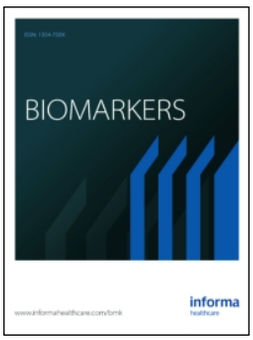

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ORIGINAL PAPER

Fabrication of an improved amperometric creatinine biosensor based on enzymes nanoparticles bound to Au electrode

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ABSTRACT

An improved amperometric creatinine biosensor was fabricated that dependent on covalent immobilisation of nanoparticles of creatininase (CANPs), creatinase (CINPs) and sarcosine oxidase (SOxNPs) onto gold electrode (AuE). The CANPs/CINPs/SOxNPs/AuE was characterised by scanning electron microscopy and cyclic voltammetry at various stages. The working electrode exhibited optimal response within 2 s at a potential of 0.6 V, against Ag/AgCl, pH 6.5 and 30 °C. A linear relationship was observed between creatinine concentration range, 0.1–200μM and biosensor response i.e. current in mA, under optimum conditions. Biosensor offered a low detection limit of 0.1 μM with long storage stability. Analytical recoveries of added creatinine in blood sera at 0.5 mM and at 1.0 mM concentrations, were 92.0% and 79.20% respectively. The precision i.e. within and between-batch coefficients of variation were 2.04% and 3.06% respectively. There was a good correlation ($R^2 = 0.99$) between level of creatinine in sera, as calculated by the colorimetric method and present electrode. The CANPs/CINPs/SOxNPs/Au electrode was reused 200 times during the period of 180 days, with just 10% loss in its initial activity, while being stored at 4 °C, when not in use.

HIGHLIGHTS

1. Prepared and characterised creatininase (CA), creatinase (CI) sarcosine oxidase (SOx) nanoparticles and immobilised them onto gold electrode (AuE) for fabrication of an improved amperometric creatinine biosensor.
2. The biosensor displayed a limit of detection (LOD) of 0.1 μM with a linear working range of 0.1 μM–200 μM.
3. The biosensor was evaluated and applied to measure elevated creatinine levels in sera from whom suffering from kidney and muscular disorders.
4. The working electrode retained 90% of its initial activity, while being stored dry at 4 °C for 180 days.

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Enzyme nanoparticles; creatinine; creatinine biosensor; Sera

1. Introduction

Creatinine (2-amino-1-methyl-5H-imidazol-4-one) is the final result of creatine metabolism, which is present in human blood and expelled from the blood eminently by the kidneys, primarily by glomerular filtration. Creatine is produced mainly in the liver from methylation of glycocyamine. Creatine, at that point is transferred to various organs such as muscles, brain and get phosphorylated to form high energy compound such as phosphocreatine. Creatinine is synthesised in this reaction as a byproduct (Taylor 1989). The concentration of creatinine in blood sera and urine of a healthy person is 45–140 μM and 0.8–2.0 g/day respectively (Kumar *et al.* 2017). Creatinine level is higher in men comparable to women due to its high muscles mass.

Clinically, it is essential to measure creatinine level in blood sera and urine samples. Renal failure, diabetic nephropathy, preeclampsia, glomerulonephritis, and urinary tract obstruction signify high level of creatinine (Reddy and Gobi 2013), whereas muscular dystrophy and myasthenia proves low level of creatinine in blood (Polinder-Bos *et al.* 2017). Creatinine level also varies in different sex as men have more creatinine level, because of more muscle mass. With increasing in life standard and advancement in technologies, there is 30% increase in prevalence of chronic kidney disease (CKD) during last decade (Coresh *et al.* 2007). The major cause of CKD is an expressively rises in the concentration of metabolic waste products in the blood, including urea and creatinine (Vaidya *et al.* 2008).

