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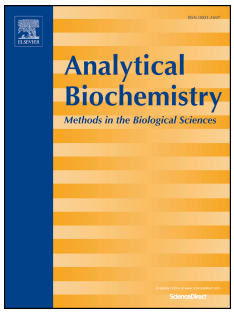

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An improved amperometric creatinine biosensor based on nanoparticles of creatininase, creatinase and sarcosine oxidase

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Abstract

An improved amperometric biosensor for detection of creatinine was developed based on immobilization of nanoparticles (NPs) of creatininase (CA), creatinase (CI), and sarcosine oxidase (SOx) onto glassy carbon (GC) electrode. Transmission electron microscopy (TEM) and fourier transform infrared spectroscopy (FTIR) were employed for characterization of enzyme nanoparticles (ENPs). The GC electrode was characterized by scanning electron microscopy (SEM), cyclic voltammetry (CV) and electrochemical impedance spectra (EIS) at different stages of its amendment. The biosensor showed optimum response within 2s at pH 6.0 in 0.1M sodium phosphate buffer and 25˚C, when operated at 1.0V against Ag/AgCl. Biosensor exhibited wider linear range from 0.01µM to 12µM with a limit of detection (LOD) of 0.01µM. The analytical recoveries of added creatinine in sera were 97.97±0.1% for 0.1mM and 98.76±0.2% for 0.15mM, within and between batch coefficients of variation (CV) were 2.06% and 3.09% respectively. A good correlation (R^2=0.99) was observed between sera creatinine values obtained by standard enzymic colorimetric method and the present biosensor. This biosensor measured creatinine level in sera of apparently healthy subjects and persons suffering from renal and muscular dysfunction. The ENPs electrode lost 10% of its initial activity within 240 days of its regular uses, when stored at 4^{o}C.

Keywords: Creatinine, Biosensor, Enzyme nanoparticles, Glassy carbon electrode, Serum.

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1. Introduction

Creatinine (2-amino-1-methyl-5H-imidazol-4-one) is a metabolic product of creatine phosphate in muscles. Creatine provides energy to muscle tissues. The measurement of creatinine level in human blood and urine is decisive and affected by renal, muscular and thyroid dysfunction [1]. Normal level of creatinine in the serum and urine of apparently healthy individuals is 45-140 μM and 0.8-2.0 gm per day respectively. The level of creatinine reaches more than 1000 μM in serum during renal, thyroid kidney dysfunction or muscle disorder. The creatinine concentration goes less than 45 μM during decreased muscle mass [2]. Men usually have slightly higher creatinine level compared to women, because women have less muscle mass. There are various analytical methods/techniques for creatinine determination such as HPLC [3], mass spectroscopy [4], IR spectroscopy [5], capillary zone electrophoresis [6], flow injection analysis systems with electrochemical and spectrometric detection [7], nuclear magnetic resonance [8]. However, these techniques had problems such as complicated, require time-consuming sample pre-treatment, expensive instrumental set-up and skilled persons to operate. Biosensor technology provides many advantages over these techniques for routine serum creatinine analysis, such as simplicity, fast response time and the reduced cost. In the biosensors, enzyme is required to be immobilized onto the working electrode. However the direct immobilization of enzyme onto the supports leads to its denaturation and subsequently its reduced activity and stability. This drawback has been overcome using nanoparticles of enzyme(s) (ENPs) in place of native/free enzyme molecules [9]. Applications of nanomaterials have increased the large surface area to volume ratio, high surface reaction activity, great catalytic efficiency, more adsorption ability for immobilization of biosensing molecules. Enzyme nanoparticles have an exclusive ability to boost the quick electron transfer between the working electrons and the enzyme [10]. The present report describes the fabrication of an improved amperometric creatinine biosensor employing unique properties of NPs of creatininase (CA), creatinase (CI), and sarcosine oxidase (SOx).

2. Materials and Methods

2.1 Chemicals and reagents

Creatininase amidohydrolase from *Pseudomonas sp.*expressed in *Flavobacterium sp.* was from SISCO Research lab. Mumbai, India and creatinase amidinohydrolase from *Pseudomonas sp.*expressed in *Escherichia coli,* sarcosine oxidase from *Bacillus sp.* from Sigma-Aldrich, USA and glassy carbon (GC) electrode from Metrohm, The Netherland were used. All other chemicals

used were of analytical reagent (AR) grade. The blood/serum samples of apparently healthy individuals and persons suffering from renal disorders were collected from the hospital of Pt. BDS Post Graduate Institute of Medical Science, Rohtak and stored at -20˚C until use. Double distilled water (DW) was used throughout the study.

2.2 Instruments used

Potentiostat/Galvanostat (Make: Autolab, model: AUT83785, manufactured by Eco Chemie, The Netherland) with a three electrode system consisting of a platinum (Pt) wire as a auxillary electrode, an Ag/AgCl electrode as a reference electrode and ENPs modified glassy carbon (GC) electrode as a working/enzyme electrode, transmission electron microscope (TEM) (JEOL 2100F, Japan), scanning electron microscope (SEM) (Zeiss EV040, USA) and Fourier transform infra-red spectrometer (FTIR) (Thermo Scientific, USA), were used.

2.3 Assay of mixture of CA, CI and SOx

The assay of creatininase (CA), creatinase (CI), and sarcosine oxidase (SOx) was carried out as described by Fossati et al. (1983) [11] with some modifications. To measure the activity of (CA, CI and SOx) enzymes, 1 ml of chromogen reagent (consisting of CI (8 kU), SOx (1.3 kU), peroxidase (1.5 kU), 0.2 mM 4-aminophenazone and 3 mM 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) of 0.1M sodium phosphate buffer (PBS buffer) (pH 7.0) per liter, was added to 50 µl of creatinine solution (0.175mM) and then 10µl of CA solution (9-10 units) was added. After incubation at 30˚C for 30 min, the absorbance of pink coloured solution was measured at 510 nm in a Spectronic-20 against control. The chromogen reagent was stored at 4˚C in an amber coloured bottle, when not in use.

2.4 Preparation of nanoparticles of creatininase (CA), creatinase (CI) and sarcosine (SOx) oxidase

Creatininase, creatinase and sarcosine oxidase-NPs were prepared separately by desolvation method as described by Kundu et al. (2012) [12]. To one ml of each enzyme solution (1 mg/ml, 0.1M PBS buffer pH 7.0) 2 ml of absolute ethanol was added with caution at a dropping rate of 0.1-0.2 ml/min under constant stirring at 500 rpm. The ENPs were formed by aggregation of native enzyme molecules. After 24 hrs, 1.8 ml of 2.5% glutaraldehyde was added to these nanoparticles suspension under continuous stirring at 500 rpm at 4˚C. Amino groups ($-NH_2$) were introduced onto enzyme-NPs by addition of 0.12g of cysteamine dihydrochloride under

constant stirring, after 5-6 hrs of the addition of glutaraldehyde. At last, enzyme nanoparticles (ENPs) were separated from native/free enzyme solution by centrifugation at 1200 rpm in cold (4˚C) for 10 min followed by dispersion in 0.1 M PBS (pH 7.0) and sonication for 5 min. All the ENPs were stored separately at 4˚C, when not in use.

