Supplementary Information

Pectin coated polyaniline nanoparticles for amperometric glucose biosensor

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1. Experimental section

A) FITC tagging procedure.

0.25 mg FITC was added to 10 mg each of PAni- Pec and GOx- PAni- Pec NPs dispersed in PBS (pH 7.4) in two different vials. The vials were kept on a shaker for 1 h at RT. The particles were centrifuged, washed twice with PBS and finally re- dispersed in 1ml PBS. 10 µl of dispersed particles were casted uniformly on a clean glass slide for recording the fluorescence microscope images.

B) Colorimetric assay for estimation of the amount of GOx loaded on PAni- Pec NPs.

The amount of GOx loaded on PAni-Pec NPs was determined by colorimetric enzyme assay[1]. GOx -PAni- Pec NPs were incubated with 1mM glucose in PBS solution (pH 7.4) for ten min at room temperature and then a mixture of HRP (0.001 wt %), 4-Aminoantipyrine (0.006 wt %) and of N,N- diethyl aniline (0.0045 wt %) were added to the above solution. The H_2O_2 released on catalytic oxidation of glucose by GOx, leads to oxidative coupling of N, N-diethylaniline with 4-amino-antipyrine resulting in the formation of a purple dye which absorbs at 553 nm. A standard curve [Absorbance at 553 nm for a constant value of glucose (1 mM) versus different GOx concentration] was plotted and used to determine the amount of GOx loaded on PAni- Pec NPs.

C) Biocompatibility studies

Polyurethane (PU) films were used as substrate to coat PAni- Pec NPs. PU was soaked in aqueous solution contains PEC, aniline, HCl for 24 h and later initiator ammonium persulfate was added in the mixture to initiate the formation of PAni-PEC on the PU films.

Homogeneous green color on the PU film indicated the formation of PAni-PEC on the PU films (PU-PAni-PEC) and used for the biocompatibility studies.

The biocompatibility of polymer films was assessed with L6 rat myoblast cells. L6 cells were cultured in DMEM medium supplemented with 10 % FBS and 1 % antimycotic-antibiotic solution in an incubator humidified with 5 % CO_2 at 37 °C. Polymers films were sterilized with 70 % ethanol and equilibrated with phosphate buffered saline (PBS) for 1 h. Approximately 10^4 L6 cells were counted and added to the polymer films and incubated for 24 h. The cell viability was determined by MTT assay[2] and the percentage cell viability was determined by using the formula,

% cell viability = [Absorbance of cells cultured with polymer/ absorbance of cell cultured alone] x100