Creatinine analyser in clinical biochemical laboratories are available which based on spectrophotometric detection of Jaffe's reaction (Jaffé 1886). However, this reaction is not very specific, as it is interfered by many blood substances like pyruvate, glucose, bilirubin, dopamine and other's. There are number of analytical methods available to determine creatinine concenteration like spectrophotometry (Krishnegowda *et al.* 2017, Saidi *et al.* 2018), chromatography (Langsi *et al.* 2017), capillary electrophoresis (Grochocki *et al.* 2017, Vitali *et al.* 2017), liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Suzuki *et al.* 2016, Brozmanová *et al.* 2017),

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chemiluminescence (Hanif *et al.* 2016), enzyme reaction (Duong and Rhee 2017), molecular imprinted polymer (MIP) assays (Ang *et al.* 2016, Diouf *et al.* 2017), pH sensors (Pal *et al.* 2016, Roy *et al.* 2017). All these methods are laborious, complex, time consuming and require expensive instruments and require skilled persons to operate. There has been considerable interest in the design of electrochemical biosensors specific for creatinine detection. The immobilised biomolecules and analyte molecules undergo chemical reactions and procreate or consume electrons or ions/particles. This brings a variation in electric current or potential which relating to the concentration of the analyte. To measure the change of electrical parameters, conductometric, potentiometric, amperometric and capacitive transducers have been utilised in electrochemical biosensors (Thevenot *et al.* 1999, Hasan *et al.* 2014). Howsoever, direct immobilisation of enzymes onto these nanomaterials by cross linking, covalent binding or adsorption gain their denaturation, prompting loss of their activity as well as shelf life of biosensor. To overcome these issues, enzymes molecule have been aggregated into their NPs and then immobilised onto electrode (Sharma *et al.* 2011, Narwal *et al.* 2018). Recently, an excellent approach has been reported by Kumar *et al.* (Kumar *et al.* 2017, Kumar *et al.* 2018) for determination of seum creatinine and sarcosine concenteration. In this, ENPs of different enzymes (CA, CI and SOx) were prepared and immobilised onto glassy carbon electrode (GCE) which resulted into a good response time and long life of biosensor. Gold electrode (Au) has been used for immobilisation of ENPs as it provides attractive features, for example, good/great conductor of electricity, low cost, low background current and easy availability which makes it a superior choice than other electrode. Further, it reacts with pirhana solution (H_2SO_4:H_2O_2, 3:1) to form polycrystalline Au (AuO) which reacts with –SH– group of ENPs to form thio-sulfate bond on the surface of Au and ENPs (Narwal *et al.* 2018, Antony *et al.* 2019). We are describing here, in the immobilisation of CANPs/CINPs/SOxNPs on to Au electrode for the construction of an improved amperometric creatinine biosensor and its application.

2. Material and methods

2.1 Chemicals and reagents

Creatininase amidohydrolase (EC 3.5.2.10) from *Pseudomonas sp.* communicated in *Flavobacterium sp.,* and creatinase amidinohydrolase (EC 3.5.3.3) from *Pseudomonas sp.* expressed in *Escherichia coli*, sarcosine oxidase (EC 1.5.3.1) from *Bacillus sp.* were purchased from SISCO Research lab Mumbai, India and Sigma-Aldrich, USA, respectively. Gold electrode (AuE) (2 mm × 20 mm, diameter × height) was purchased from local market, Rohtak were used. All other chemicals utilised in this work were of analytical reagent (AR) grade. All through this study, double distilled (DW) water was used. The blood/serum samples of evidently healthy individuals and persons who suffering from kidney/renal disorders were collected from the hospital of Pt. BDS Post Graduate Institute of Medical Science, Rohtak and kept at −20 °C till use.

2.2 Instruments used

Potentiostat/Galvanostat (Make: Autolab, model: AUT83785, manufactured by Eco Chemie, The Netherland), connected with a three electrode system, comprising of a platinum (Pt) wire as a auxiliary electrode, an Ag/AgCl electrode as a reference electrode and ENPs modified gold electrode (CANPs/CINPs/SOxNPs/AuE) as a working/enzyme electrode. Transmission electron microscope (TEM) (Talos, F200C, Thermo Scientific USA), scanning electron microscope (SEM) (Zeiss EVO18, USA), Fourier transform infra-red spectrometer (FTIR) (Thermo Scientific, USA) and UV Spectrophotometer (Make: Shimadzu, Japan, Model 1700), were used. Waving balance, pH metre, centrifuge and water bath were also used in this study.