2.5 Characterization of ENPs by TEM and FTIR

Creatininase, creatinase and sarcosine oxidase nanoparticles were characterized by recording their transmission electron microscopic (TEM) images and Fourier transform infra-red (FTIR) spectra. The TEM provided the size of ENPs in the range 2nm-100nm. FTIR spectra of ENPs were recorded for functional groups of the O–H stretching, C=C stretching, COOH stretching, C-C stretching, C–O (epoxy/ether) stretching and N-containing bioligand vibrations.

2.6 Preparation of working electrode

The bare GC electrode was dipped into pirhana solution (H_2SO_4:H_2O_2, 3:1) for 20 min, cleaned by alumina slurry and then dipped into ethanol for 5-6 hrs and washed with DW. Now the GC electrode was dipped into mixture of ENPs (in 1:1:1 ratio) for 48 hrs for immobilization. The creatininase, creatinase and sarcosine oxidase-NPs aggregates got bound onto GC electrode. The ENPs modified GC electrode was washed with 0.1 M PBS, pH 7.0.

2.7 Characterization of working electrode (NPs/GCE) by SEM, FTIR, CV and EIS

The GC electrode, at different stages of its construction was characterized by scanning electron microscopic (SEM), Fourier transform infrared spectra (FTIR) and electrochemical impedance spectroscopic (EIS) study in a potentiostat/galvanostat with three electrode system, GC electrode as a working electrode, a Pt wire as the auxiliary electrode and an Ag/AgCl electrode as the reference electrode. The EIS measurement were carried out in 5mM $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ in the 0.1 M PBS pH 7.0 at ambient temp at the polarization potential 0.1 V. Cyclic voltametry (CV) of working electrode was recorded in potentiostat/galvanostat in the potential range, 0.3 to 1.0 V in 25 ml of 0.1 M PBS (pH 7.0) containing 0.1ml 0.1 M creatinine. The current (in mA) was measured at different time (in seconds).

To record FTIR spectra of ENPs deposited onto GC electrode, the immobilized material was scrapped off the GC electrode and these pellets were kept into the socket of the FTIR spectrometer and its spectrum was recorded.

2.8 Construction and response measurement of creatinine biosensor

To construct the creatinine biosensor, ENPs modified GC as working electrode, Ag/AgCl as reference electrode, Pt wire as counter electrode were connected through potentiostat. Fig 1 provides the schematic representation of fabrication of CA/CI/SOx/GC electrode response measurement. Cyclic voltametry (CV) of working/enzyme electrode was recorded in potentiostat in the potential range, -0.3 to 1.0 V in 25 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1ml 1M creatinine. The current (in mA) was measured at different time (in seconds).

2.9 Optimization of creatinine biosensor

To optimize working creatinine biosensor, effect of pH, temperature, time and substrate (creatinine) concentration on biosensor response was 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 buffers, each at a final concentration of 0.1M, sodium acetate buffers (pH 4.0 to 5.0) and sodium phosphate buffer (pH 5.5 to 8). Identically to determine optimum temperature the reaction mixture was incubated at different temperature (5-80ºC at an interval of 5˚C). The effect of creatinine concentration on biosensor response was determined by varying the concentration of creatinine in the range 0.01mM-12mM.

2.10 Application of creatinine biosensor for determination of serum creatinine

The intravenous blood (1ml each) was drawn from apparently healthy male and female (20 each) and persons suffering from renal disorders at Pt. BDS PGIMS, Rohtak using sterilized needle and syringe(5ml), transferred it to a plastic vial and kept at room temperature for 30 min for coagulation. It was then centrifuged at 1200 rpm for 10–12 min and its supernatant (serum) was collected. Each serum sample was analyzed for creatinine content by measuring cyclic voltametry (CV) of working/enzyme electrode in potentiostat in the potential range, -0.3 to 1.0 V in 25 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1ml serum samples under optimum condition. The creatinine content in serum was interpolated from standard curve between creatinine concentrations vs current in mA prepared under optimal assay conditions of biosensor.

Determination of serum creatinine by enzymic colourimetric method: Serum (100µl) was diluted 100-fold with water, incubated with 100µmol of Tris/HCI buffer (pH 8.5) and 1.21µmol of N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (TOOS), 1.48µmol of 4-aminoantipyrine,

100µmol of KCI,10µmol of MgCl2,10µmol of ATP, 2 units of creatininedeiminase,0.04 units of N-methylhydantoin amidohydrolase, 0.01 units of N-carbamoylsarcosine amidohydrolase, and 2.5 units each of sarcosine oxidase and peroxidase, in a total volume of 1.0ml. After 30min of incubation at 37°C, the color produced was measured at 555nm against a reagent blank and creatinine content was calculated from standard curve between creatinine vs A_{555} [13].

3. Results and discussion

3.1 Characterization of creatininase, creatinase and sarcosine oxidase nanoparticles

The size of monomeric enzymes size is between 2-8nm. However the electron microscopic images of CA, CI and SOx ENPs showed that aggregate of enzyme molecules have hollow spherical structures. The average size of NPs of CA, CI and SOx as measured by TEM, was 28.8 nm, 18.64 nm, 14.4 nm respectively (Fig.3). The fundamental modes of FTIR in NPs of CA, CI and SOx were stretching and bending of N-H bond of the amine group. The primary aromatic amines the N-H stretching frequency occurs in the region 1080-2300 cm-1 for CA, 1020-2250 cm-1 for CI and 1050-2200 cm^{-1} for SOx. The FTIR spectrum showed characteristic peaks at 1040-1560 cm^{-1} for CA, 1000-1600 cm^{-1} for CI and 1060-1720 cm^{-1} for SOx due to symmetric and one asymmetric N-H stretching vibrations. Broad bands indicate the presence of the amine form in the structure. The observed low values of bands are due to the participation in H-bonding interactions. The band observed at 1640 cm-1 for CA, 1680 cm-1 for CI & 1720 cm-1 for SOx is due to the C=O stretching mode. The C-H stretching wave numbers of N bonded methyl group are lower than methyl groups attached to carbon atom. The N-methyl C-H stretch occurs from 1905-2080 cm-1 for CA, 1880-2100 cm-1 for CI & 1840-2020 cm-1 for SOx (Fig. 4).

3.2 Characterization of working glassy carbon (GC) electrode creatinine during construction

3.2.1 By SEM

The SEM images of the surface of bare GC electrode and CANPs/CINPs/SOxNPs/GC electrode are shown in Fig 5. The modification of bare GC electrode into working GC electode was seen clearly from these SEM images (Fig. 5). The SEM image of the bare GC electrode showed a smooth and featureless morphology. The CANPs/CINPs/SOxNPs/GC electrode exhibited granular, globular structural morphology, which appeared due to absorption of CANPs/CINPs/SOxNPs/GC onto GC electrode. The porous electrode revealed larger and

effective surface area after absorption of CANPs/CINPs/SOxNPs/GC NPs, granular and globular structure changed to regular form (Fig.5). Absorption of enzymes gives a favorable environment for high loading of enzymes.