2. Figures and Table

Figure S1: FT-IR spectra of (a) PAni- Pec NPs (b) GOx- PAni- Pec NPs

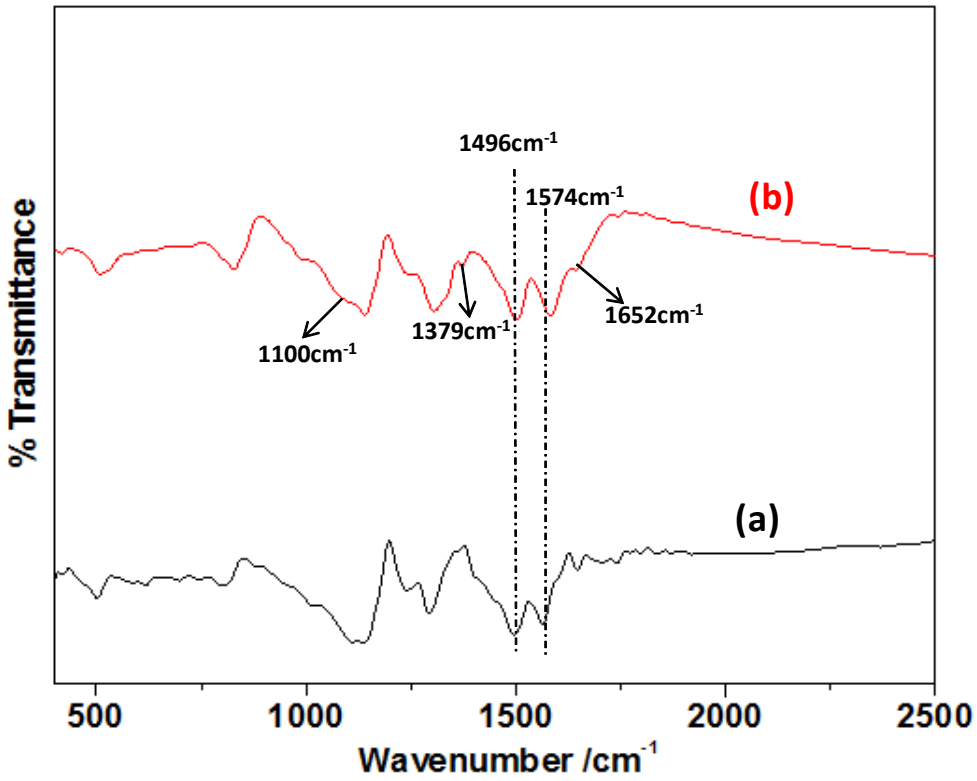


Figure S2: Hydrodymanic radius distribution plot of PAni- Pec and GOx-PAni- Pec NPs.

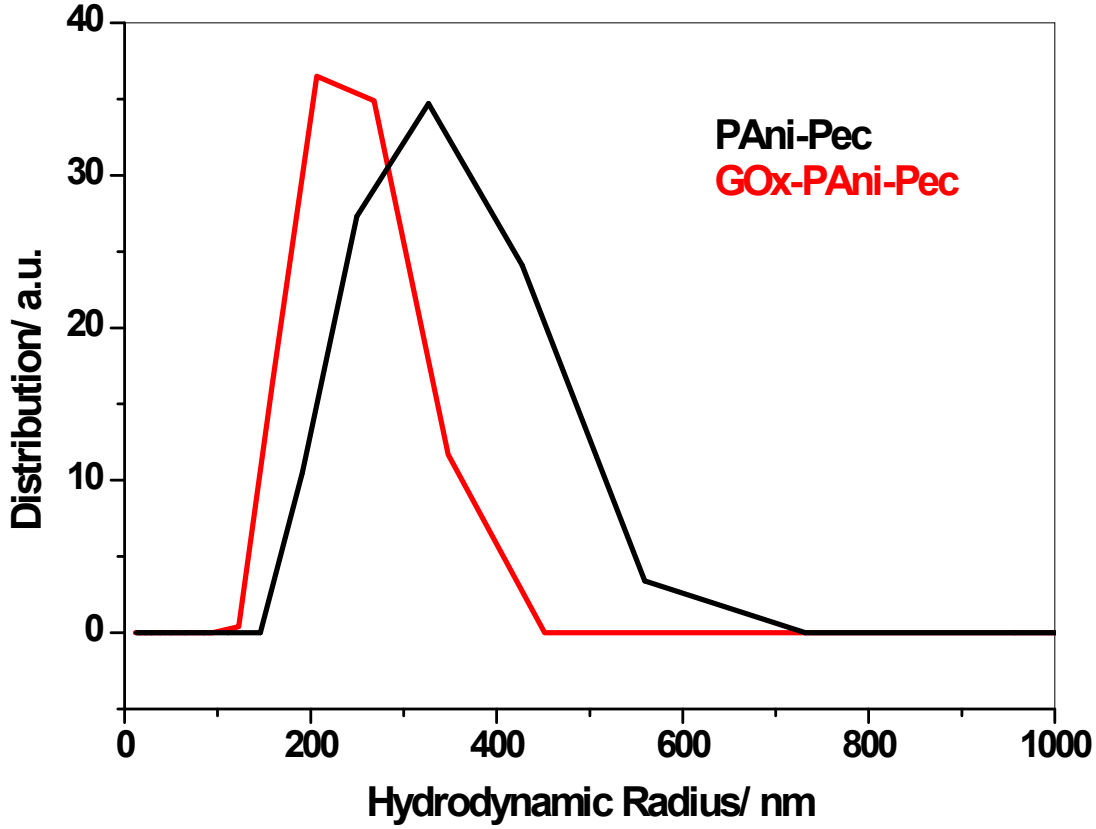


Figure S3: CV of a) PAni- Pec NPs and b) GOx- PAni- Pec NPs in 1 M HCl at a scan rate of 50 mVs^{-1}.

