Supplementary Information

3D phosphorus doped mesoporous graphitic carbon nitride based immunosensor for swine flu detection

Vishakha Nirbhaya[a], Yogesh Kumar[a], Ramesh Chandra[b], Suveen Kumar[a*]

[a]Department of Chemistry, University of Delhi, Delhi-110007, India.

[b]Institute of Nano Medical Sciences, University of Delhi, Delhi-110007, India.

*Corresponding author: suveendev@gmail.com

1. Reagents and Materials:

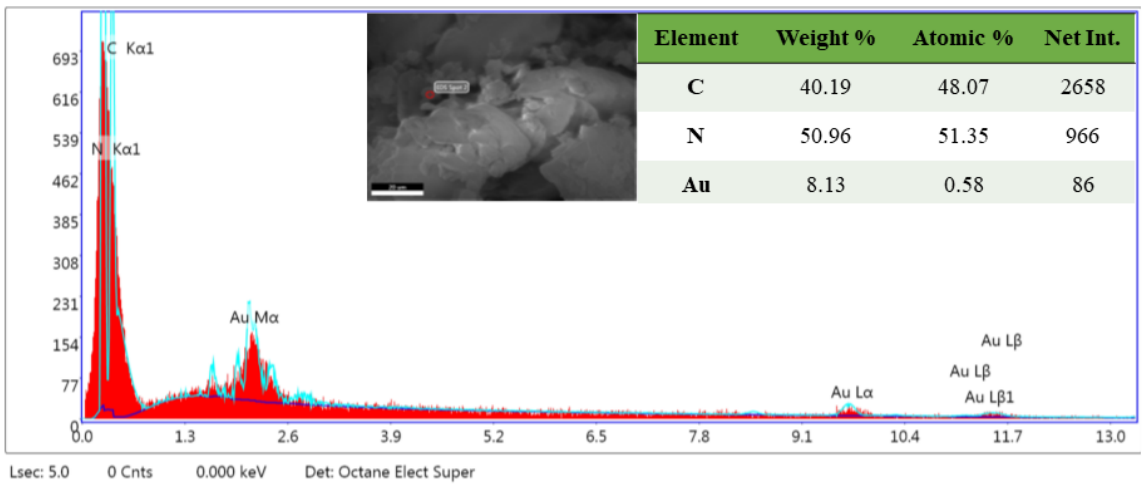
Melamine [$C_3H_6N_6$] was purchased from Loba Chemie Pvt. Ltd., cyanuric acid [$C_3H_3N_3O_3$] purchased from GLR innovations, N, N-Dimethylformamide (DMF) [C_3H_7NO] was purchased from SRL Pvt. Ltd., Dimethyl sulfoxide (DMSO) was procured from Fisher Scientific. Bovine serum albumin (BSA), 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC) [$C_8H_{17}N$] and L-serine were procured from Sigma-Aldrich. N-hydroxy succinimide (NHS), sodium dihydrogen phosphate dehydrate [$NaH_2PO_4.2H_2O$], disodium hydrogen phosphate dihydrate [$Na_2HPO_4.2H_2O$], potassium hexacyano ferrate (II) trihydrate [$K_4(Fe(CN)_6)3H_2O$)], potassium hexacyano ferrate (III) [$K_3(Fe(CN)_6)$] and sodium chloride [NaCl] were procured from Merck Life Science Pvt. Ltd. Phosphate buffer saline (PBS) solutions of different pH were prepared using 0.2 M $NaH_2PO_4.2H_2O$ and 0.2 M $Na_2HPO_4.2H_2O$ with NaCl using deionized water. Serum Amyloid A monoclonal antibody (anti-SAA) and Serum Amyloid A (SAA) protein biomolecules were purchased from Wuhan Xinqidi Biological Technology Co., Ltd. (Wuhan, China). All the chemicals were of analytical grade and were used without any purification. All electrochemical studies were conducted in triplicate.

1.2 Instrumentations

The structural, morphological and surface characterization was carried out via X-ray diffraction studies (XRD; Rigaku Miniflex 600), Scanning electron microscope (SEM; JEOL JSM 6610LV, Japan), Field emission scanning electron microscope (FESEM; Zeiss Gemini), Transmission electron microscope (TEM; FEI Tecnai G220 S-Twin), and Fourier transform infrared spectroscopy (FT-IR; Nicolet iS50), Brunauer-Emmett-Teller (BET) theory studies (Micromeritics Instrument Corp.). The electrochemical measurements were performed on an Autolab Potentiostat Galvanostat (AUT204 Netherlands).