3.2.2 By electrochemical impedance spectroscopy *(*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 CA, CI and SOx ENPs onto glassy carbon (GC) 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 Warburg diffusion process. The Nyquist plot (Fig. 6) displays EIS of bare GC, immobilized GC electrode in 5mM $K_3Fe(CN)_6/K_4Fe(CN)_6$. The R_{CT} value for the CANPs/CINPs/SOxNPs/GC electrode was obtained as 480 Ω, which was higher compared to bare electrode (R_{CT}=270 Ω). These results showed that the increased R_{CT} value of CANPs, CINPs and SOxNPs/GC electrode was due to the immobilization of enzyme nanoparticles onto GC surface. This increase in R_{CT} is attributed to the fact that most biological molecules, including enzyme nanoparticles, are poor electrical conductors at low frequencies and cause hindrance to the electron transfer.

3.3 Construction and optimization of creatinine biosensor

Amperometric creatinine biosensor based on immobilization of ENPs of CA, CI, SOx onto glassy carbon (GC) electrode was developed. The maximum current was observed with in 2s at 1.0V against Ag/AgCl. The biosensor showed both anodic and cathodic peaks with equal slope in presence of substrate (Fig. 7). Hence, in subsequent electrochemical studies, the current was recorded within 2s at 1.0V, at a scan rate of 20V/s. The biosensor showed optimum current at pH 6.0 at 25°C in 0.1 M sodium phosphate buffer, which is closer to the physiological pH (Fig. 8a, 8b). The optimum pH 6.0 at 25°C of present biosensor is comparable to earlier biosensors (optimum pH 6.7, temp. 25°C) [14]; (optimum pH 6.0, temp. 25) [15] (Table 4). Hence, the subsequent electrochemical experiments were carried out in 0.1M sodium phosphate buffer at pH 6.0 and temperature 25°C. The biosensor offered optimum response of 2s (Fig. 8c), which was better than earlier reported creatinine biosensors i.e 4s [2]; 240s [16]. There was a hyperbolic relationship between biosensor response and creatinine concentration in the range 0.01µM-12µM (Fig. 2).

3.4 Evaluation of biosensor

There was a linear relationship between the biosensor response i.e. current (in mA) and the creatinine concentration in the range of 0.01µM-12µM (Fig. 2), which is comparable to earlier biosensors i.e 0.5-1000 µM [2]; 5-2000 µM [15]; 9-100 µM [17]. The detection limit of the developed biosensor was 0.01µM, which is also better than earlier biosensors, (1µM) [18]; [19] (0.5 µM); (2.4 µM) [14]; (3.2 µM) [20]; (5 µM) [21]. The analytic recoveries of creatinine added in sera at levels of 0.1mM and 0.15mM were 97.97±0.1 and 98.76±0.2% respectively demonstrating the reliability of the developed biosensor (Supplementary Table 1). Within and between batch coefficients of variation for the determination of creatinine in sera on the same day and after one week of storage were 2.06% and 3.09%, respectively (Supplementary Table 2). These high precisions highlight the good reproducibility and consistency of the present method, which can be attributed to the electrostatic attraction of CA, CI, SOx onto the glassy carbon electrode (Table 4).

3.5 Correlation

The creatinine level in sera of apparently healthy subjects and persons suffering from renal disorders, as measured by present biosensor were compared with those obtained by standard enzymic colorimetric method. A good correlation (R^2 = 0.99) was found between these two methods (Fig. 9). This shows the high accuracy of the method.

3.6 Applications of creatinine biosensor

The creatinine level in sera samples of apparently healthy persons, as measured by the present biosensor was in the range of 78.694±0.03 µM (n=20) which is in normal established range the serum creatinine level was 298.417±0.02 µM (n=20) for persons suffering from kidney disease which is significantly higher than those in apparently healthy persons (p>0.1) (Supplementary Table 3). The present biosensing method is much more simple, sensitive, specific and rapid than colorimetric method.

3.7 Interference study and selectivity

Ascorbic acid, uric acid, urea, glycine, glucose, lactose and sarcosine at their physiological concentrations had practically no effect on biosensor response under the standard assay conditions.

3.8 Storage stability of ENPs/GC electrode

The present ENPs electrode lost 10% of its initial activity after 250 times uses during the span of 240 days of its regular uses, when stored dry at 4˚C (Fig. 10). This biosensor is better than earlier biosensors (15% loss in its initial response after 180 days) [22]; (58% loss in its initial response after 20 times utilization in 60 days) [23] and (50% loss in its initial response after used for 100 times in 180 day) [2]. A comparison of analytical properties of this creatinine biosensor with earlier biosensor is summarized in Table 4. These observations indicate the better analyte performance of present biosensors over earlier biosensors (Table 4).

4. Conclusion

The use of nanoparticles of CA, CI and SOx (bound to GC electrode) in construction of amperometric creatinine biosensor has resulted into its better analytical performance in terms of faster response time (2s), lower detection limit (0.01 µM), broad linear range (0.01 to 12µM) and higher storage stability (240 days) compared to earlier creatinine biosensors. The study could be extended to promote other amperometric biosensors, employing ENPs and GC electrode.

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List of Tables

Supplementary Table: 1 Analytical recovery of added creatinine in the serum samples (n=5), as measured by creatinine biosensor based on CANPs/CINPs/SOxNPs/GC electrode (CA=Creatininase, CI=Creatinase, SOx=Sarcosine Oxidase, NPs=Nanoparticles, GC=Glassy carbon electrode).

Supplementary Table: 2 Within and between assay coefficients of variation for determination of creatinine in the serum samples, as measured by creatinine biosensor based on CANPs/CINPs/SOxNPs/GC electrode (CA=Creatininase, CI=Creatinase, SOx=Sarcosine Oxidase, NPs=Nanoparticles, GC=Glassy carbon electrode).

Supplementary Table: 3 Serum creatinine levels in apparently healthy persons and kidney patients, as measured by creatinine biosensor based on CANPs/CINPs/SOxNPs/GC electrode (CA=Creatininase, CI=Creatinase, SOx=Sarcosine Oxidase, NPs=Nanoparticles, GC=Glassy carbon electrode).

Table: 4 A comparison of various analytic parameters of creatinine biosensors.