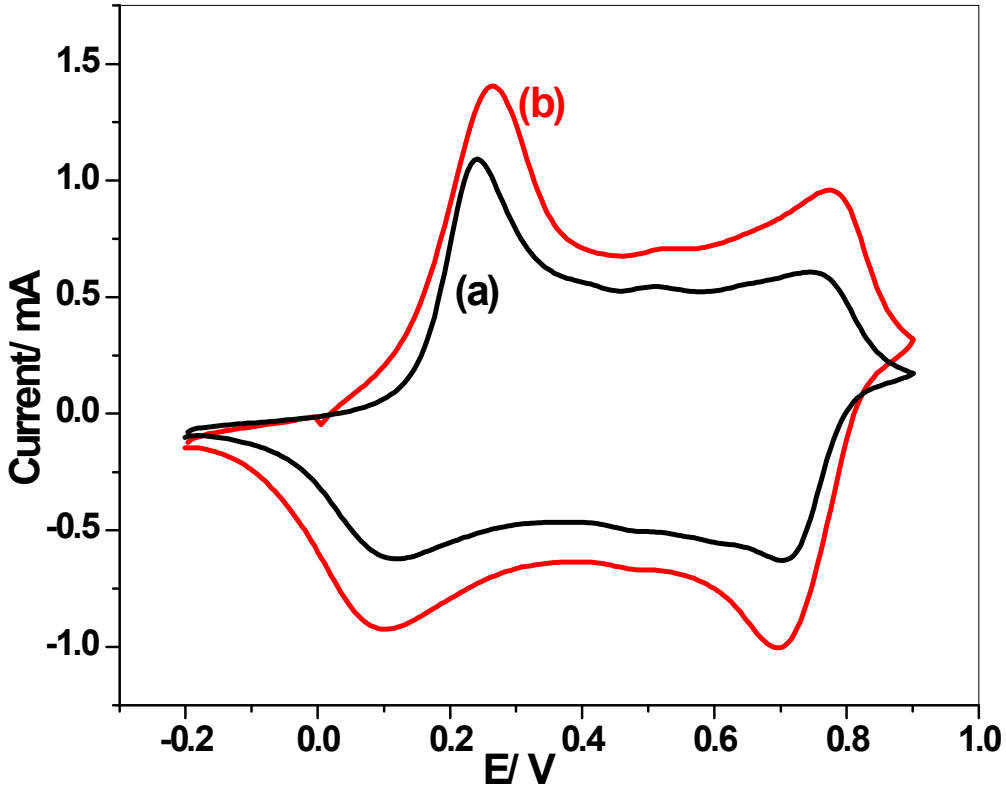


Figure S4: Cyclic voltammogram of PAni modified electrode with increasing H_2O_2 concentration.