2.3 Assay of mixture of CA, CI and sox

The assay of enzymes (CA, CI and SOx) was done as demonstrated by Fossati *et al.* (Fossati *et al.* 1983) with certain modifications or adjustments. To quantify the action of (CA, CI and SOx) catalysts, 1 mL of chromogen reagent (comprising 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 0.1 M sodium phosphate buffer (PBS buffer) (pH 7.0) per litre, was dissolved into 50 μL of creatinine mixture (0.175 mM) and after that 10 μL of CA solution (9–10 units) was included. After incubation for 30 min at 30 °C in Spectronic-20, the absorbance of pink coloured solution was recorded in Spectronic-20D + at 510 nm against control. The chromogen reagent was stored in dark Coloured bottle at 4 °C, when not in use.

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

The ENPs of CA, CI and SOx were prepared by dissolvation method as described by Kumar *et al.* (Kumar *et al.* 2017). In which 2.0 mL of each dissolved enzyme solution (CA,CI and SOx) in 0.1 M sodium phosphate (PBS) buffer pH 7.0 (1.0 mg/mL) were taken into 50 mL conical flask. Under constant stirring of 500 rpm, add 4.0 mL of absolute ethanol in to flask, dropwise at the rate of 0.1–0.2 mL/min in cold (4–6 °C). The ENPs were formed by aggregation of native enzyme molecules. After 24 h with constant stirring, add 1.8 mL of glutaraldehyde (2.5%) was added to this mixture suspension under continuous stirring at 500 rpm at 4 °C. After 5–6 h of the addition of glutaraldehyde, add 0.12 g of cysteamine dihydrochloride under constant stirring which was introduced amino groups ($–NH_2$) onto enzyme-solution. The enzyme nanoparticles (ENPs) were isolated solution by centrifugation for 10 min. at 1500 rpm in low temperature (4 °C) followed by scattering in 0.1 M PBS (pH 7.0) and sonication for 5 min. After that ENPs were stored gently at 4 °C, when the ENPs were not in use.

2.5 Characterisation of ENPs by TEM, FTIR and UV spectra

The NPs of CA, CI and SOx were characterised/identified by recording their transmission electron microscopic (TEM)

images, Fourier transform infra-red (FTIR) and UV spectra. The TEM gives the size and shape, ENPs of CA, CI and SOx. Functional groups such as O–H stretching, C = C stretching, COOH stretching, CC stretching, C–O (epoxy/ether) stretching and N-containing biological vibrations of ENPs were recorded by FTIR spectra. Shapes and optical properties of the ENPs were studied by recording UV spectra absorbance at different wavelength.

2.6 Preparation of working electrode

To prepare ENPs based working or constructed electrode, ENPs were covalently immobilised onto surface or upper layer of Au electrode. To achieve this, firstly bare gold (Au) electrode was rubbed by silica gel with muslin cloth followed by washing in distilled water. Then it was kept in pirhana mixture (H_2SO_4:H_2O_2, 3:1) for 20 min, and cleaned by silica gel. After that it was dipped in to ethanol for 5–6 h and followed by sonication for 2–4 min, to remove adsorbed particles. Now, washed polycrystaline Au electrode was placed into the mixture of ENPs (1:1:1) solution for 48 h at 4 °C so that ENPs aggregates was attached onto Au electrode by covalent immobilisation. In between, the ENPs mixture was disturbed, to avoid settling of ENPs in mixture. Finally, the ENPs bound working Au electrode was washed with 0.1 M PBS buffer (pH = 7.0) and stored in the same buffer at 4 °C, until use. A covalent immobilisation could be possible between –OH groups of polycrystalline Au electrode and –SH groups of ENPs, with concomitant removal of one water molecule (Narwal *et al.* 2018).