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Table 1

Creatinine added (mM)	Creatinine found (mM)	% Recovery
-	0.38	-
0.1	1.017	97.97±0.1
0.15	1.519	98.76±0.2

Table 2

N	Creatinine (µM)	CV (%)
Within assay (5)	86.6	2.06
87		
88		
85		
86		
87		
Between assay (5)	85.4	3.09
86		
88		
83		
84		
86		

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Table 3

Sex	Age (year)	Apparently healthy persons (µM)	Sex	Age (year)	Diseased persons (µM)
M	75	97.26±0.4	M	37	450.94±0.2
M	48	79.58±0.6	M	25	185.68±0.4
M	50	88.42±0.3	M	60	530.52±0.1
F	70	79.58±0.6	M	50	468.63±0.2
F	70	88.42±0.3	F	43	503.99±0.5
F	NB	70.74±0.2	F	66	300.63±0.2
M	26	70.74±0.2	F	94	176.84±0.3
M	42	79.58±0.6	M	70	141.47±0.5
M	50	97.26±0.4	M	55	176.84±0.2
M	45	88.42±0.3	F	16	503.99±0.5
M	48	70.74±0.2	M	62	185.68±0.4
F	57	79.58±0.5	M	50	468.63±0.2
F	33	70.74±0.2	M	40	176.84±0.3
F	70	61.89±0.5	M	62	176.84±0.2
F	80	61.89±0.5	M	62	185.68±0.4
M	15	61.89±0.5	M	58	238.73±0.2
M	NB	61.89±0.5	F	63	291.79±0.6
M	40	79.58±0.6	F	82	229.89±0.5
M	24	88.42±0.3	F	28	389.05±0.1
M	21	97.26±0.4	F	58	185.68±0.4
		(n=20) = 1573.88/20 = 78.694±0.2			(n=20) = 5968.34/20 = 298.417±0.4

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Table 4

Sr. No.	Support for immobilization	Enzyme(s) used	Electrode	Method of immobilization	Potential applied	Optimum pH	Optimum temp.	Response time (sec.)	Detection limit	Linear range	Storage ability (days)	Ref.
1	Polyethylene tetephthalate (PET)	CA, CI, SOx	Screen printed carbon electrode	Adsorption	0.1 V	6.7	25	10 s	2.4 µM	5-1000 µM	180	[14]
2	AuNP/CHIT/c-MWCNT	CA, CI, SOx	Glassy carbon	Cross-linking	0.2	7.5	25	4 s	0.5 µM	0.5-1000 µM	180	[2]
3	Ag NPs	CI	NR	NR	NR	10.0	NR	300 s	0.003 µM	0.01-1.0 µM	NR	[24]
4	Nafion/Pd	CI	Carbon electrode/Pd-Ni/SiNW	Adsorption	0.4 V	7.0	25	NR	NR	10-1700 µM	NR	[25]
5	AuNP/ Calix arene	CI, SOx	Nafion coated copper plating	Covalent	NR	4.0	NR	60 s	0.01 µM	0.01-0.1 µM	35	[26]
6	AuNP	CI	NR	Cross-linking (CI & AuNP)	NR	6.0	25	144 s	80 µM	100-20000 µM	NR	[15]
7	PANi	CA, CI, SOx	Carbon	Adsorption	0.4 V	7.25	34	10 s	50 µM	50-1000 µM	30	[23]

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8	pH sensitive field-effect transistors (pH-FET)	CA	Silical-ite	Adsorption	NR	7.4	NR	240 s	5 µM	5-2000 µM	390	[16]
9	Poly(ethyl eneimine	CA	Phosp-hotun-gstic	NR	NR	NR	NR	NR	0.06 µM	0.125-62.5 µM	NR	[27]
10	Molecular imprinting polymers (MIP)	CI	Epoxy graphi-te matrix	Adsorption	NR	5.0	NR	NR	7 µM	9-100 µM	28	[17]
11	Rhodamin e B dye	CA	Au	Covalent	NR	NR	NR	NR	0.005 µM	0.01 - 120 µM	NR	[28]
12	Nano-gold NPs	CA	Zeolite	Cross-linking	NR	NR	NR	240 s	10 µM	NR	NR	[29]
13	Calyx(4) pyrrole	CA	Ion-selecti-ve	Non-covalent entrapme nt	NR	NR	NR	NR	10 µM	1-10 µM	NR	[30]
14		CA, CI, SOx	GC	Adsorption	0.1 V	6.0	25	2 s	0.001 µM	0.01-1000 µM	240	Present biosensor

Table 4 Comparison of different analytic parameters of present creatinine biosensor with those of earlier biosensors. (CA = Creatininase, CI = Creatinase, SOx = Sarcosine Oxidase, PANi = Polyaniline, MWCNT = Multiwalled carbon nanotubes, CHIT = Chitoson, PET = Polyethylene terephthalate)

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Legends to figures

Figure: 1 Schematic representation of fabrication of CANPs/CINPs/SOxNPs/GC electrode and electro-chemical reactions involved in its response measurement, (CA=Creatininase, CI=Creatinase, SOx=Sarcosine Oxidase, NPs=Nanoparticles, GC=Glassy carbon electrode).

Figure: 2 Standard curve of creatinine concentration in (a) lower concentration and (b) higher concentration, by creatinine biosensor based on CANPs/CINPs/SOxNPs/GC glassy carbon (GC) electrode, prepared in 25ml 0.1 M sodium phosphate buffer (pH=7.0) containing 0.1ml (0.1 M) creatinine solution under standard conditions, (CA=Creatininase, CI=Creatinase, SOx=Sarcosine Oxidase, NPs=Nanoparticles, GC=Glassy Carbon electrode).

Figure: 3 Transmission electron microscopic (TEM) images of nanoparticles (NPs) of (a) CA, (b) CI, (c) SOx , (CA=Creatininase, CI=Creatinase, SOx= Sarcosine Oxidase).

Figure: 4 FTIR spectra of (a) native CA (b) CANPs, (c) native CI , (d) CI NPs, (e) native SOx (f) SOx NPs, (CA=Creatininase, CI=Creatinase, SOx= Sarcosine Oxidase, NPs= Nanoparticles).

Figure: 5 Scanning electron microscopic (SEM) images of (a) bare GC electrode and (b) CANPs/CINPs/SOxNPs modified GC electrode, (GC=Glassy Carbon electrode, CA=Creatininase, CI=Creatinase, SOx=Sarcosine Oxidase, NPs=Nanoparticles,).

Figure: 6 Electrochemical Impedance Spectra (EIS) of (a) Bare GC electrode, (b) CANPs/CINPs/SOxNPs modified GC electrode, (GC=Glassy Carbon electrode, CA=Creatininase, CI=Creatinase, SOx=Sarcosine Oxidase, NPs=Nanoparticles).

Figure: 7 Cyclic voltammogram (CV) of CANPs/CINPs/SOxNPs/GC electrode in 25ml 0.1 M sodium phosphate buffer (pH=7.0) containing 0.1ml 0.1 M creatinine solution at a scan rate of 20V/s, (CA=Creatininase, CI=Creatinase, SOx=Sarcosine Oxidase, NPs=Nanoparticles, GC=Glassy carbon electrode).

Figure: 8 Influence of (a) pH, **(b)** incubation temperature and **(c)** time on the current response of CANPs/CINPs/SOxNPs/GC electrode, in 25ml 0.1 M sodium phosphate buffer (pH=7.0) containing 0.1ml 0.1M creatinine solution, (CA=Creatininase, CI=Creatinase, SOx=Sarcosine Oxidase, NPs=Nanoparticles, GC=Glassy carbon electrode).

Figure: 9 Correlation curve between sera creatinine values as measured by standard colorimetric (x axis) and the current biosensor (y axis) based on CANPs/CINPs/SOxNPs/GC electrode, (CA=Creatininase, CI=Creatinase, SOx=Sarcosine Oxidase, NPs=Nanoparticles, GC=Glassy carbon electrode).