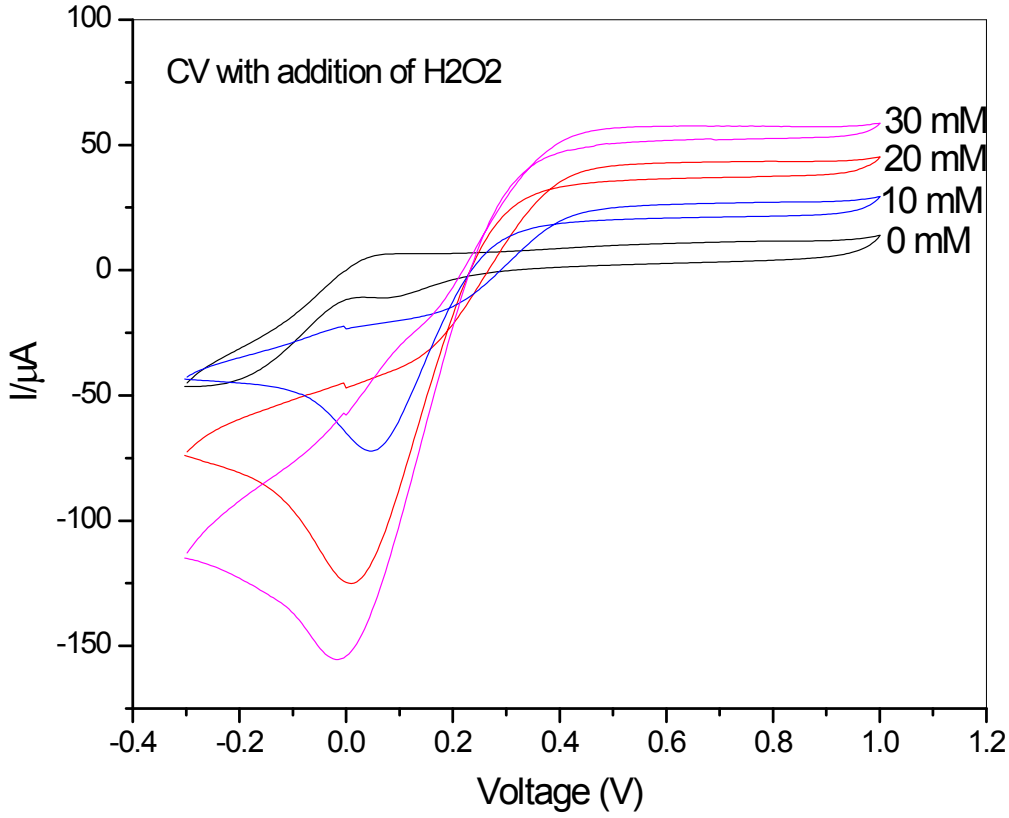


Figure S5: Cyclic voltammogram of GOx- PAni- Pec NPs in 2mM glucose (PBS) at different scan rates. Inset: Plot of current at 0.6 V vs. scan rate.