2.7 Characterisation of working electrode (ENPs/AuE) by SEM, FTIR and CV

The surface morphology of Au electrode before and after (bare and working) immobilisation of ENPs was characterised by scanning electron microscope (SEM) and Fourier transform infra-red spectra (FTIR). FTIR spectra of ENPs bound Au electrode was recorded by scrapping off the Au electrode and keeping the powder into the socket of the FTIR spectrometer. Cyclic voltametry (CV) of working electrode was recorded in potentiostat/galvanostat in the potential range 0.0 V–0.6 V in 25 mL 0f 0.1 M PBS buffer (pH = 6.5) containing 0.1 mL 0.1 M creatinine. The current was measured in mA at different time (in seconds).

2.8 Construction of creatinine biosensor and its response measurement

An amperometric creatinine biosensor was formed using ENPs/Au electrode as working electrode, Ag/AgCl as references electrode and Pt wire as auxiliary electrode connected through potentiostat. A graphic representation of construction of CANPs/CINPs/SOxNPs/Au electrode and its response analysis is shown in Figure 1. Biosensor response was measured by cyclic voltammetry (CV) in a potentiostat in the range, 0.0–0.6 V in 25 mL of 0.1 M sodium phosphate buffer (pH 6.5) containing 0.1 mL 1.0 M creatinine. Variations in current (in μA) were measured at different time ranges 1–5 s.

2.9 Optimisation of creatinine biosensor

The ENPs based creatinine biosensor was apprised for various optimisation parameters such as effect of pH, incubation temperature, response time and effect of substrate (creatinine) concentration was studied. To get optimum pH, the pH was differed in the range pH 5.0–9.0, at an interval of pH 0.5 using a suitable buffer (strength = 0.1 M) such as: pH 5.0–5.5 sodium acetate buffers, pH 5.5–8.5 sodium phosphate buffer and for pH 8.0–9.0 sodium glycine buffer. Identically, creatinine concentration on biosensor response was studied by varying the concentration of creatinine in the range of 0.1 mM–10.0 mM. To get optimum tempture, the reaction mixture was brooded at different temperatures in the temp. range 5–80 °C at an interval of 5 °C.

2.10 Evaluation of creatinine biosensor

The ENPs based creatinine biosensor was assessed for various analytical parameters such as detection limits, analytical recovery, substrate concentration wide range, response time and storage stability. Furthermore, the correlation of the present method was also done with the commercialise method i.e. Immuno chromatography pack technique, which depends on the rule that two sorts of specific antibodies against antigen are utilised. One of the antibody was immobilised on the chromatographic paper, also the other is marked with colloidal gold and penetrated into the sample pad at the end of the membrane. At the point, when the fluidic sample was dropped on the sample pad, the antigen in the sample forms an immunocomplex with the counter acting agent named colloidal gold. Its complex moves alongside the fluidic sample and reach the immune response immobilised on the layer, followed by forming in immunocomplex with the immobilised antibody, it resulted in creating a shaded red-purple line. The appearance of red-purple line on the membrane demonstrates the presence of antigen of interest for the sample. Since the fluid of the sample moves through the layer quick, it makes it conceivable to identify the presence or absence of antigen within 15 min.

2.11 Application of creatinine biosensor

The intravenous blood (1 mL each) samples drawn from healthy person and person suffering from kidney disorders (200 total) at Pt. BDS PGIMS, Rohtak, transferred it to a centrifuged tube and kept at room temperature for 30 min for coagulation. Thereafter, it was centrifuged at 1500 rpm for 12–15 min to get their supernatant (sera) and kept at 4 °C until use. The level of creatinine in sera was determined by measuring cyclic voltammetry (CV) of working/enzyme electrode in potentiostat in the potential range, 0.0–0.6 V in 25 mL of 0.1 M sodium phosphate buffer (pH 6.5) containing 0.1 mL serum samples under optimum condition. The current (μA) was recorded and the quantity of creatinine in serum

