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Fabrication of an amperometric sarcosine biosensor based on sarcosine oxidase/ Chitosan/CuNPs/c-MWCNT/Au electrode for detection of prostate cancer

Running title: Amperometric determination of sarcosine

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Highlights

- Constructed an improved amperometric sarcosine biosensor based on covalent immobilization of SarOx onto CHIT/CuNPs/c-MWCNT/Au electrode.
- The biosensor showed optimum current within 2 s, at 0.5V, pH 7.0 and temp 35ºC.
- The limit of detection and working range of biosensor were 0.1pM and 0.1- 100 µM respectively.
- Biosensor measured sarcosine in sera, which were significantly higher in prostate cancer patients compared to apparently healthy persons.
- The biosensor was evaluated and compared with earlier sensors.

Abstract

An amperometric sarcosine biosensor was fabricated based on covalent immobilization of sarcosine oxidase (SarOx) onto the nanocomposite of carboxylated multi-walled carbon nanotubes (cMWCNT)/chitosan (CHIT) and copper nanoparticles (CuNPs), electrodeposited on gold (Au) electrode. The SarOx/CHIT/CuNPs/c-MWCNT/Au electrode was characterized by scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The enzyme electrode exhibited optimum current within 2 s at a potential of 0.2 V against Ag/ AgCl, pH 7.0 and 35 °C. A linear relationship was obtained between sarcosine concentration in the range, 0.1-100 μM and current (mA) under optimum conditions. The biosensor exhibited a high sensitivity of 277.5 μA/μM/cm^2, a low detection limit of 0.1 pM and excellent storage stability (180days). The analytical recoveries of added sarcosine in sera at 0.5 μM and at 1.0 μM concentration were 95.5% and 97.30 respectively. The precision i.e. within and between-batch coefficients of variation (CVs) were 1.08% and 1.70% respectively. There was a good correlation (R^2=0.99) between the level of sarcosine in sera as measured by the standard immuno kit method and the present biosensor. The biosensor measured sarcosine level in sera of prostate cancer patients, which was significantly higher than those of apparently healthy persons (p value<0.01).

Keywords: Sarcosine; Sarcosine biosensor; Sarcosine oxidase; Nanomaterials; Serum; Prostate cancer.

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Introduction

Sarcosine (N-methyl glycine), a natural amino acid, has been found in the muscles and other body tissues. It is an intermediate by-product of glycine metabolism. Sarcosine is generated from glycine by glycine-N-methyl transferase and degraded to glycine by sarcosine dehydrogenase [1]. It is also found naturally, as an intermediate in the metabolism of choline to glycine. The level of sarcosine in blood is 1.4 ± 0.6 μM under normal conditions [2]. Previously, sarcosine had been related to mental illness and schizophrenia. Sarcosinemia, an inborn error of

sarcosine metabolism, occurs due to deficiency of folate, which is required for conversion of sarcosine to glycine [3]. Recently, sarcosine has been found to activate prostate cancer cells and thus its occurrence in urine and blood indicates the malignancy of prostate cancer cells [4]. Although the increased level of sarcosine in tissues has been identified as a biomarker for prostate cancer progression to metastasis, it is yet to be detected in blood and urine [5]. Various methods are available for determination of sarcosine such as enzymic colorimetric method [6], enzymatic method [7], spectrometric and electrochemical analysis method [8] and liquid chromatography with tandem mass spectroscopy [9]. All these methods have one or the other drawbacks such as poor specificity, low sensitivity, time-consuming sample preparation and requirement of skilled persons to operate. Biosensing methods overcome these drawbacks as these are simple, sensitive, specific, rapid and easy to operate. A few sarcosine biosensors based on SarOx/screen printed electrode (SPE) [10], molecularly imprinted polymer (MIP) synthesized by emulsion polymerization using sarcosine as template, methacryloylamido histidine (MAH) as functional polymer and glycol dimethacrylate (EDMA) as crosslinking agent[11], paramagnetic nanoparticles based immuosensor [12], SarOx/graphene/chitosan/silver nanoparticles (AgNPs)/ glassy carbon electrode (GCE) [13] and MIP/AuNPs/ screen printed carbon electrode (SPCE) [14] have been reported. But, SPE requires a laborious and time-consuming process for working electrode, which is toxic, unstable and affected by weather [15]. MIP based biosensors require long extraction time and a large amount of solvent and are temperature sensitive and troublesome, because MIP activation suffers from incomplete removal of the template, collapsing of the cavity, distortion of binding points, and rupture of cavity [16]. To the best of our knowledge, there has been no report on biosensing of sarcosine in blood/urine.

During the past decade, a variety of nanomaterials have been employed in the construction of biosensors to improve their analytical performance. The immobilization of enzymes onto nanocomposite has enhanced their catalytic activity, as the nanoparticles (NPs) are generally surrounded by oxygen lattice [17]. CuNPs not only acts as an antibiotic, anti-microbial, and anti-fungal agent but also have high biocompatibility with the biomolecules [18]. Chitosan (CHIT) is an important biopolymer for immobilization of biomolecules, due to its excellent film-forming ability, high permeability, ease of chemical modification, mechanical strength, non-toxicity, low cost and easy availability. Further, $-NH_2$ groups of CHIT provides the hydrophilic environment

for the biomolecules. [19]. Owing to their extraordinary thermal conductivity and mechanical and electrical properties, carbon nanotubes have also been employed as additives to various structural materials. Carboxylated multiwalled carbon nanotubes (c-MWCNT) are endowed with exceptionally high material properties, very close to their theoretical limits, such as electrical and thermal conductivity, strength, stiffness, toughness and low density [20]. Gold electrode (AuE) offers many attractive features over earlier electrodes, used in sarcosine biosensors, due to their high electrical conductivity, low background current, no requirement of any pretreatment and easy availability [21]. Thus, the use of a nanocomposite of CHIT/CuNPs/cMWCNT is expected to promote the electron transfer rate of redox enzyme remarkably and retain its bioactivity. We describe herein the construction of an amperometric sarcosine biosensor based on covalent immobilization of SarOx onto CHIT/CuNPs/c-MWCNT modified Au electrode with better analytic performance. The biosensor has been employed for measurement of sarcosine level in serum, which is required for diagnosis and medical management of prostate cancer.