Figure: 10 Storage stability of enzyme nanoparticles modified glassy carbon (GC) electrode in 0.1 M sodium phosphate buffer (pH=7.0) at 4°C.

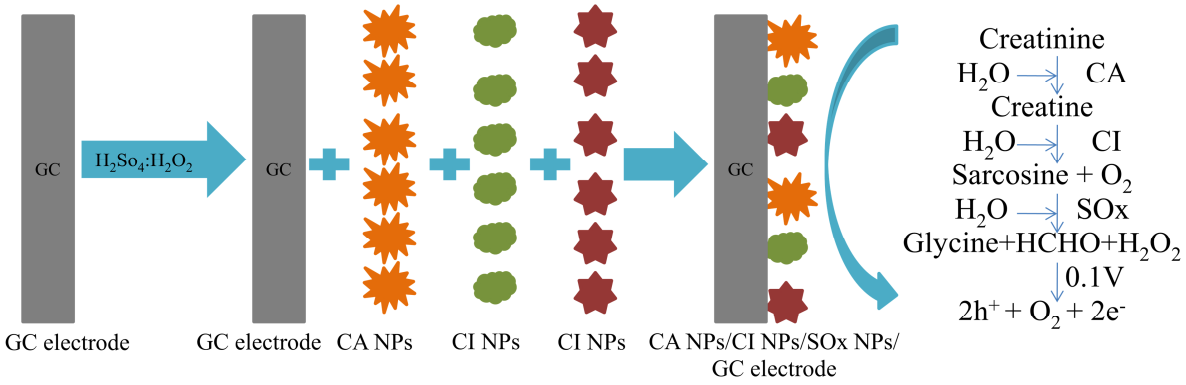

GC
$II_2So_4:II_2O_2$
GC
GC
GC electrode
GC electrode
CA NPs
CI NPs
CI NPs
CA NPs/CI NPs/SOx NPs/
GC electrode
Creatinine
H_2O
CA
Creatine
H_2O
CI
Sarcosine + O_2
H_2O
SOx
Glycine+HCHO+H_2O_2
0.1V
$2h^+ + O_2 + 2e^-$

Fig. 1

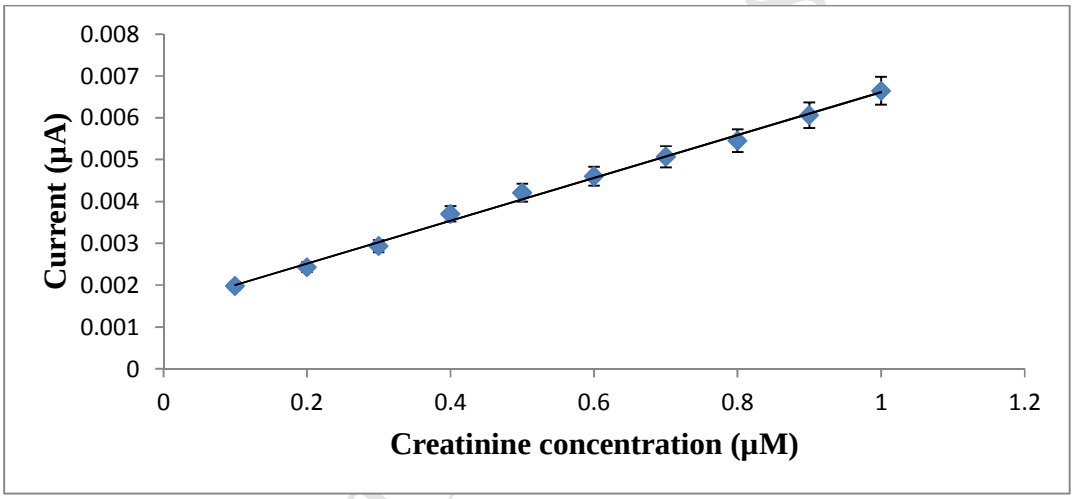

0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
Current (µA)
0
0.2
0.4
0.6
0.8
1
1.2
Creatinine concentration (µM)

Fig. 2 a

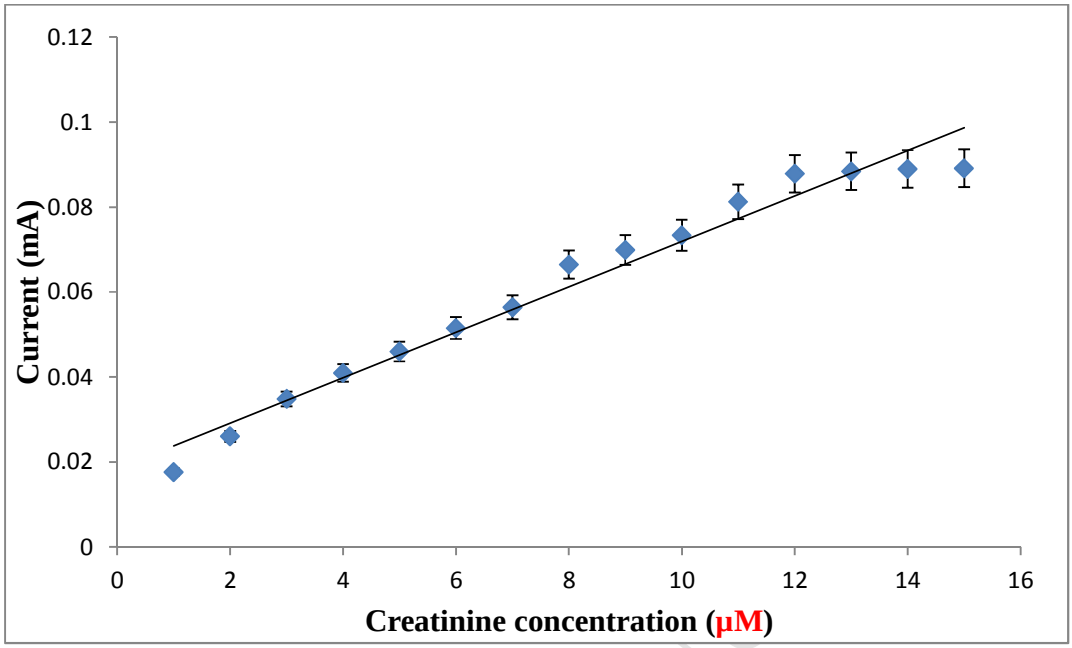
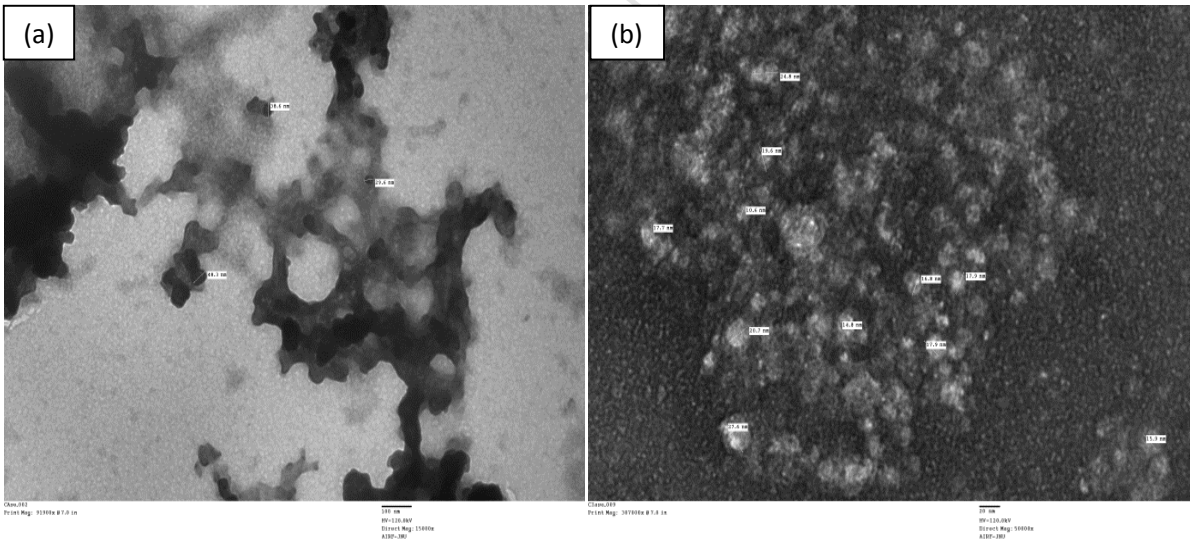
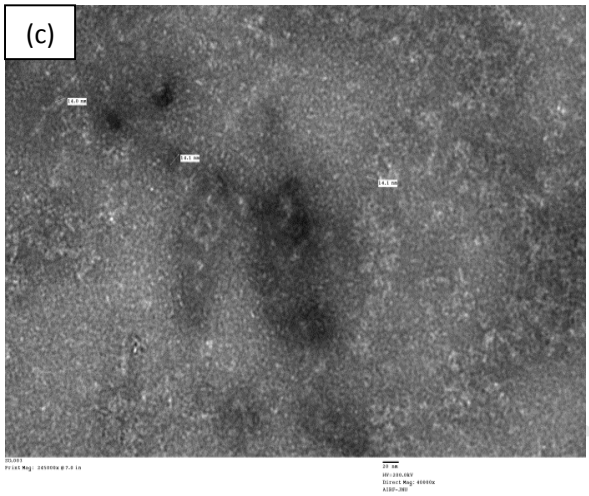