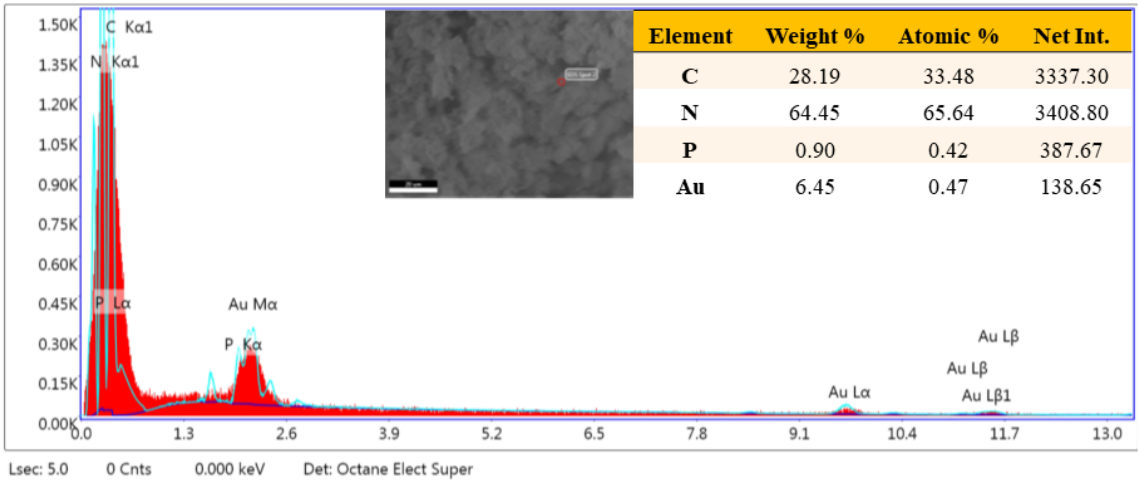
Table S1: BET surface areas and average pore size of g-C_3N_4 and P-C_3N_4.

Surface Analysis parameter/ Compound Name		**g-C_3N_4**	**P-g-C_3N_4**
Surface Area (m^2/g)	BET Surface Area	114.5	92.65
Pore Volume (cm^3/g)	Adsorption & Desorption	0.154656 0.207379	0.204753 0.208754
Pore Size (nm)	Adsorption & Desorption	6.57 5.83	8.63 8.62
	average pore diameter (4V/A by BET)	5.5	8.59
	Mean pore size	2.34	2.6



Element	Weight %	Atomic %	Net Int.
C	40.19	48.07	2658
N	50.96	51.35	966
Au	8.13	0.58	86

Figure S1: Energy dispersive X-Ray spectroscopy results of g-C_3N_4.



Element	Weight %	Atomic %	Net Int.
C	28.19	33.48	3337.30
N	64.45	65.64	3408.80
P	0.90	0.42	387.67
Au	6.45	0.47	138.65

Figure S2: Energy dispersive X-Ray spectroscopy results of P-g-C_3N_4.

Table S2: List of Heterogenous electron transfer rate (HET k°) of the fabricated electrodes.

S. No.	Electrode	R_{ct} (Ω)	HET rate (k^o)[i] (cm s^{-1})
1.	g-C_3N_4/ITO	132.7	1.605×10^{-6}
2.	P-g-C_3N_4/ITO	66.8	3.18×10^{-6}

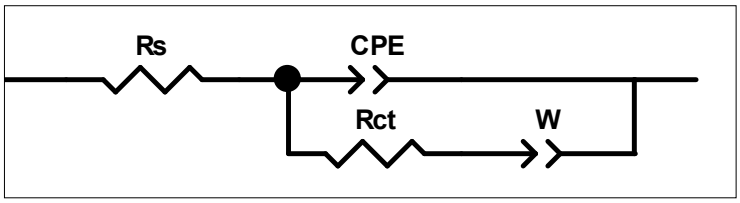


Figure S3: Randles equivalent circuit model

[i] HET = Heterogenous electron transfer rate (k°),

$$R_{ct} = \frac{RT}{n^2F^2Ak^{\circ}C}$$

where, R = molar gas constant, T = temperature, n = number of electrons (n = 1), F = Faraday constant, A = active surface area of the electrode, C = concentration of the electroactive species and R_{ct} = charge transfer resistance of electrode surface.
R_s= resistance of solution, CPE= constant phase element and W= Warburg impedance.