Experimental Methods

Materials

Sarcosine oxidase (SarOx) from *Bacillus* sp.(25U/mg) from Sigma-Aldrich, sarcosine, potassium chloride, copper sulfate, sodium phosphate monobasic, sodium phosphate dibasic, potassium ferrocyanide, potassium ferricyanide, sodium acetate and silica gel from SRL, Mumbai and sulphuric acid and hydrochloric acid from Qualigens Fine Chemicals, Mumbai and cMWCNT from Intelligent chemicals, Panchkula were used. Au wire (2mm x20mm, diameter x height) was purchased from local jeweler's shop, Sera of apparently healthy subjects and persons suffering from prostate cancer were collected from local Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Science (Pt. BDS PGIMS) hospital. All other chemicals were analytic reagent (AR) grade. Double distilled water (DW) was used throughout the experimental studies.

Instruments Used

Potentiostat/Galvanostat (Make: Autolab, model: AUT83785, manufactured by Eco Chemie .The Netherland) with a three electrode system consisting of a Pt wire as an auxiliary electrode, an Ag/AgCl electrode as standard/reference electrode and SarOx/ CHIT/CuNPs/c-MWCNT/AuE as

a working/enzyme electrode. All electrochemical activities were performed by general purpose electrochemical software (GPES). Scanning electron microscope (SEM) (Zeiss EV040, USA). UV Spectrophotometer (Make: Shimadzu, Japan, Model 1700), X-ray diffractometer (XRD), (Make: 122 Rigaku, D/Max2550, Tokyo, Japan) were used. The electrochemical impedance spectroscopic (EIS) study was done by GPES (FRA) software in 5mM $K_3Fe(CN)_6/K_4Fe(CN)_6$, the 25ml solution with in frequency range of 0.01 Hz–15 kHz.

Materials and methods

Assay of sarcosine oxidase: It was based on the following chemical reactions:

$$\text{Sarcosine}+O_2+H_2O \xrightarrow[\text{Oxidase}]{\text{Sarcosine}} \text{Glycine} + \text{Formaldehyde} + H_2O_2$$

$$2H_2O_2+\text{4-aminoantipyrine}+\text{phenol peroxidase} \longrightarrow \text{Quinoneimine dye (Pink)} + 4H_2O$$

The assay was carried out in a 15 ml test tube wrapped with a black paper. The reaction mixture contained 1.8 ml of 0.1M sodium phosphate buffer (PBS) (pH 7.8), 0.1 ml $SarO_x$ (2 mg/mL sodium phosphate buffer (PBS), 0.05 M pH 7.0) and 0.1 ml sarcosine (0.01 M). After incubation at 37 °C for 15 min, 1.0 ml color reagent was added and kept at the same temperature for 30 min to develop the color. The blank was also run in the same manner except that enzyme solution was replaced by reaction buffer. A_{520} was read against blank and concentration of H_2O_2 generated in the assay was extrapolated from the standard curve of H_2O_2. The color reagent consisted 50 mg 4-aminophenazone, 100 mg phenol and 1.0 mg horseradish peroxidase per 100 ml of 0.4 M sodium phosphate buffer, pH 7.0 and stored in an amber colored bottle at 4°C and prepared fresh every week. The protein content in dissolved SarOx was measured by Lowry method. One unit of enzyme is defined as the amount of enzyme required to generate 1 nmol H_2O_2/min/ml [22].

Fabrication of SarOx/CHIT/CuNPs/c-MWCNT/Au electrode

Synthesis of copper nanoparticles (CuNPs)

CuNPs were prepared by chemical reduction method [16]. NaOH (0.1 M, 10 ml) was added to a mixture of aqueous solution of $CuSO_4$ (0.4 M, 100 ml) and ethylenediamine-tetraacetic acid (EDTA, 0.1 M, 10 ml). $NaBH_4$ (0.3 M, 100 ml) was added to this mixture/solution under constant stirring and heated to 60 °C. After stirring for 45 min, the product i.e. CuNPs were

washed three times with ethanol (30%) and acetone (70%) separately to improve the evaporation and collected by centrifugation and finally dried in a vacuum oven at 35 – 45 °C.

Electrodeposition of CHIT/CuNPs/c-MWCNT onto AuE

Prior to modification, the surface of the Au wire (diameter 2 mm) was polished manually by alumina slurry (diameter 0.05 µm) with a polishing cloth, followed by thorough washing with DW. Au wire was then treated with piranha solution (H_2SO_4: H_2O_2 in the ratio 3:1) to clean the adsorbed moieties. The procedure is followed by placing it into ethanol solution (30%) for 5-6 hrs and sonicated to remove adsorbed particles. Finally, the polycrystalline Au wire washed with DW for 3-4 times. The c-MWCNT (1.0 mg) were dispersed into 4 ml mixture of concentrated H_2SO_4 and HNO_3 in 3:1 ratio and ultrasonicated for 3-4 h to get its finely dispersed black colored solution. The dispersed c-MWCNT (0.1 ml) was mixed into a mixture of 0.5 ml 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.2 M) and 0.5 ml N-hydroxysuccinimide(NHS) (0.2M). The pH was adjusted to 6.0 and kept at room temperature for 1h. Chitosan (0.2%, 200 µl) was added to 25 ml 1N KCl and finally electropolymerized onto Au electrode through cyclic voltammetry by applying 20 successive polymerization cycles at -1.1 to 0.1 V. CuNPs suspension (200 µl) and 1.0 ml of cMWCNT suspension were added into 25 ml 1N KCl and finally electrodeposited onto Au electrode by applying 20 successive polymerization cycles at -1.1 to 0.1 V at a scan rate of 20 mV/s. During the electrochemical polymerization, the surface of Au electrode became green gradually, indicating the deposition of CHIT/CuNPs/cMWCNT film on Au wire. The resulting CHIT/CuNPs/cMWCNT modified Au was washed thoroughly with DW to remove unbound matter.

Characterization of CuNPs and its composites

By UV spectroscopy

To study the optical properties and dispersion of CuNPs and fabricated materials (CHIT/CuNPs, CHIT/CuNPs/c-MWCNT), UV spectra were recorded in a UV spectrophotometer in the range, 200 to 600 nm.

By X-ray diffraction (XRD)

XRD of CuNPs was carried out with X-ray diffractometer in reflection mode. The sample was scanned from 20º to 80º (2 Â) in steps of 0.05º. The crystallite domain sizes (D) of CuNPs was examined from XRD peaks based on the Scherrer's equation: D = 0.9 / (W cos Â), where the

wavelength of X-ray was 0.15418 nm, Â is the Bragg's diffraction angle and W is the true half-peak width of the X-ray diffraction lines.

By transmission electron microscopy (TEM)

The size and shape of CuNPs were measured by a high-resolution transmission electron microscope (Zeiss EM 912), at an accelerating voltage of 120 kV. The samples of CuNPs for TEM were prepared by placing the product solution onto the carbon-coated copper grids and allowing the solvent to evaporate in air.