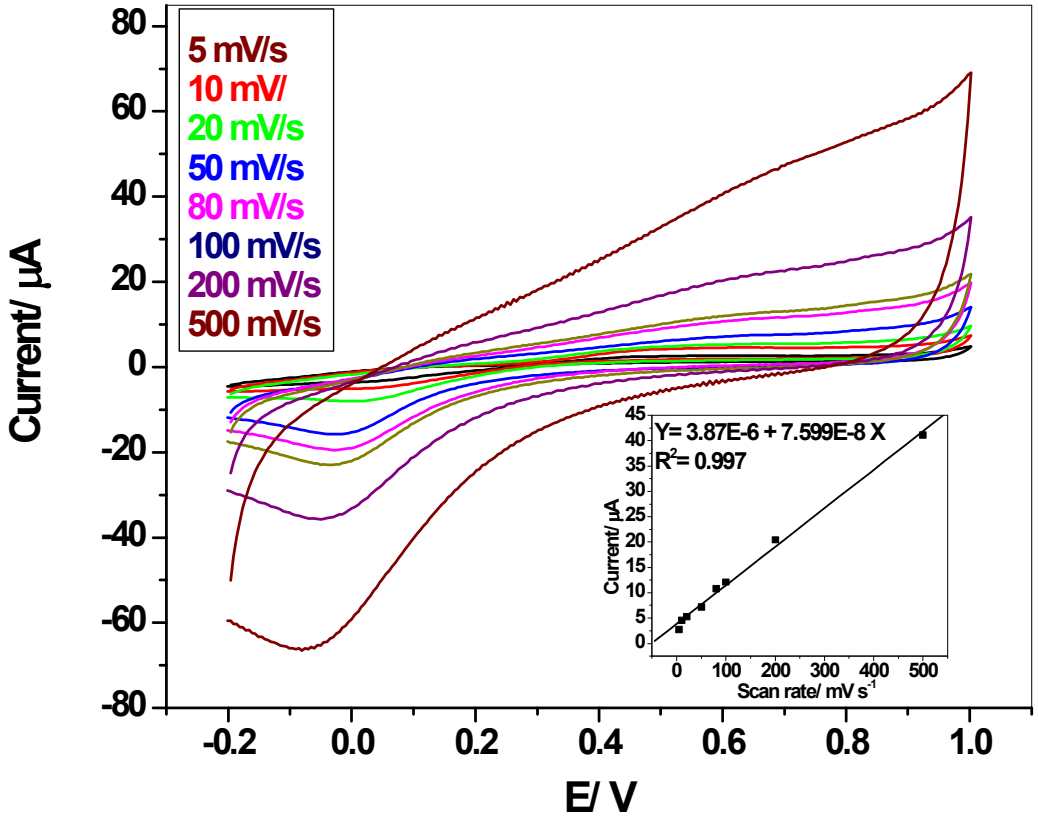


Figure S6: Amperometric response of the biosensor electrode towards glucose at various working potentials. Inset: The corresponding amperograms on application of pulse of different voltages.

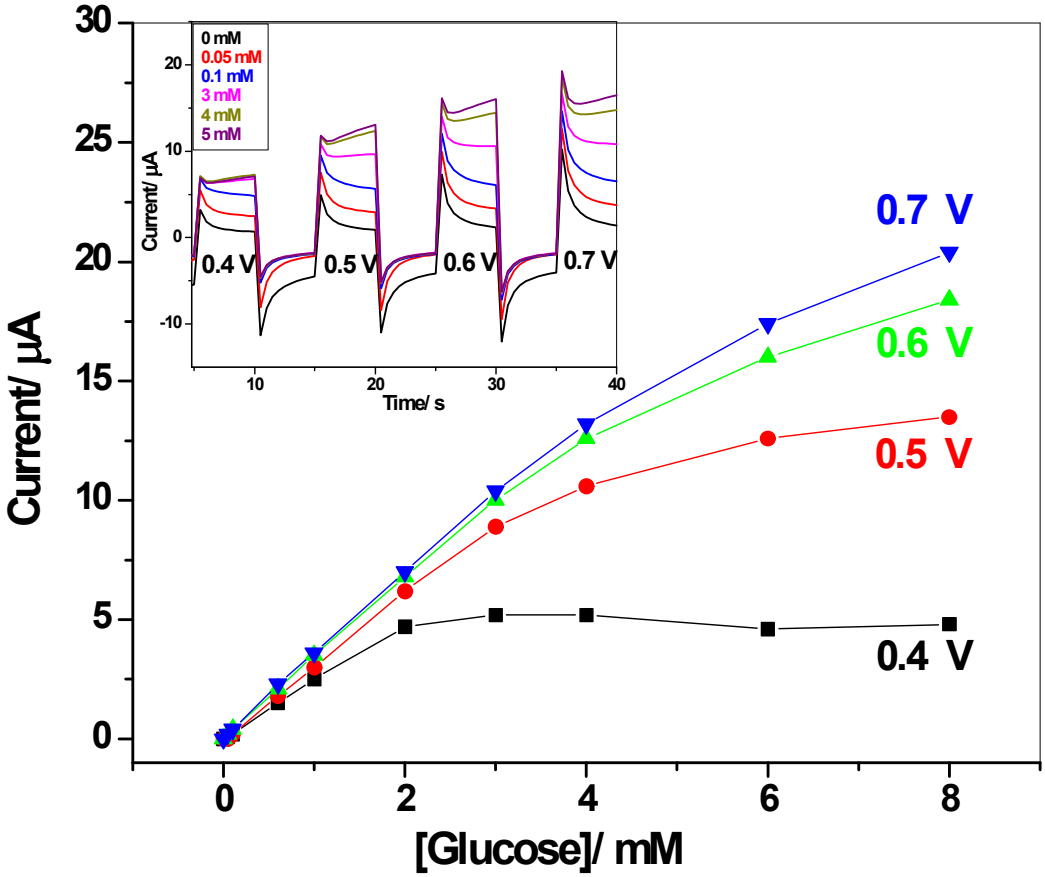
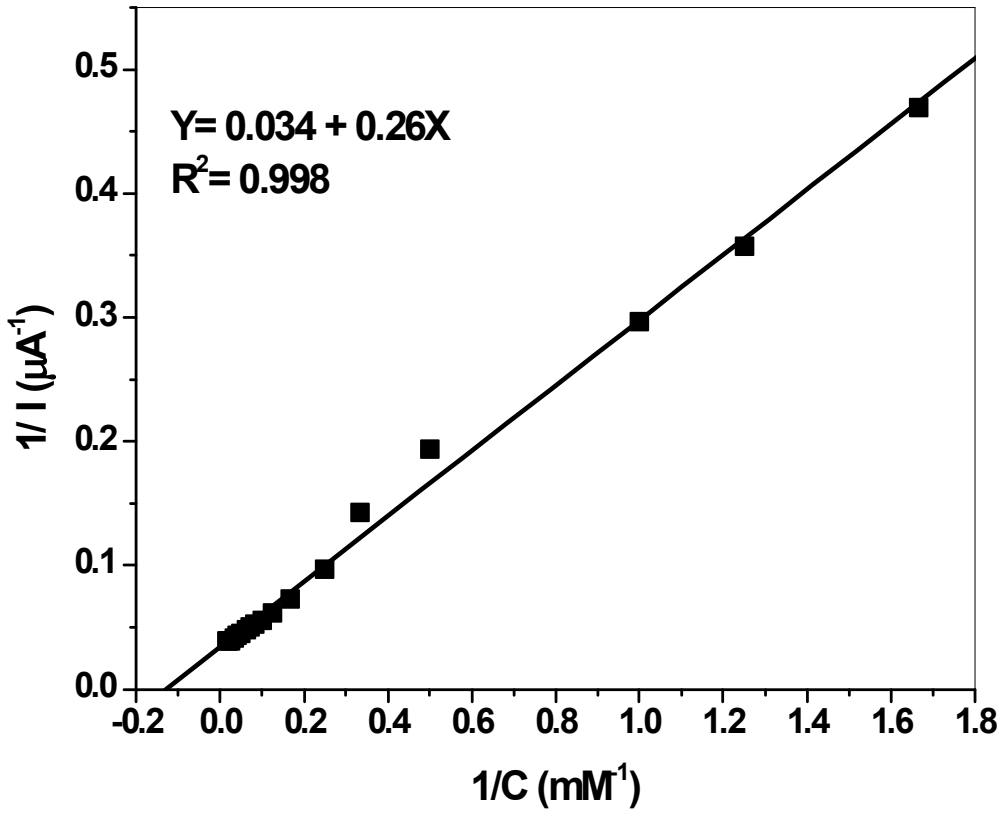