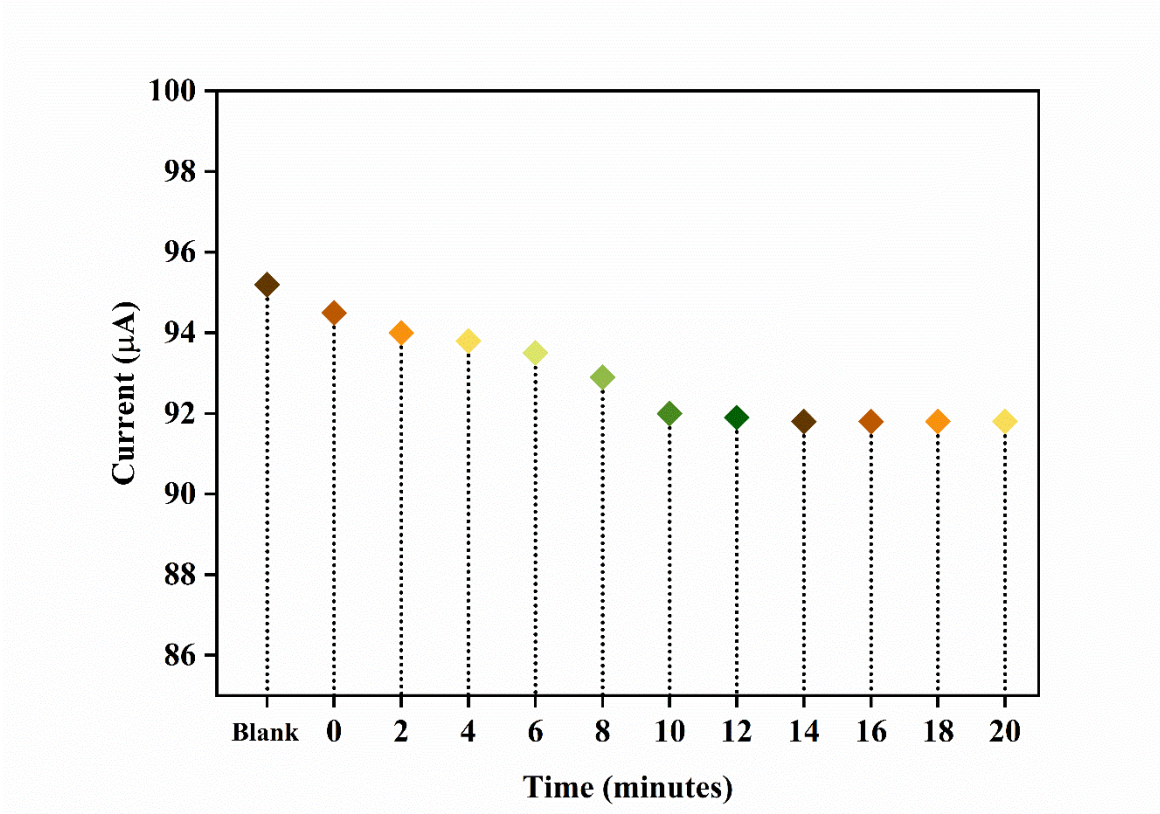


Figure S4: Incubation study plot of peak current (μA) vs time (min.) for BSA/anti-SAA/Ser/P-g-C_3N_4/ITO bioelectrode against SAA protein using DPV technique.