Fig. 2 b

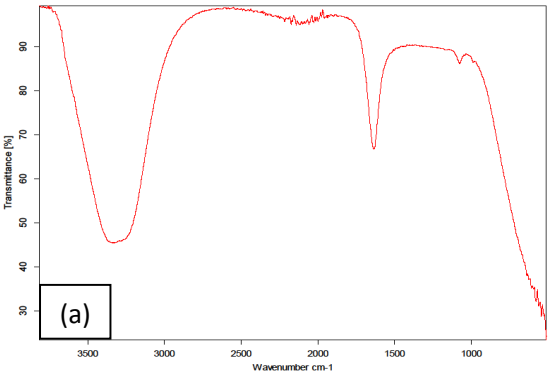


TEM CA **TEM CI**

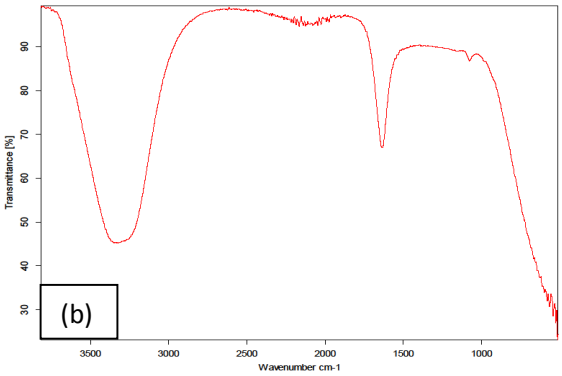


TEM SOx

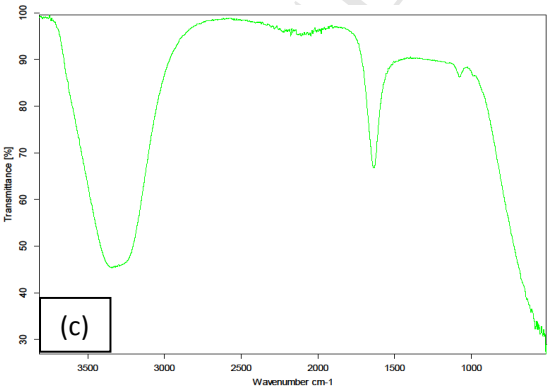
Fig. 3



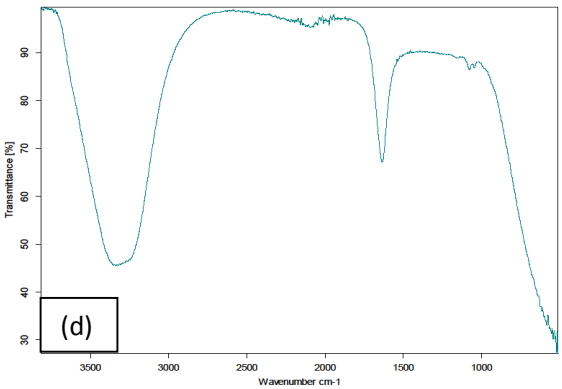
CA native enzyme



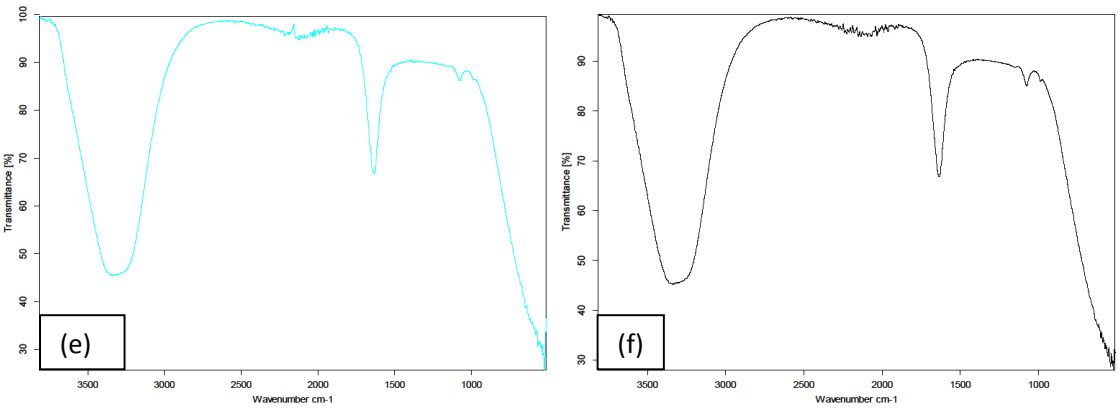
CA ENPs



CI native enzyme



CI ENPs



SOx native enzyme SOx ENPs

Fig. 4