Figure S7: Lineweaver-Burk plot for amperometric response of GOx- PAni- Pec NPs biosensor towards glucose addition.



2A: Advantages and drawbacks of various methods used for synthesis of PAni nanostructures

No.	Polymerization Method	Advantages	Drawbacks	Reference
1	Template and seeded growth	Controlled morphology	Need to remove the template	3
2	Electrospinning/ Electrochemical polymerization	Affords uniform fibres, gives good adherence to the substrate	Requires conducting substrate	3
3	Surfactant assisted polymerizations	Controlled morphology	Difficulty in removing surfactants from resulting nanostructure during post-synthetic treatments	3
4	Interfacial polymerizations	Nanofibered morphology	Involves the use of organic solvent during synthesis and low yield	4
5	Sonochemical synthesis	Smooth surface with uniform morphology	Requires additional equipment	5
6	Solution polymerization	Tuneable morphology	Intractable product	5
7	Enzymatic synthesis	Eco-friendly	Expensive	5
8	Our method (GOx-PAni-Pec NPs)	Controlled morphology, no organic solvent used, highly biocompatible environment for the entrapped biomolecule due to biopolymer pectin	-	Present work

Table S1: Overview of sensor characteristics for the various glucose sensors reported in literature. The characteristics nano-structured polyaniline based glucose sensors are highlighted in grey.