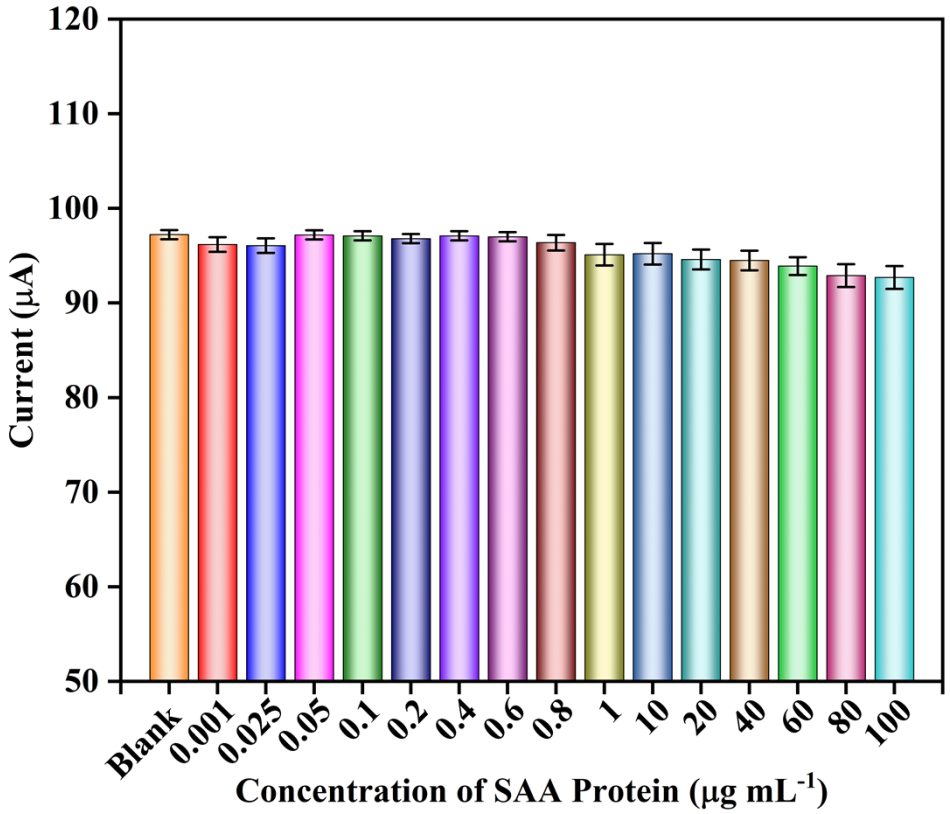


Figure S5: Control study plot of peak current (μA) vs concentration of SAA protein (10 ng mL^{-1}-100 μg mL^{-1}) for Ser/P-g-C_3N_4/ITO electrode using DPV technique.