Immobilization of SarOx onto CHIT/CuNPs/cMWCNT modified Au electrode

The CHIT/CuNPs/cMWCNT modified wire was placed into 1.5 ml of dissolved SarOx (1mg/1 ml) and kept overnight at 4 °C. The resulting CHIT/CuNPs/cMWCNT/AuE was washed 3-4 times with 0.1 M sodium phosphate buffer, pH 7.3 to remove unbound enzyme. The CHIT/CuNPs/cMWCNT/AuE was used as a working electrode and stored dry at 4 ºC, when not in use. This working/enzyme electrode was characterized by SEM, FTIR, and EIS at different stages of its construction. The schematic representation of the construction of working electrode is shown in Fig. 1.

Construction and response measurement of sarcosine biosensor

An amperometric sarcosine biosensor was constructed by connecting, CHIT/CuNPs/cMWCNT/AuE, as the working electrode, Ag/AgCl as the reference electrode, Pt wire as the counter electrode through potentiostat. Cyclic voltammetry (CV) of working/enzyme electrode was recorded in Potentiostat–Galvanostat in the potential range, 0 to 0.7 V in 25 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1 ml of 10 μM sarcosine. The current (in mA) was measured at different time (in seconds).

Optimization of sarcosine biosensor

To optimize working conditions of the biosensor, effects of pH, incubation temperature, time of incubation and substrate (sarcosine) concentration on biosensor response were studied. To determine optimum pH, the pH was varied between pH 4.0 to 8.0 at an interval of pH 0.5 using the following buffer, each at a final concentration of 0.1 M: pH 4.0 to 5.0 sodium acetate buffers and pH 5.5 to 8 sodium phosphate buffer. Similarly to determine optimum temperature and time the reaction mixture was incubated at different temperature (5–70 ºC) and time (1 to 20 s). The

effect of sarcosine concentration on biosensor response was determined by varying the concentration of sarcosine in the range 0.1 μM-100 μM.

Application of sarcosine biosensor

Blood samples (1 ml each) were drawn from apparently healthy male and female (5 each) and persons with a diagnosis of prostate cancer at Pt. BDS PGIMS), Rohtak hospital and centrifuged at 2000 rpm for 10–15 min and their supernatant (serum) was collected. Sarcosine content in sera was determined by the present biosensor in the similar manner as described above for its response measurement, under its optimal working conditions except that sarcosine was replaced by serum sample. The sarcosine level in sera was interpolated from standard curve between sarcosine concentrations (μM) versus current in mA prepared under optimal assay conditions of biosensor and analyzed by Student's t-test.

Results and discussion

Characterization of CuNPs

By UV spectra

UV and visible spectra exhibited broad absorbance peak at optical range i.e 300 nm, showing the small size and high degree of optical conductivity. The presence of the single absorption peak implied that the formed nanoparticles were nearly spherical attributed to the coherent oscillation of the conduction electrons. The conjugates prepared with CuNPs also showed absorbance at 350nm, with strong absorption peaks, due to the carboxyl group of EDC-NHS and amino group of chitosan, which enhances the absorption. UV absorption peak of conjugates (CHIT/CuNPs, CHIT/CuNPs/c-MWCNT) at 300 nm showed no effect on size, morphology and dielectric function of CuNPs (Fig.2a).

By XRD

The characteristic diffraction rings, which correspond to the reflections at (110), (111), (200), (211), and (301) showed clearly the polycrystalline nature of the CuNPs, which tend to increase the surface area of the working electrode. Diffraction peaks indexed the pure face-centered cubic (f.c.c) lattice of CuNPs without any adulteration of impurities (Fig. 2b).

By TEM

The average size of dispersed CuNPs, as measured by TEM was 50 nm. It showed the spherical morphology of CuNPs without any aggregation (Fig. 2c). The size conveyed the facile synthesis of the CuNPs at the nanoscale.

Characterization of enzyme electrode during construction

By SEM

The SEM images of the surface of bare AuE, CHIT/CuNPs/AuE, CHIT/CuNPs/c-MWCNT/AuE and SarOx/ CHIT/CuNPs/c-MWCNT/AuE are shown in Fig. 3a. The stepwise modification of electrode could be seen clearly from these SEM images. The SEM image of the bare AuE showed a smooth and featureless morphology. The CHIT/CuNPs/c-MWCNT /AuE composite film exhibited a net structure. The film was more uniform and porous, hence effective surface area was larger for the entrapment of biological material. On immobilization of SarOx, a globular structural morphology was appeared due to the interaction of SarOx with CHIT/CuNPs/c-MWCNT /AuE.

By EIS

EIS provides the useful information on impedance changes of the electrode surface during the fabrication process. It was carried out to investigate immobilization of SarOx onto Au electrode. The diameter of the semicircle at higher frequencies corresponds to the electron transfer resistance (R_{CT}) which controls the electron transfer kinetics of the redox probe at the electrode interface, while the linear part at lower frequencies corresponds to Warberg diffusion process. The Nyquist plot (Fig. 3b) displays EIS of bare AuE, CHIT/CuNPs/AuE, CHIT/CuNPs /c-MWCNT /AuE and SarOx /CHIT/CuNPs/c-MWCNT /AuE in 5 mM $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$. The R_{CT} values were obtained as 0.5 kΩ, 1.5 kΩ, 1.75 kΩ, 2.75 kΩ respectively (the average of three replications). The decrease in R_{CT} value of the CHIT/CuNPs/c-MWCNT /AuE compared to bare Au electrode was due to the attachment of nanomaterials onto the electrode resulting in high electron transfer efficiency. However, the R_{CT} of SarOx/CHIT/CuNPs/c-MWCNT /AuE after immobilization of SarOx was increased due to binding of enzymes (SarOx) molecules, which are poor electrical conductors at low frequencies and cause hindrance to electron transfer.

These results also confirmed the immobilization of SarOx onto the CHIT/CuNPs/c-MWCNT modified AuE.

Construction & optimization of sarcosine biosensor

A novel amperometric sarcosine biosensor was developed based on covalent immobilization of sarcosine oxidase (SarOx) onto CHIT/CuNPs/c-MWCNT /AuE. The peaks strength revealed the layer by layer electrodeposition of CHIT/CuNPs/c-MWCNT onto Au electrode (Fig.4a). The maximum current (oxidation and reduction peaks) was observed at 0.2 V, which is lower than earlier sensor (0.6 V) [10] (Fig. 4b). The biosensor showed optimum current at pH 7.0 in 0.1 M sodium phosphate buffer, which is close to the physiological pH (Fig. 5a). The optimum incubation temperature of the biosensor was observed at 35 ºC (Fig. 5b) and the current was recorded within 2 s at this voltage (Fig. 5c), which is quite low than earlier sensors (<120 s) [11]. Hence, the subsequent electrochemical experiments were carried out in 0.1 M sodium phosphate buffer, pH 7.0 at 35 °C. There was a beeline relationship between biosensor response and sarcosine concentration in the range 0.1μM-100μM, the response was constant after 100 μM.