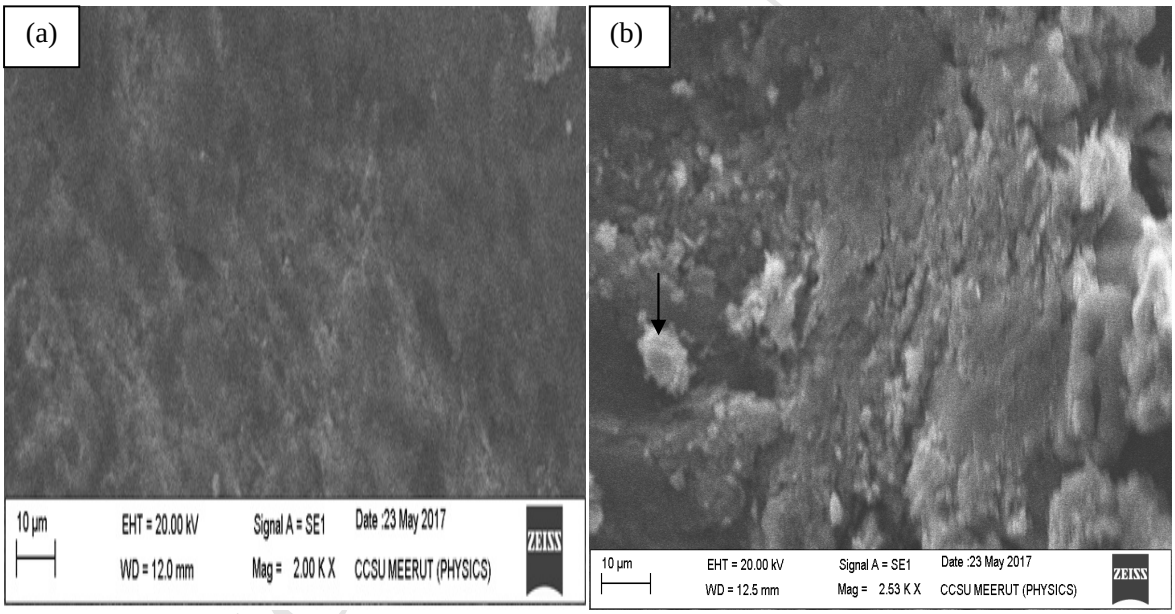


Fig. 5

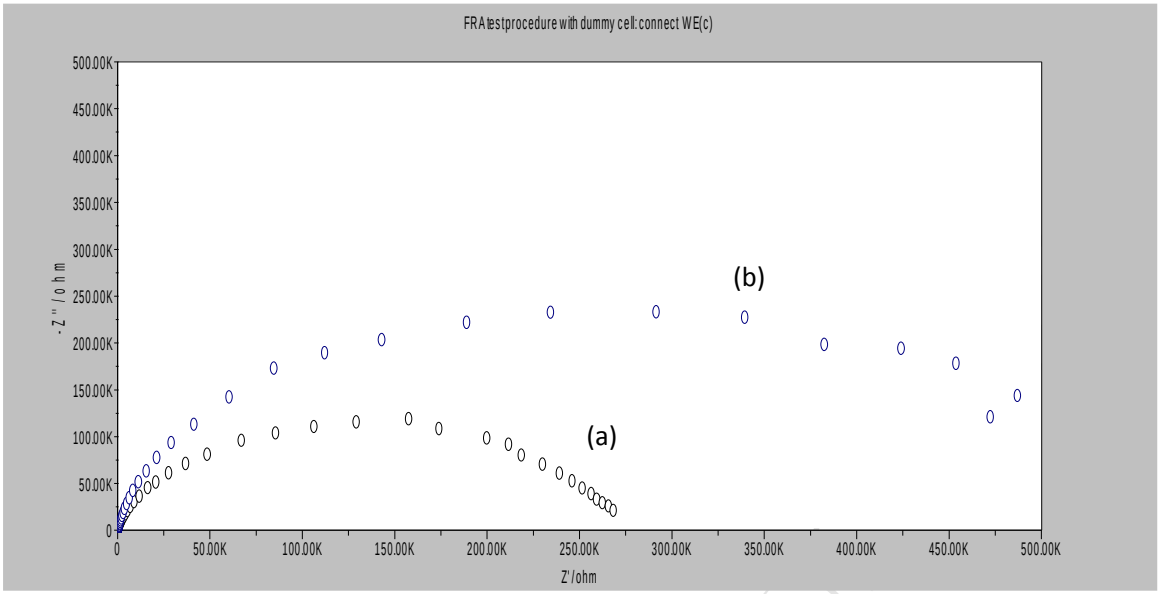

FRA test procedure with dummy cell: connect WE(c)
- Z '' / ohm
Z' / ohm
(a)
(b)

Fig. 6

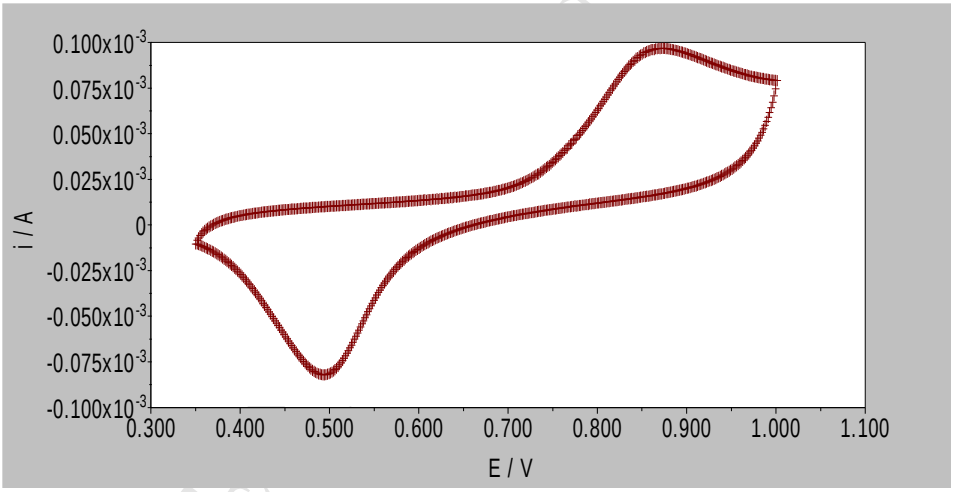

i / A
E / V

Fig. 7

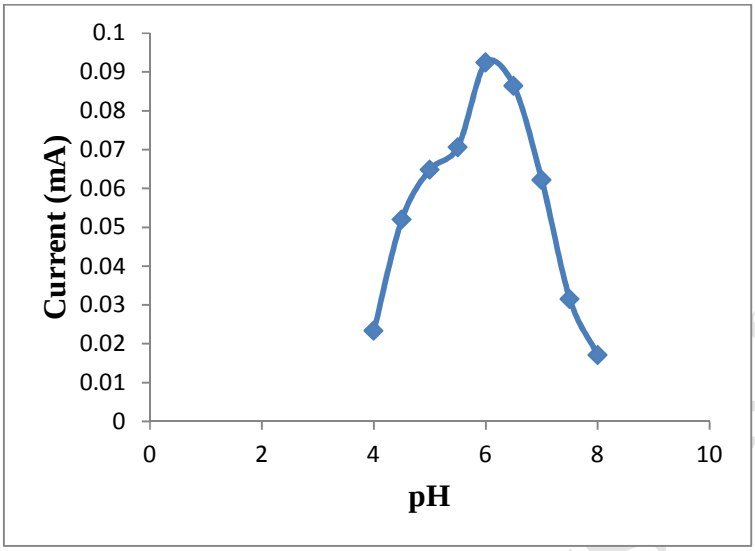

Current (mA)
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
2
4
6
8
10
pH

Fig. 8(a)