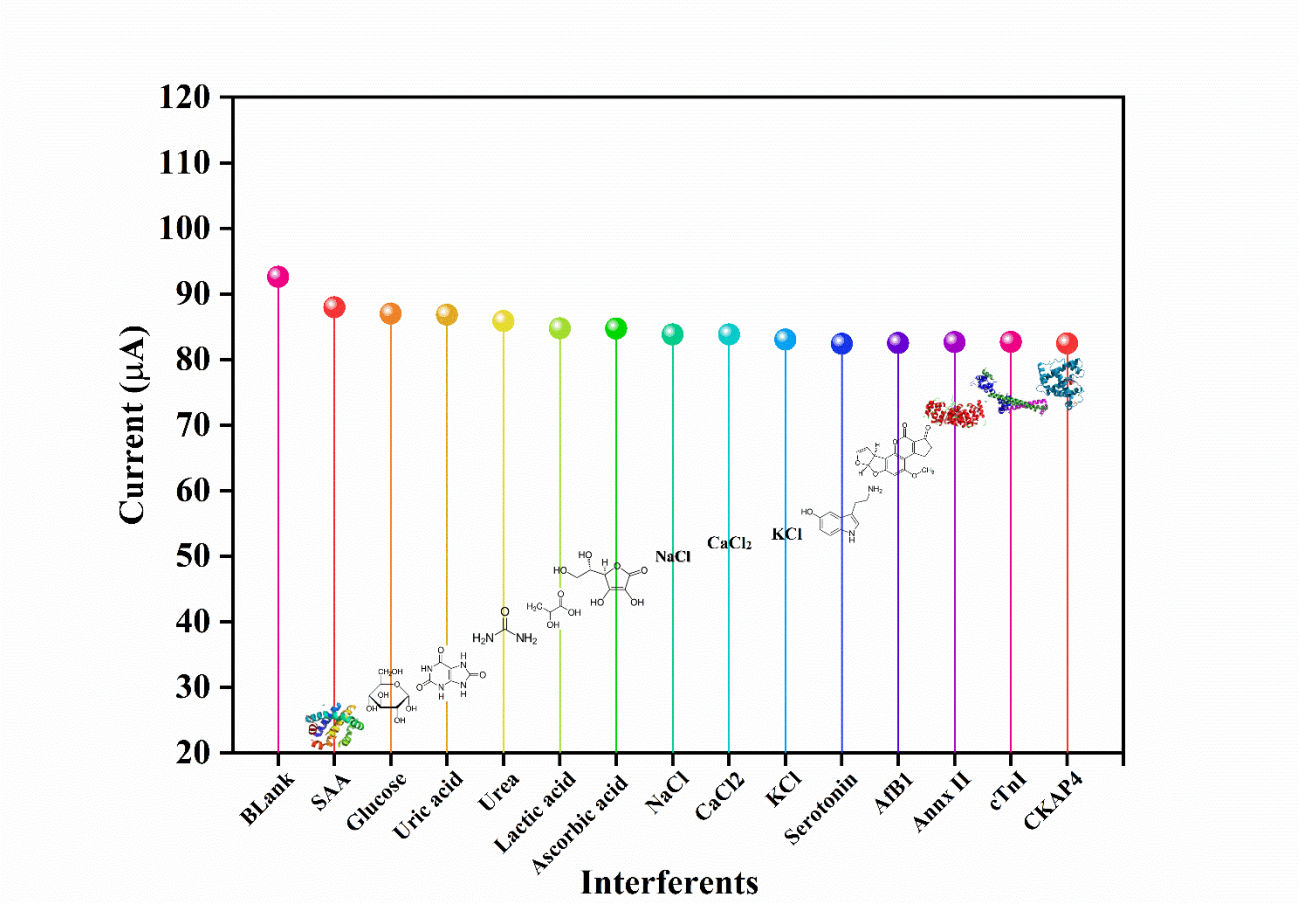


Figure S6: Interferent study plot of peak current (μA) vs various analytes present in the human blood sample (SAA = serum amyloid A, glucose, uric acid, urea, lactic acid, ascorbic acid, NaCl, CaCl2, KCl, serotonin, AfB1= Aflatoxin B1, Annx II= Annexin A2, cTnI = cardiac troponin I and CKAP4= Cytoskeleton-associated protein 4) for BSA/anti-SAA/Ser/P-g-C_3N_4/ITO bioelectrode using DPV technique.

Studies of T-test calculation for interferent studies:

The statistical comparison of the interferent studies were determined using the t-test and p-test. Therefore, we have obtained a confidence level of 95% and its calculation are as follows:

Table S3: Data values for interferent study:

S. No.	Interferents	Peak current (I_p/ µA)	%RSD
1.	Blank electrode	92.6	-
2.	Serum Amyloid A (SAA)	87.9	0
3.	Glucose	87.0	0.73
4.	Uric Acid	86.8	0.86
5.	Urea	85.8	1.71
6.	Lactic Acid	84.7	2.62
7.	Ascorbic Acid	84.7	2.62
8.	NaCl	83.8	3.38
9.	$CaCl_2$	83.8	3.38
10	KCl	83.04	4.02
11	Serotonin	82.4	4.57
12	AfB1	82.5	4.48
13	Annexin II	82.6	4.4
14	cTnI	82.7	4.31
15	CKP4	82.4	4.57
	Mean I_p ($\bar{x}$)	**84.8**	**Average %RSD 2.97**

We chose the null hypothesis $H_o = \mu = 80$

Alternate hypothesis $\mu \neq 80$

To calculate t-score using eq. S1

$$t = \frac{\bar{x} - \mu}{\sigma/\sqrt{N}} \quad \text{.....eq. S1}$$

where μ = Population means

$\bar{x}$ = Mean I_p

σ = Standard deviation of samples (i.e., Interferents)

N = No. of samples

Calculated sample standard deviation according to eq. S2

$$\sigma = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x-\bar{x})^2}$$ …… eq. S2

σ = 2.725

Standard error of mean (SEM) $\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}}$

= 0.7037

Based on the SEM, a confidence level of 95% (or statistical significance of 5%) is typically used for the data representation.