Evaluation of biosensor

There was a linear relationship between the biosensor response i.e. current (in mA) and the sarcosine concentration in the range 0.1 μM-100 μM (Fig. 5d), which is better than earlier biosensors [10-14].The detection limit of the present biosensor was 0.1 pM, which is better than earlier sensors (10^5 pM)[10], (1.35×10^4 pM)[10-14]. The analytical recoveries of added sarcosine in sera at levels of 0.5 μM and 1.0 μM were 97.30 and 95.58% respectively, demonstrating the reliability of the present biosensor. Within and between-batch coefficients of variation for the determination of sarcosine in sera on the same day and after one week of storage were 1.08% and 1.70%, respectively. These high precisions highlighted the good reproducibility and consistency of the present biosensor compared to already reported biosensors [10-14]. This can be attributed to the covalent immobilization of SarOx onto the CHIT/CuNPs/c-MWCNT /AuE.

Correlation

The sarcosine level in 10 sera of apparently healthy subjects and persons suffering from prostate cancer, as measured by present biosensor were compared with those obtained by standard

immuno kit method. There was a good correlation (r = 0.99) between these two methods (Fig. 5e).

Applications of sarcosine biosensor

The sarcosine content in sera as measured by the present biosensor was in the range 1.3±0.02-3.2±0.04 µM (n=10) mM for apparently healthy persons and 8.2 ± 0.05-31±0.04 µM (n=10) for persons suffering from prostate cancer, which is significantly higher than healthy ones (p value<0.01) (Table 1).The increased level of sarcosine in blood might be due to mutations at the gene level, which could either increase the expression of sarcosine synthesizing enzyme (glycine N-methyl transferase) or decrease the expression of sarcosine metabolizing enzyme (sarcosine dehydrogenase and pipecolate oxidase)[21]. Due to the small number of patients tested, these results would need to be confirmed in a larger patient cohort.

Interference study and selectivity

Citric acid, glucose, glutamic acid, urea and ascorbic acid at their physiological concentrations had practically no effect on biosensor response under the standard conditions (Fig. 5f).

Storage stability of enzyme electrode

The enzyme electrode maintained 90% of its initial activity even after 180 days of regular uses when stored dry at 4 ^{0}CsThis stability of present enzyme electrode/biosensor was higher than earlier sensors (Table 2).

Conclusion

The detection of sarcosine is a major concern nowadays, as it is a potential biomarker for prostate cancer. The methods available for sarcosine determination are complicated, costly and time-consuming. Hence, the present biosensor was designed. The use of CHIT/CuNPs/c-MWCNT nanocomposite in construction of sarcosine biosensor has resulted in its better analytical performance in terms of faster response time (2 s), lower detection limit (0.1 pM), wider linear range (0.1to 100 µM) and higher storage stability (180 days) as compared to earlier biosensors. This nano-composite could also be used for the improvement of other biosensors

[23]. Moreover, the enzyme nanoparticles based biosensor could be designed to make the construction of biosensor very simple [24-26]. In addition to the improvements in biosensing methods, the paper based electrode could be used for construction of biosensor, which will help in accomplishing the targets of cost effectiveness and real time monitoring of analytes at the besdside of patients [27-30].

Conflict of Interest: The authors have no conflict of interest.

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Figure: 3a Scanning electron microscopic (SEM) images of (a) bare Au electrode (b) CHIT/CuNPs/AuE (c) CHIT/CuNPs/c-MWCNT (d) SarOx/CHIT/CuNPs/c-MWCNT.

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Figure: 5(a) Influence of pH on the current response of SarOx/CHIT/CuNPs/c-MWCNT/AuE **(b)** Influence of temperature on the current response of SarOx/CHIT/CuNPs/c-MWCNT/AuE **(c)** Influence of time on the current response of SarOx/CHIT/CuNPs/c-MWCNT/AuE **(d)** Standard curve of sarcosine concentration by sarcosine biosensor based on SarOx/CHIT/CuNPs/c-MWCNT/AuE. **(e)** Correlation between serum sarcosine level measured by immuno kit method (x axis) and the current method (y axis) employing the sarcosine biosensor based on SarOx/CHIT/CuNPs/c-MWCNT/AuE. (f) Effect of various interferents on the current response of SarOx/CHIT/CuNPs/c-MWCNT/AuE.