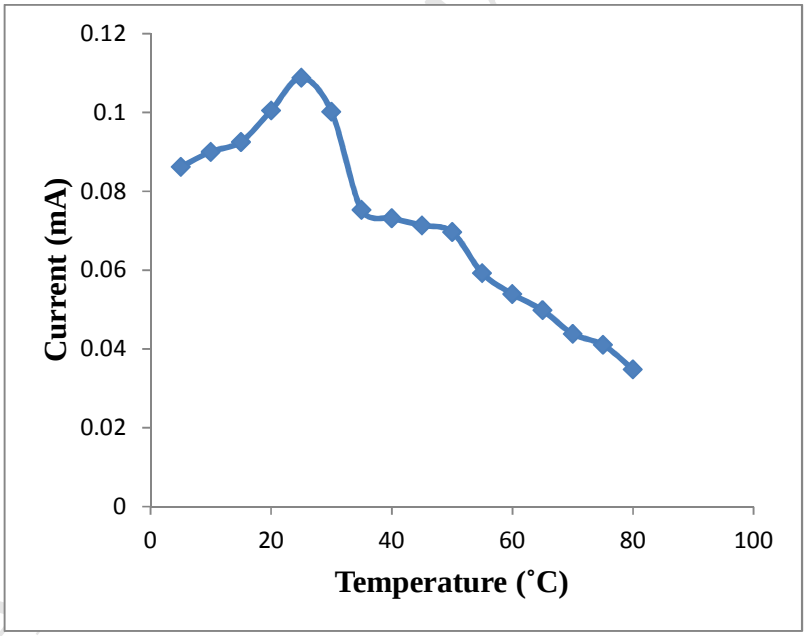

Current (mA)
0.12
0.1
0.08
0.06
0.04
0.02
0
0
20
40
60
80
100
Temperature (˚C)

Fig. 8(b)

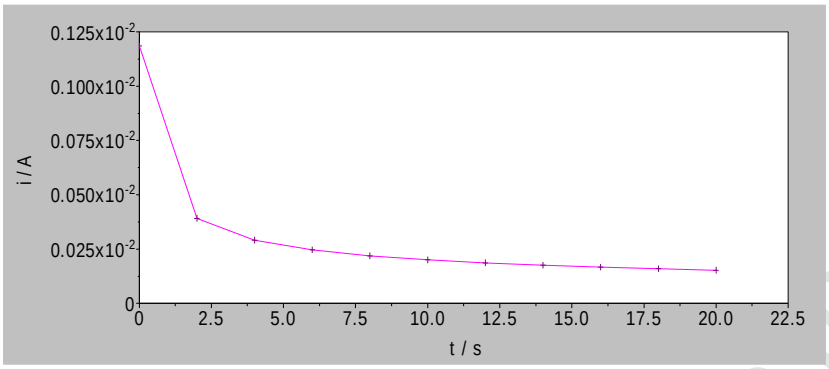


Fig. 8(c)

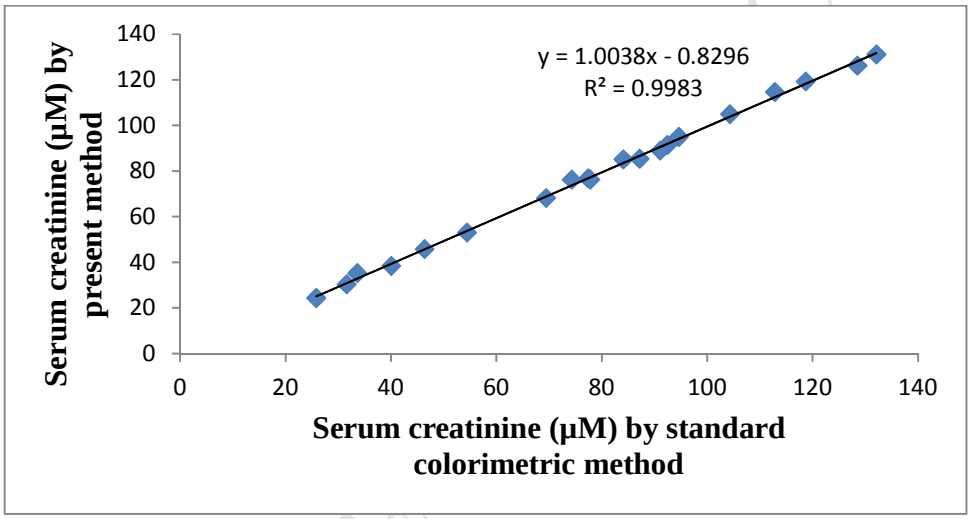


Fig. 9

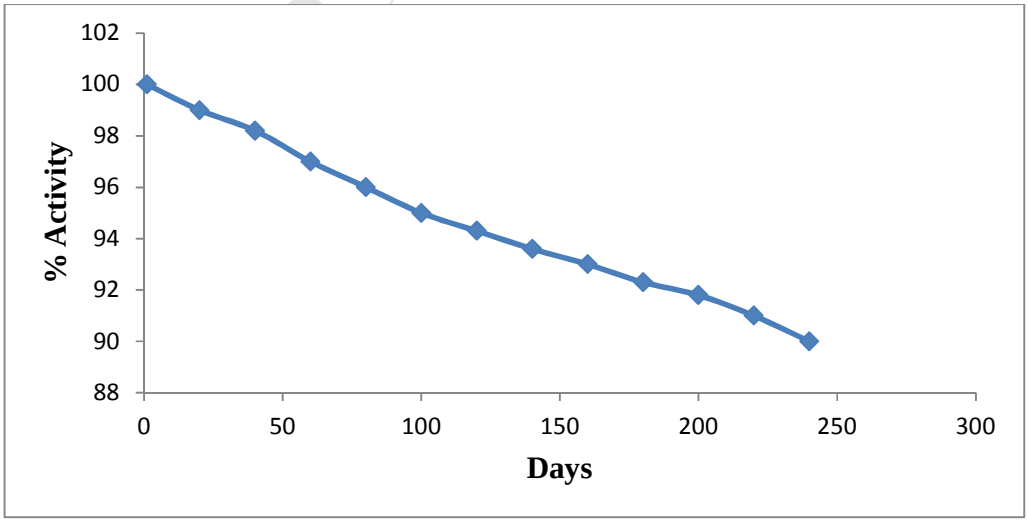


Fig. 10