In the given data,

$\bar{x}$ = 84.8; σ = 2.725; μ = 80; N = 15

Using eq. S1,

$$t = \frac{(\bar{x} - \mu)}{\sigma/\sqrt{N}}$$

$$t = \frac{84.8 - 80}{2.725/\sqrt{15}}$$

t-score = 6.82

In denominator N-1 for sample, but for population it will be N.

Now to calculate or find p value,

(i) one-tailed t test was applied to check whether μ > 80 or not.

(ii) Two tailed test is used to check symmetrical distribution

So, for p test

Degree of freedom = N-1 = 14

Significance level = 0.05

t score = 6.82

By checking t-table for DOF= 14 and significance level = 0.05, we have found

t-value = ± 2.145 (for two tailed)

or

= 1.761 (for one tailed)

On comparing t score > t test value at 0.05 significant level.

(The p value is less than the significant level: $p < 0.05$. we can reject the null hypothesis with 95% confidence level that there is no difference between means).

So, we can say with 95% confidence level that our null hypothesis is wrong and mean of sample is greater than $\mu > 80$.

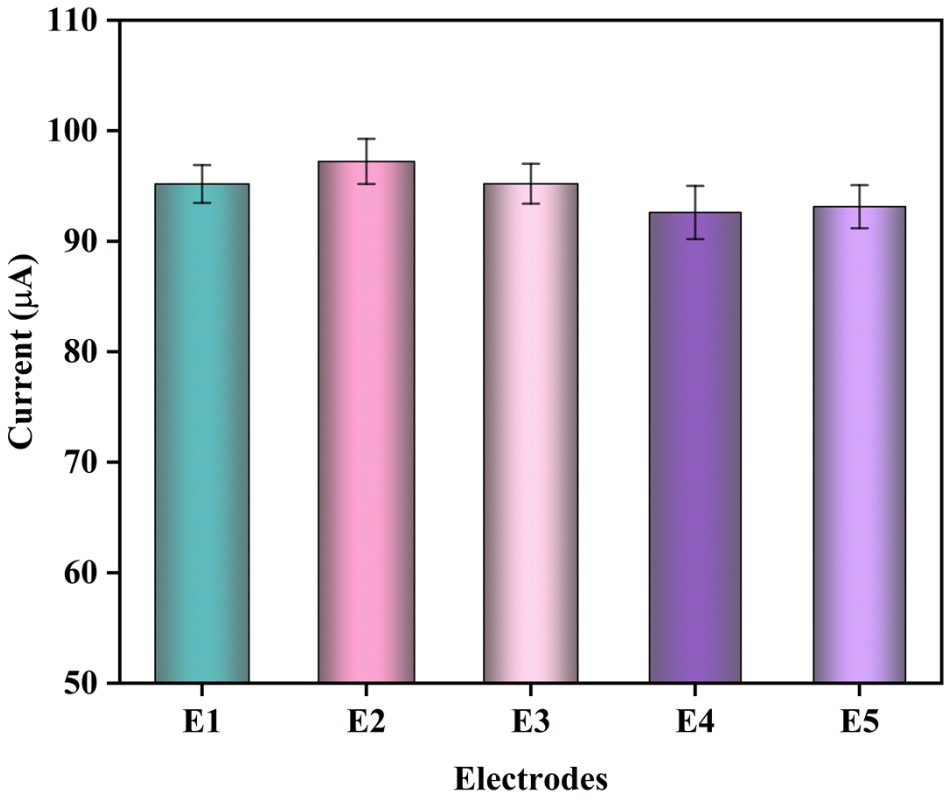


Figure S7: The bar graph of peak current (μA) vs electrodes for DPV response of five different fabricated BSA/anti-SAA/Ser/P-g-C_3N_4/ITO bioelectrodes recorded under optimized experimental condition using DPV technique.