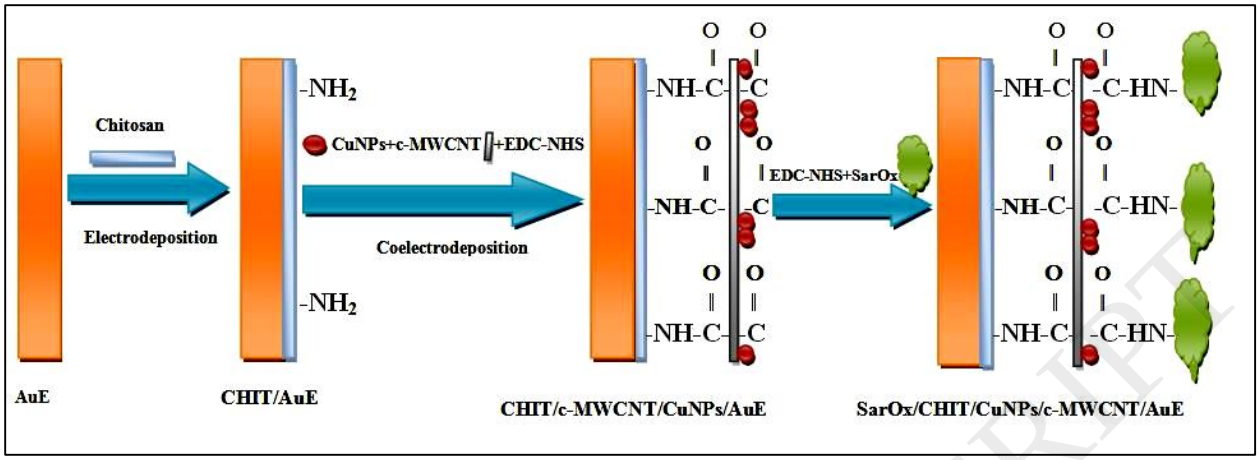


Fig.1

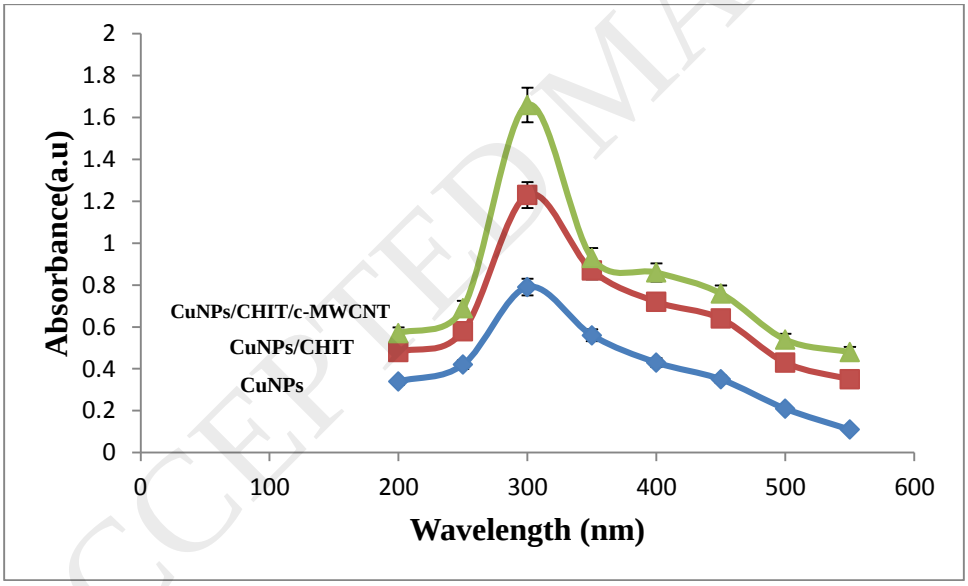


Fig.2a

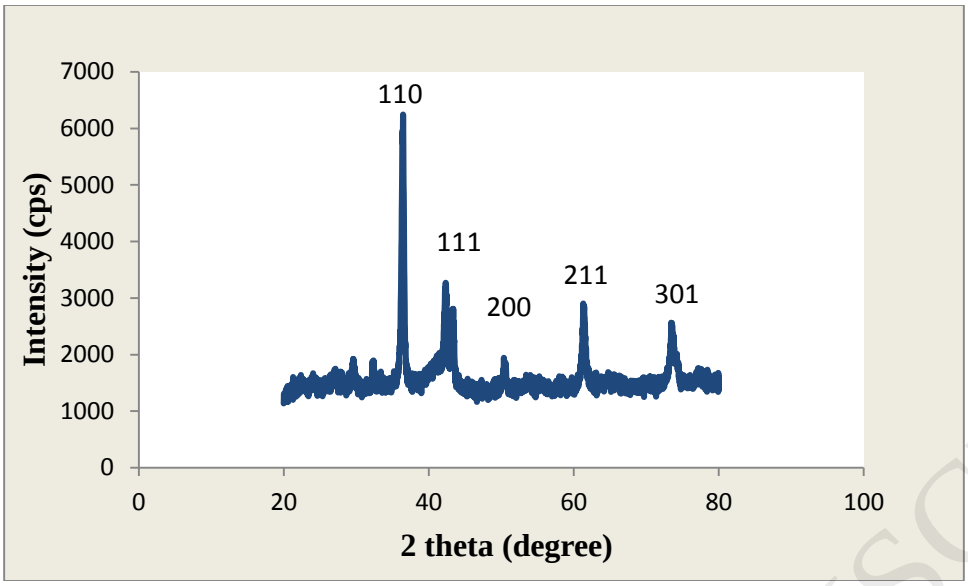


Fig. 2b

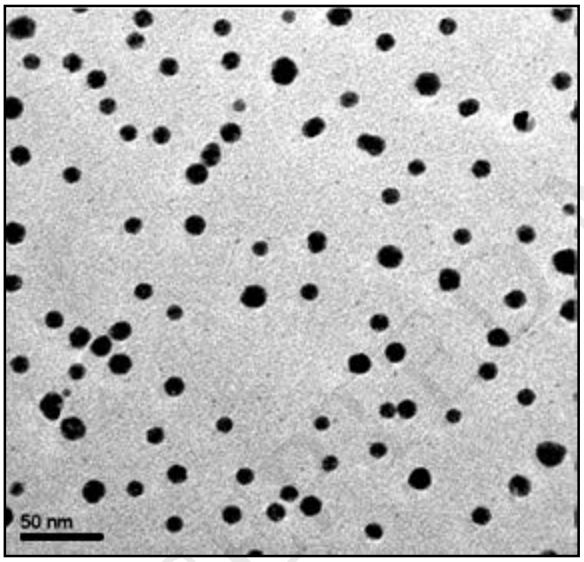


Fig. 2c

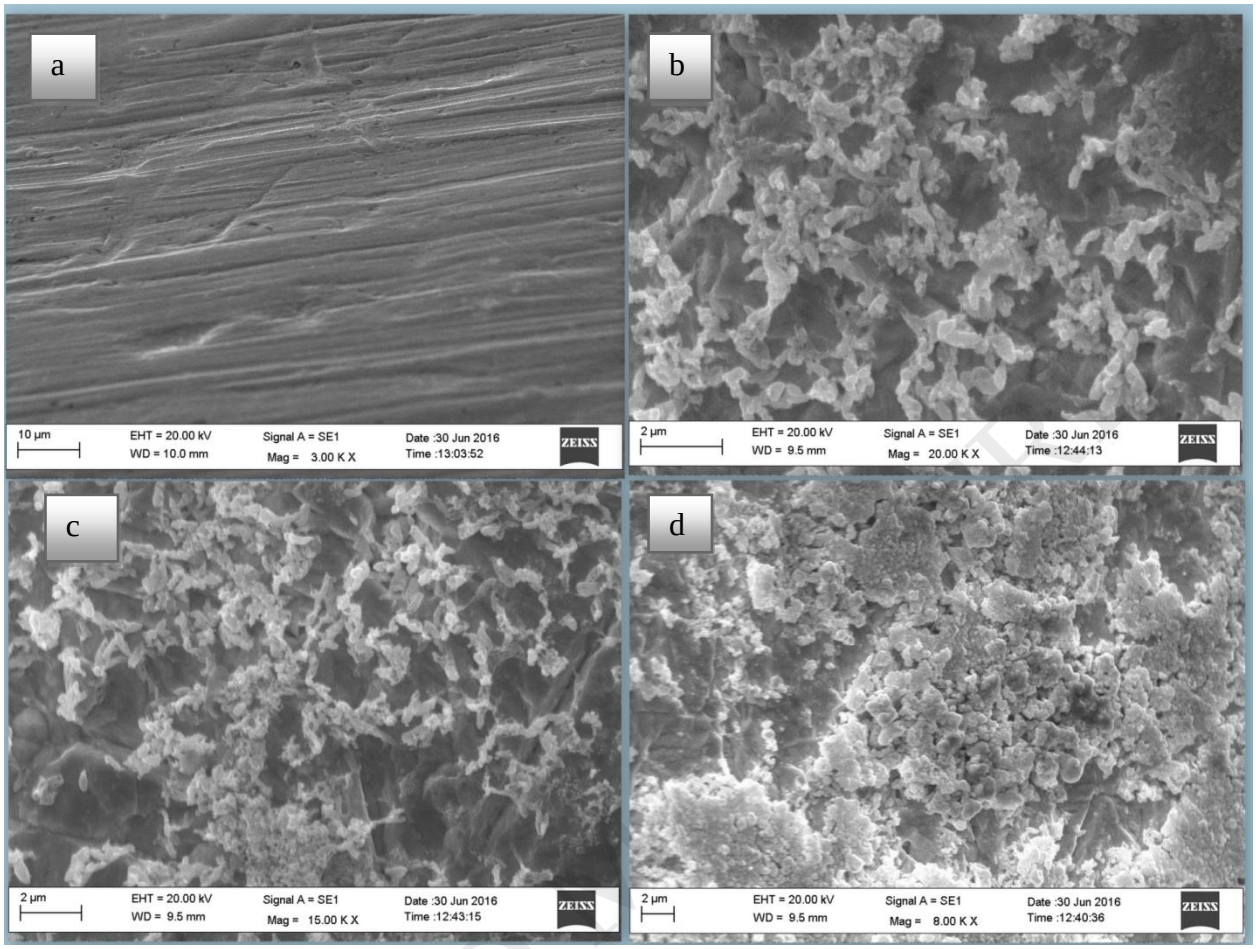


Fig. 3a

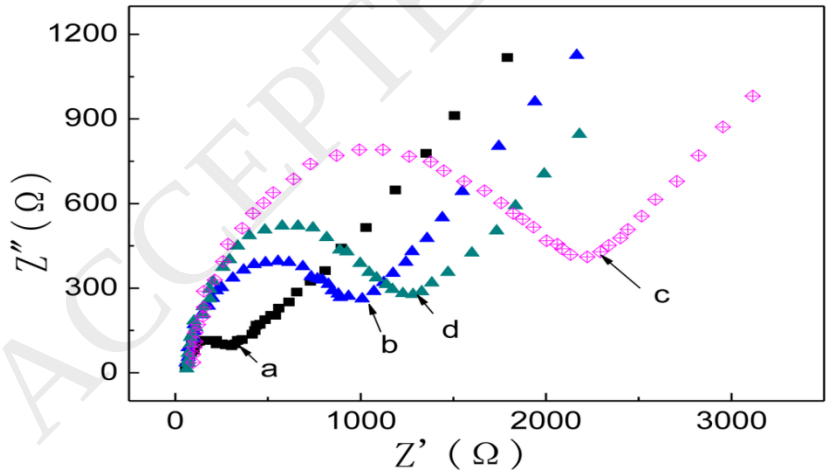


Fig. 3b

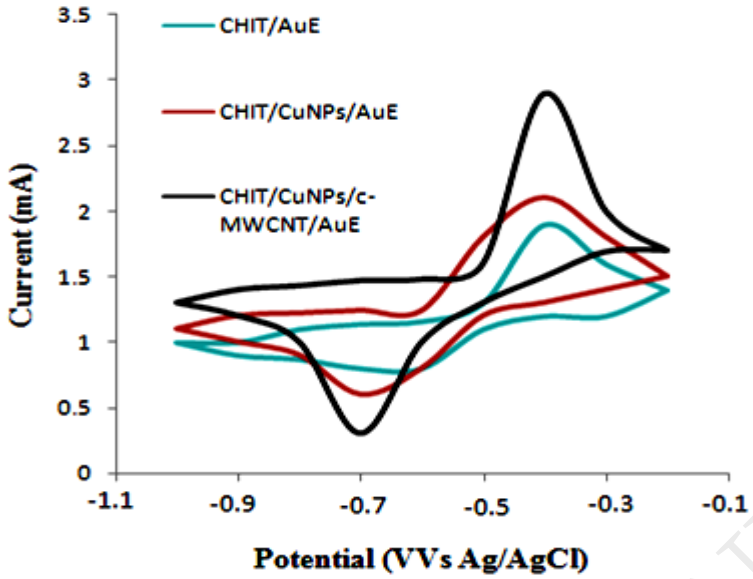


Fig.4a

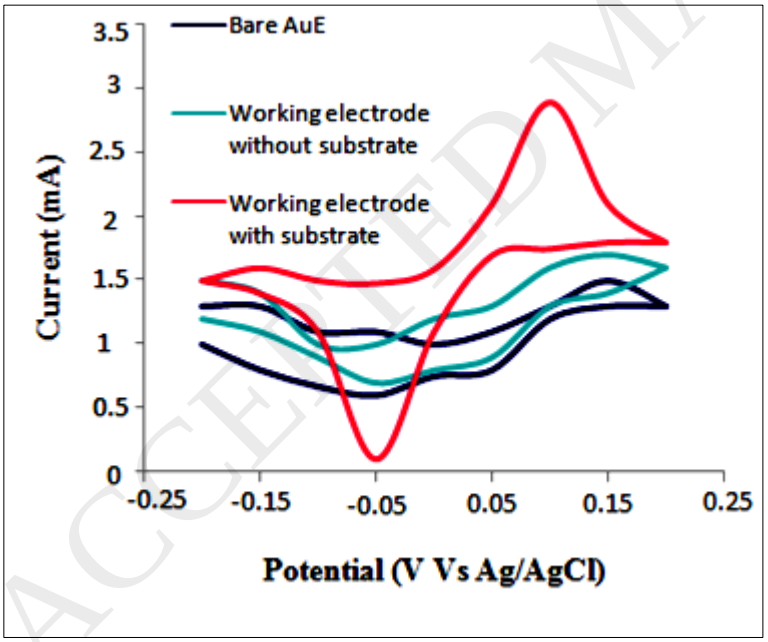


Fig. 4b

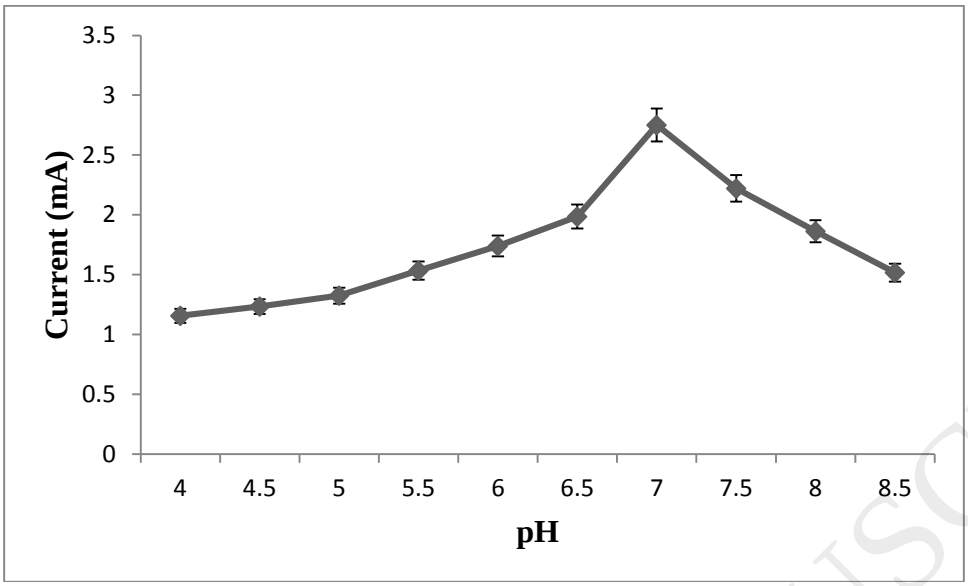


