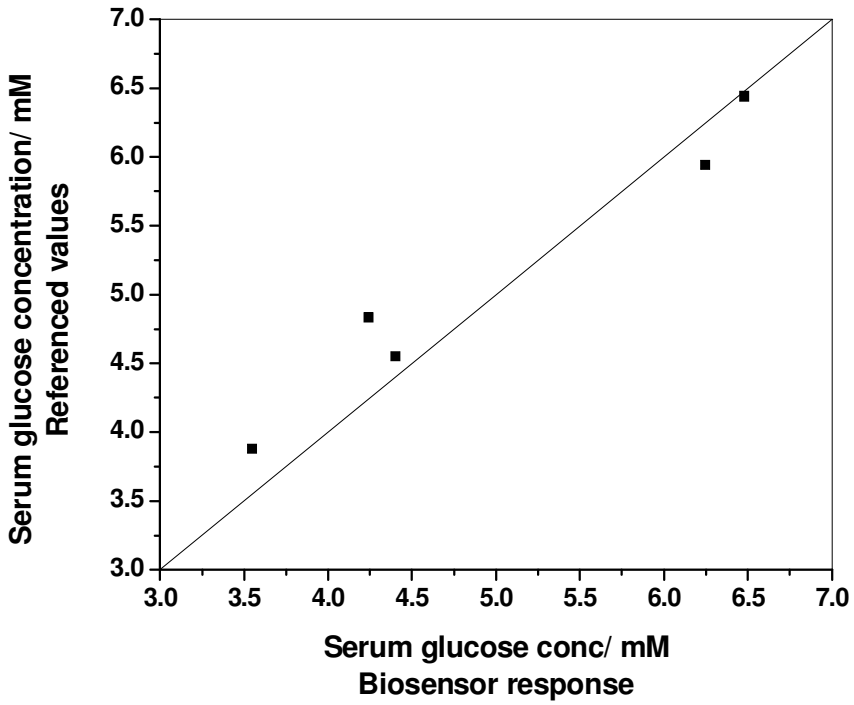

Fig.10: Comparison of the serum glucose concentration estimated using the GOx- PAni- Pec NP biosensor with the reference values as estimated by pathology lab.

Efficiency of the biosensor for analysis of real samples was tested by estimating glucose in human blood serum samples. The serum samples were diluted 5 times with 0.1M PBS (pH 7.4) and the measurements were carried out using a single biosensor electrode for a given set of samples. The concentration of glucose in the serum sample estimated based on biosensor response was plotted against the respective glucose concentrations estimated by pathology lab (reference value). A representative set of data is depicted in **Fig. 10**. All the data points are close to the line of unit slope indicating that the experimental data values measured using the biosensor match very well with the reference value demonstrating reliability of the biosensor. The % bias and relative standard deviation values for estimation of glucose in blood serum samples are presented in **Table S3** in ***Supplementary Information.***

Thus the PAni-Pec NPs based glucose sensor exhibited excellent performance in terms of sensitivity, stability and reliability. Immobilization of GOx on biocompatible polyaniline nanoparticles helped to improve and retain its effective catalytic activity towards glucose resulting in three time's higher sensitivity as compared to conventional polyaniline. The sensitivity can be further improved by using high surface area conducting electrodes like carbon cloth as the substrate instead of platinum. The biosensor design presented herein can be extended for bioanalysis of other analytes by selection of the appropriate biocatalyst.

3. Conclusion

In this work, an alternate strategy for fabricating amperometric glucose biosensor based on PAni-Pec-GOx NPs has been proposed. PAni-Pec NPs were synthesized using pectin as a stabilizer and characterized by a complementary combination of UV-vis spectroscopy, FTIR, SEM and DLS studies. The nanoparticulate morphology of PAni permitted rapid electron-transfer while

providing large surface area for efficient spatial distribution of the GOx enzyme resulting in three times higher sensitivity as compared to conventional PAni. The sensitivity obtained was much higher as compared to that reported in literature for several composites of PAni with functional materials such as CNTs or metal nanoparticles. Pectin not only helped to stabilize the colloidal nanoparticles of PAni but also rendered it biocompatible as evident from the cell viability studies. In addition to this, the layer of pectin permitted covalent immobilization of the enzyme to PAni. The biosensor displayed excellent electrocatalytic response for the detection of glucose in terms of sensitivity (79.49 $\mu AmM^{-1}cm^{-2}$), detection limit (43.5μM) and stability. The linear range of detection (0.06- 4 mM) of the biosensor was well within the glucose concentration range present in real blood samples. The biosensor demonstrated satisfactory performance for glucose analysis in real samples thereby endorsing its potential for clinical application. This approach can be further extended for low level detection of other analytes by incorporating appropriate biological component during electrode fabrication.

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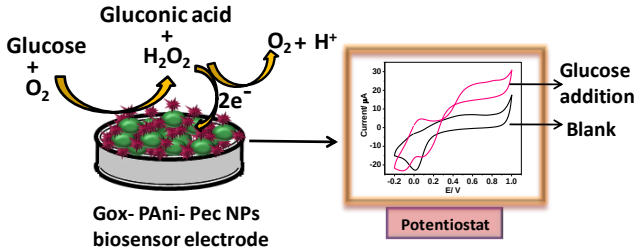

A facile strategy for synthesis of polyaniline nanoparticles and their application for development of highly sensitive amperometric glucose biosensor is demonstrated herein.