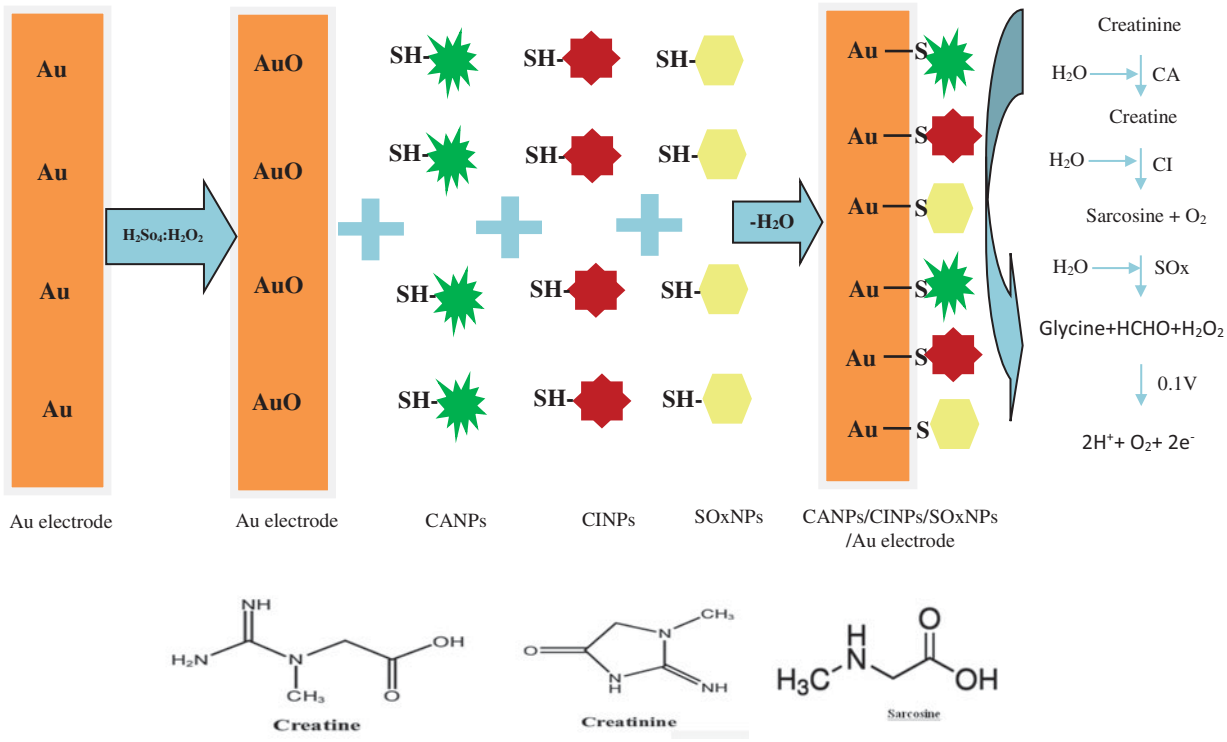

Figure 1. Schematic representation of fabrication of CANPs/CINPs/SOxNPs/Au electrode and electrochemical reactions involved in its response measurement (CA: Creatininase; CI: Creatinase; Sox: Sarcosine Oxidase; NPs: Nanoparticles; Au: Gold electrode).