Fig. 5a

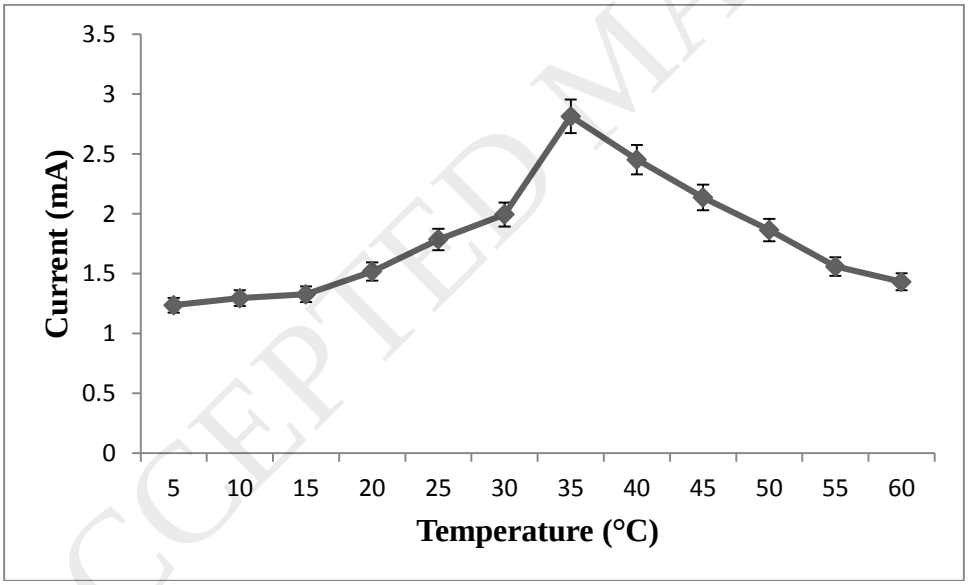


Fig. 5b

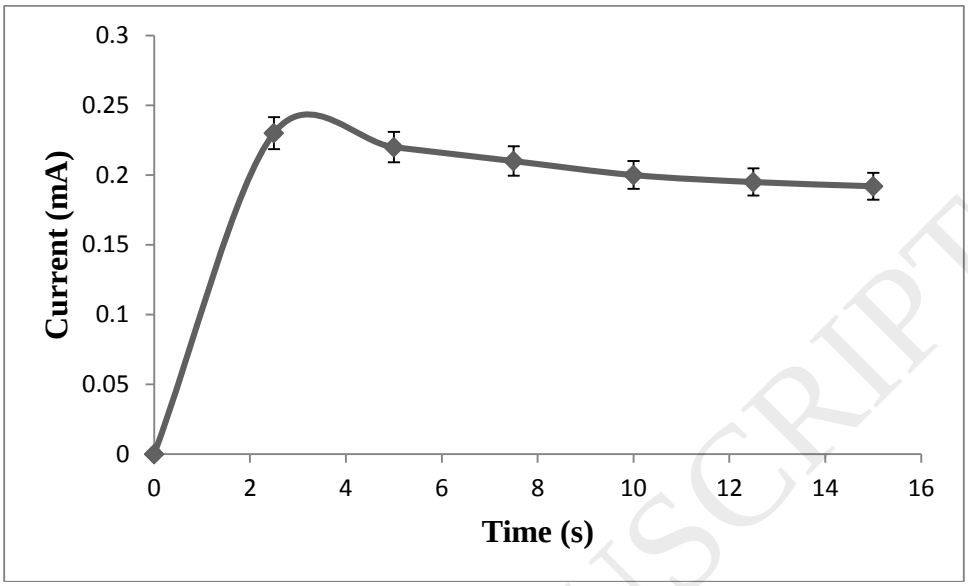


Fig. 5c

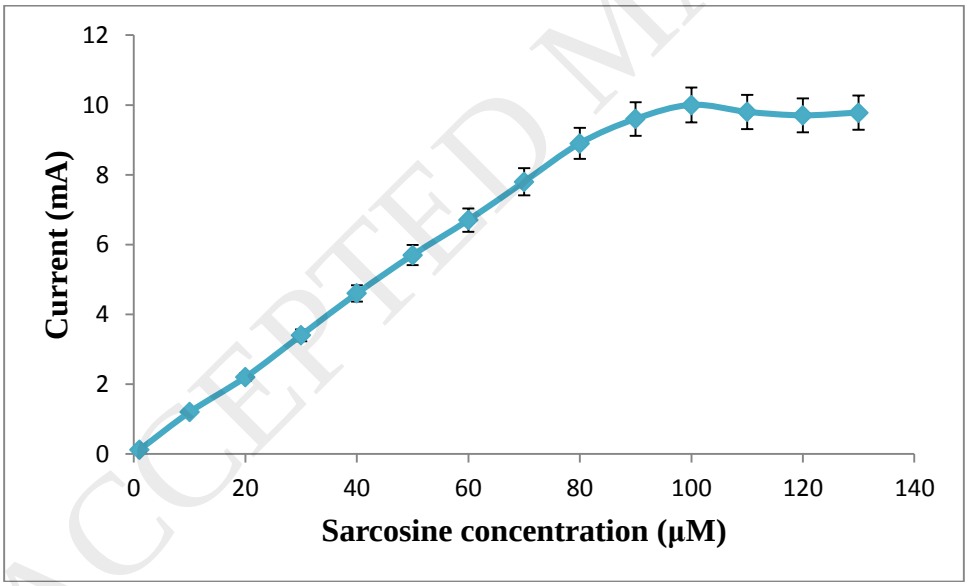
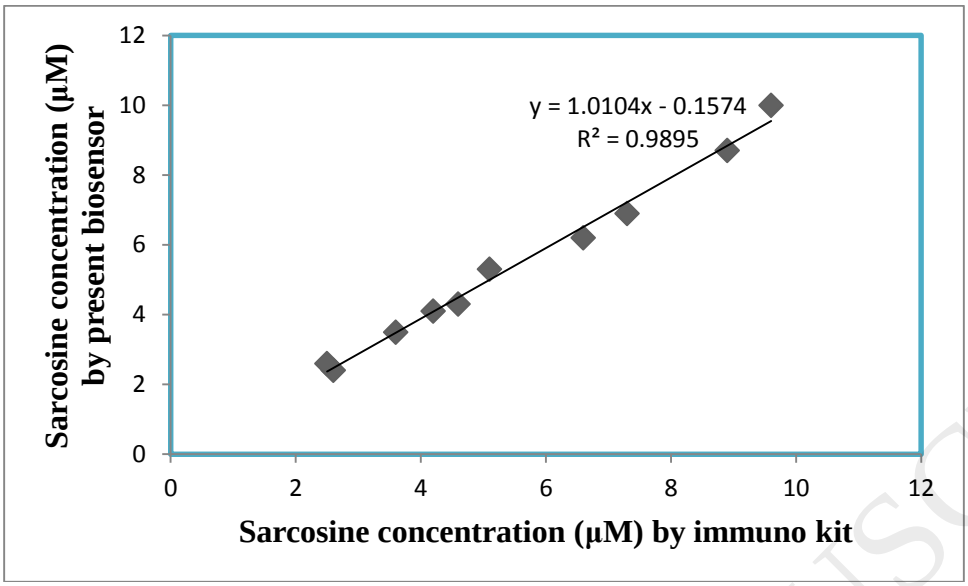


Fig. 5d



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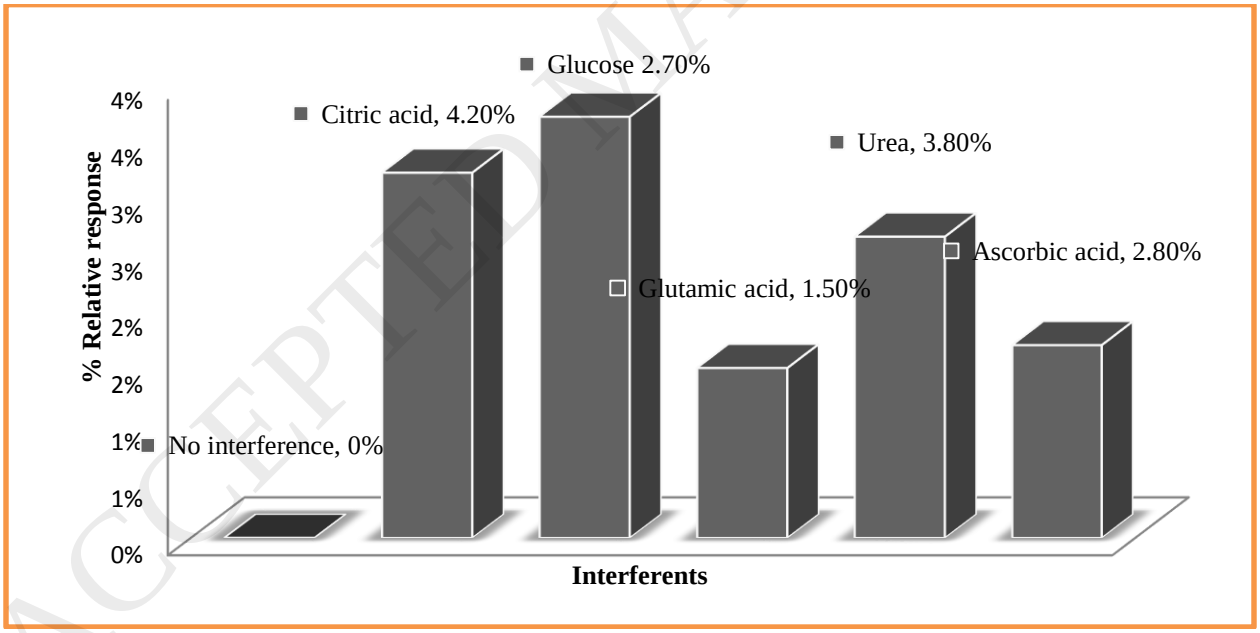

Fig. 5f

Table: 1 Serum sarcosine levels in apparently healthy persons and prostate cancer patients, as measured by sarcosine biosensor based on SarOx/CHIT/CuNPs/c-MWCNT/AuE.