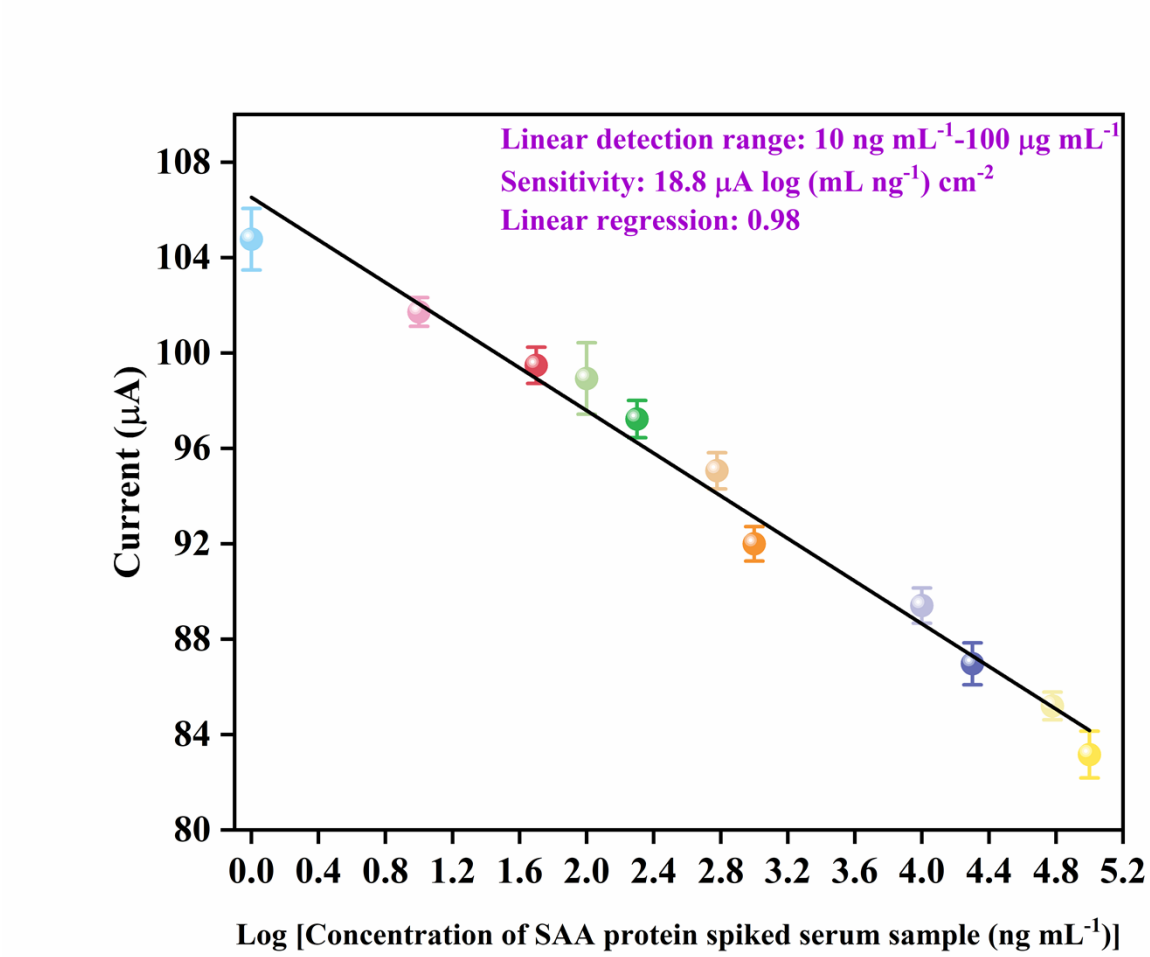


Figure S8: Calibration plot of peak current (µA) vs log of concentration of SAA protein (ng mL^{-1}) spiked serum samples for BSA/anti-SAA/Ser/P-g-C_3N_4/ITO bioelectrode using DPV technique.

Table S4: The bar graph for comparision of peak current (µA) standard SAA protein sample and SAA protein spiked serum samples

S. No.	Concentration of SAA protein (ng mL^{-1})	Peak current (µA)		% RSD
		SAA (in standard sample)	SAA (in spiked serum sample)	
1.	Blank	99.4	104.77	3.72
2.	10	98.5	101.72	2.28
3.	50	95.73	99.48	2.72
4.	100	92.89	98.93	4.45
5.	200	91.49	97.23	4.30
6.	600	89.13	95.06	4.55
7.	1000	84.62	91.58	5.58
8.	10000	81.84	89.41	6.25
9.	20000	79.71	86.97	6.16
10.	60000	76.5	85.2	7.60
11.	100000	74.21	83.16	8.04
Average %RSD				**5.06**