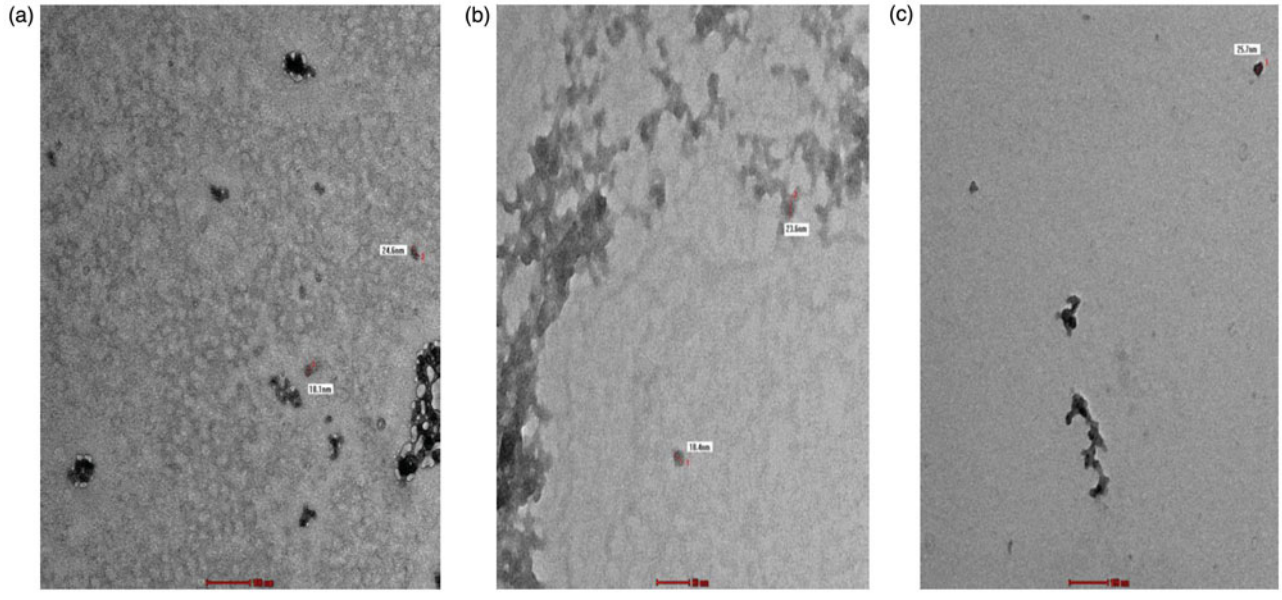

Figure 2. Transmission electron microscopic (TEM) images of nanoparticles (NPs) of (a) CA, (b) CI, (c) SOx (CA: Creatininase; CI: Creatinase; SOx: Sarcosine Oxidase).

was interpolated from the standard curve between creatinine concentrations and current (in μA) under optimal operational conditions.

3. Results and discussion

3.1 Characterisation of ENPs by TEM, FTIR and UV spectroscopy

The size of ENPs, as estimated by TEM, was between 5 and 100 nm with an average size of 20 nm (Figure 2), which shows up-gradation of the surface zone of the enzyme, as compared to bare enzyme.

FTIR of NPs of CA, CI and SOx showed the extending and twisting of N-H obligation of the amine gathering. The essential aromatic amines the N-H extending recurrence happens in the district 1080–2300 cm^{-1} for CA, 1020–2250 cm^{-1} for CI and 1050–2200 cm^{-1} for SOx. The FTIR range indicated trademark at 1077–2120 cm^{-1} for CA, 1045–2118 cm^{-1} for CI and 1076–2120 cm^{-1} for SOx, because of symmetric and one unbalanced N–H extending vibrations. Wide groups demonstrate the nearness of the