Table 2: A comparison of different analytic parameters of present sarcosine biosensor with those of earlier biosensors.

Table 1

Sex	**Age**	**Apparently Healthy Persons (µM)**	**Sex**	**Age**	**Prostate cancer patients (µM)**
M	32	2.4±.03	M	42	20.7±.04
M	41	1.6±.04	M	35	8.2±.05
M	37	1.3±.02	M	46	8.9±.02
M	29	1.9±.01	M	49	9.0±.01
M	34	2.5±.04	M	60	31.0±.04
M	32	2.4±.05	M	37	21.8±.03
M	44	3.2±.04	M	32	9.8±.02
M	46	2.7±.03	M	54	9.9±.06

Table 2

Sr no	Working Electrode	Optimum ph	Temp (˚C)	LOD (pM)	Linear range (µM)	Response time (sec)	potential	Storage stability (days)	Ref
1	Screen printed	7.2	37	16000pM	0.01-0.1 µM		0.6 V	60	10
2	Molecular imprinted polymer (MIP)			1350pM	100-0.01 µM	<120		165	11
3	Au	7.0	37	50pM	0.005-0.05 µM				12
4	Glassy carbon	7.0		1000000 pM	1.0-177 µM		0.49 V		13
5	Au			1000 pM					14
6	Au	7.0	35	0.10pM	0.1-100 µM	2	0.2 V	180	Present work