Electrode Material	Linear Range	Sensitivity	Detection Limit	Km	Interference effects	Ref.
Pt/GOx-PVA	0.3-2.0 mM	9.66 μA mM^{-1}	10 μM	12.8 mM	No interference due to Fructose, sorbitol and UA.A minimal interference from AA observed.	6
PB/MWNTs-GOx-CS-ICPTES sol–gel composite film	0.025-1.3 mM	15.2 μA mM^{-1} cm^{-2}	7.5 μM	3.67 mM	No interference due to AA and UA.	7
GOD/Pt/MWNT-PAni biosensor	0.003-8.2 mM	16.1μAmM^{-1}	1 μM	0.64 mM	No interference due to UA, Ethanol, L-cysteine, AA and L-tyrosine.	8
PAni/PAN-GOD	0.002-12mM	34.11 μA mM^{-1} cm^{-2}	2μM	13.0 mM	No interference due to AA, glutathione and acetaminophen. A negligible interference effect from UA observed.	9
GOx/Pt-DENs/PAni/CNT	0.001-12 mM	42.0 μA mM^{-1} cm^{-2}	0.5 μM	-	No interference due to UA, AA and acetaminophen.	10
The GOD/CS-PB/CS	0.002-0.4 mM	-	0.397 μM	3.73 mM	No interference due to UA and AA.	11
Pt-DENs/GOx/Pt-DENs/PAni/PSS	0.01-4.5 mM	39.63 μA mM^{-1} cm^{-2}	0.5 μM	-	No interference due to UA, acetaminophen and AA.	12
PtNPs- PAni hydrogel	0.01-8 mM	96.1 μA mM^{-1} cm^{-2}	0.7 μM	0.572 mM	No interference due to AA and glutathione. A negligible interference effect from L-cysteine observed.	13

PAni- nanowires	0-8 mM	2.5 mA mM^{-1} cm^{-2}	0.05 mM	-	-	14
PAni- nanotube	0.01-5.5 mM	97.18 ±4.62 µA mM^{-1} cm^{-2}	0.3 ± 0.1 µM	2.37 ± 0.5 mM	No interference due to AA, UA and 4-acetamidophenol.	15
PAni- nanofibre	0.01 -1 mM	0.5µA/mM	0.5 µM	1.05± 0.04 mM	No interference due to AA, UA and 4-acetamidophenol.	16
GOx- PAni-Pec NPs	0.06- 4 mM	79.49 µA $mM^{-1}cm^{-2}$ (2.5 µA mM^{-1})	43.5µM	7.65 mM	No interference due to AA, UA and urea.	Our Work
GOx loaded Normal PAni	0.8 - 4 mM	22.95 µA $mM^{-1}cm^{-2}$	780 µM	62.85 mM	-	Our Work

* Pt= Platinum, GOx- Glucose oxidase, PVA= Poly (vinyl alcohol), MWNTs= Multiwalled carbon nanotube, PB= Prussian blue, CS= Chitosan, ICPTES= 3- isocyanatopropyltriethoxysilane, PAN= Polyacrylonitrile, DENs= Dendrimers, CNT= Carbon nanotubes, PSS= Polystyrene sulphonic acid, PtNPs= Platinum nanoparticles, UA=Uric acid, AA= Ascorbic acid.

Table S2: Reproducibility & repeatability of the biosensor

Reproducibility studies: The current response of three different freshly prepared GOx- PAni- Pec NPs biosensor towards addition of 0.4 mM glucose concentration is presented below.

Electrode No.	1	2	3
Current	1.163	1.248	1.254
Mean current (μA)	1.222		
STDev	0.050		
RSD (%)	4.16 %		

Repeatability studies: The current response of GOx- PAni- Pec NPs biosensor towards addition glucose for three different measurements using the same sensor electrode.

Glucose (mM)	Measurement1 Current (μA)	Measurement2 Current (μA)	Measurement3 Current (μA)	Average Current (μA)	Std.Dev	% RSD
0.4	1.437	1.532	1.426	1.464	0.0593	4.05 %

Table S3: Determination of glucose in blood serum samples using the GOx- PAni- Pec NPs biosensor

No.	Values measured using the biosensor (mM)	Values reported by pathology lab (mM)	Bias (mM)	Relative deviation (%)
1	3.55	3.88	-0.23	-8.5%
2	4.40	4.55	-0.30	-3.29%
3	4.24	4.833	-1.55	-12.2%
4	6.25	5.94	1.20	5.20%
5	6.48	6.44	0.38	0.6%

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