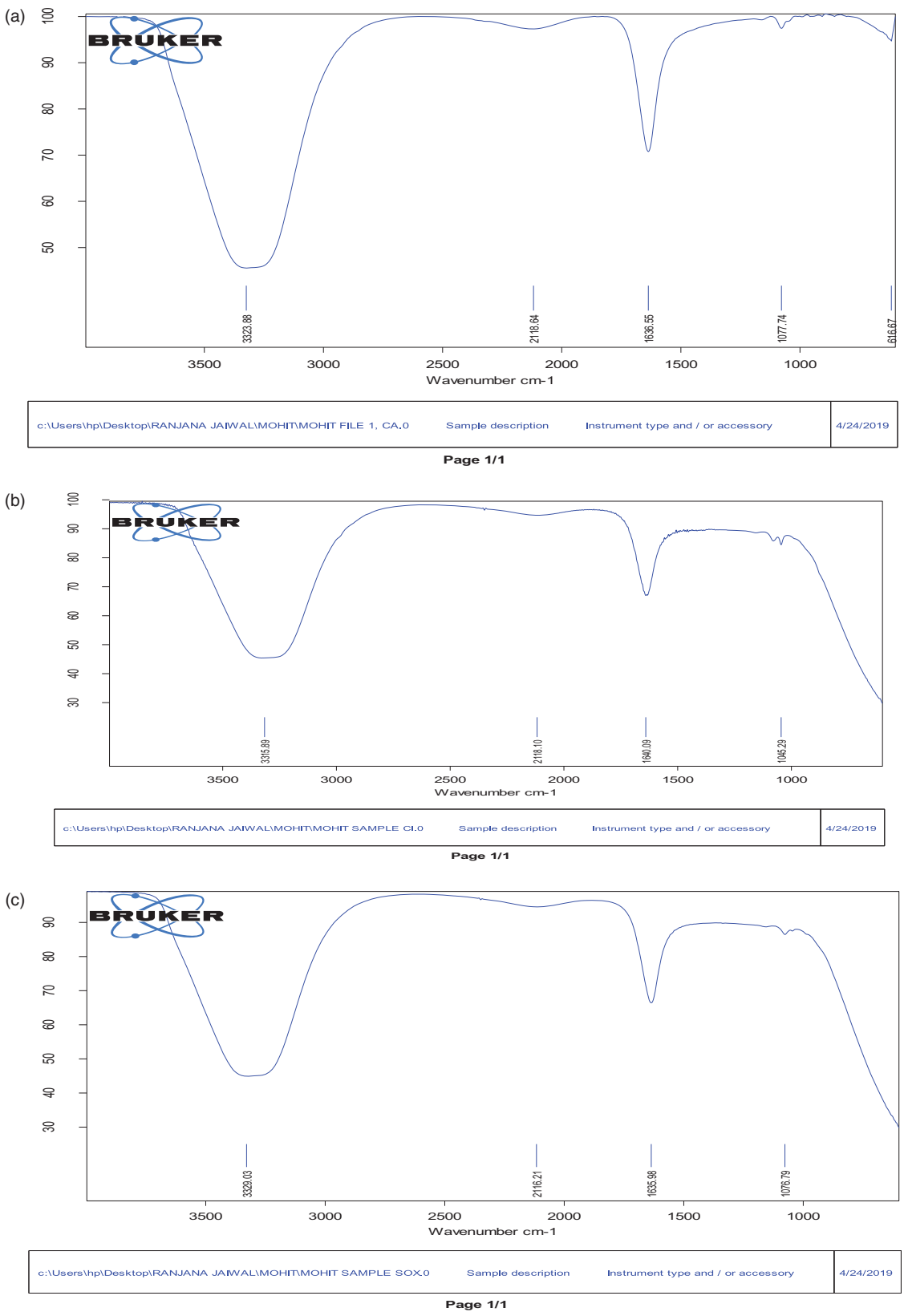

Figure 3. FTIR spectra of (a) CANPs, (b) CI NPs, (c) SOx NPs (CA: Creatininase; CI: Creatinase; SOx: Sarcosine Oxidase; NPs: Nanoparticles).

amine structure in the structure. The watched low estimations of groups were because of the investment in H-holding associations. The band seen at 1636 cm^{-1} for CA, 1640 cm^{-1} for CI and 1635 cm^{-1} for SOx was because of the C = O extending mode. The C–H extending wave quantities of N reinforced methyl gathering were lower than methyl bunches joined to carbon iota. The N-methyl C–H extend happened from 1905–2080 cm-1 for CA, 1880–2100 cm^{-1} for CI and 1840–2020 cm^{-1} for SOx, respectively (Figure 3).

UV and visible spectra exhibited solid absorbance peak at 300 nm, showing the small size and uncovering a high level of retention of fragrant amino acids of peptide chain/free enzyme, following their NPs arrangement. The nearness of the single absorption peak implied that the shaped nanoparticles were nearly spherical, which can be attributed to the coherent oscillation of the conduction electrons (Figure 4).

3.2 Characterisation of working gold (Au) electrode during construction

By SEM: Figure 5(a,b) demonstrated the surface of bare or smooth Au electrode and ENPs modified working Au electrode respectively. Surface of bare or smooth Au electrode showed smooth and featureless morphology in SEM image. The surface of ENPs/Au electrode exhibited granular, globular morphology, which showed up because of immobilisation of CANPs/CINPs/SOxNPs onto Au electrode.

3.3 Construction of amperometric creatinine biosensor

An amperometric creatinine biosensor was constructed deoended on immobilisation of ENPs of CA, CI and SOx onto gold electrode (Figure 6), which is easier compared to earlier reported amperometric biosensors (Pundir *et al.* 2019).

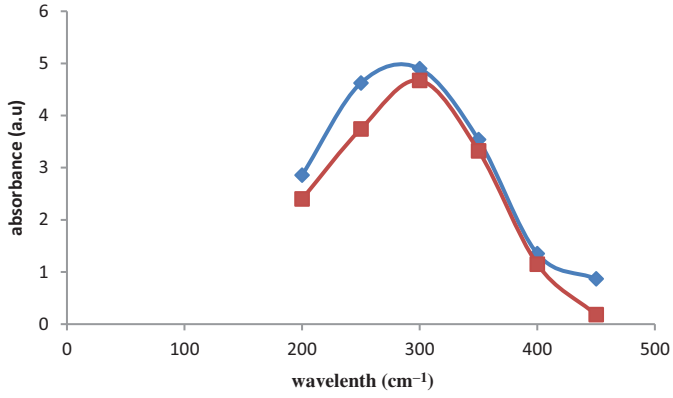

Figure 4. UV-visible Spectroscopy, CA, CI, SOx native enzymes and CANPs, CINPs and SOxNPs nanoparticles (CA: Creatininase; CI: Creatinase; SOx: Sarcosine Oxidase).

3.4 Evaluation of biosensor

There was a linear relationship between the biosensor response i.e. current μA and the creatinine concentration in the range, 0.1 μM–200μM (Figure 7). This linearity is superior to prior biosensors i.e. 0.5–1000 μM (Jain *et al.* 2015); 5–2000 μM (He *et al.* 2015); 9–100 μM (Kamel, 2016). The detection limit (LOD) of this working biosensor was 0.1 μM, which is likewise superior to earlier reported biosensors, (1 μM) (Busono *et al.* 2015); (0.5 μM) (Zhybak *et al.* 2016); (2.4 μM) (Ping *et al.* 2013); (3.2 μM) (Hsiue *et al.* 2004); (5 μM) (Sutariya *et al.* 2016). The analytic recoveries of creatinine added or included in sera at levels of 0.5 mM and 1.0 mM were 92.0 ± 0.1% and 79.20 ± 0.4% respectively exhibiting the reliability of the developed biosensor (Table 1). Within and between batch coefficients of variation for the determination of creatinine in blood sera on the same day/same time and after a week (7 days) storage at −20°C were 2.04% and 3.06%, respectively (Table 2). These high precisions feature the great reproducibility and consistency of the present method, which can be credited to the electrostatic attraction of CA, CI, SOx onto the lustrous gold electrode (Table 3). This can be credited to the covalent immobilisation of ENPs onto the Au electrode, also.

3.5 Determination of creatinine in sera

The present biosensor (working electrode) calculated the creatinine level in blood sera of healthy person which was in the range of 80.448 μM ($n = 20$) and sera of people suffering from renal disease was in range of 295.626 μM ($n = 20$), which is essentially greater than those in obviously healthy people ($p < 0.1$). The present/current biosensor is substantially more basic, touchy, explicit and quick than colourimetric technique.

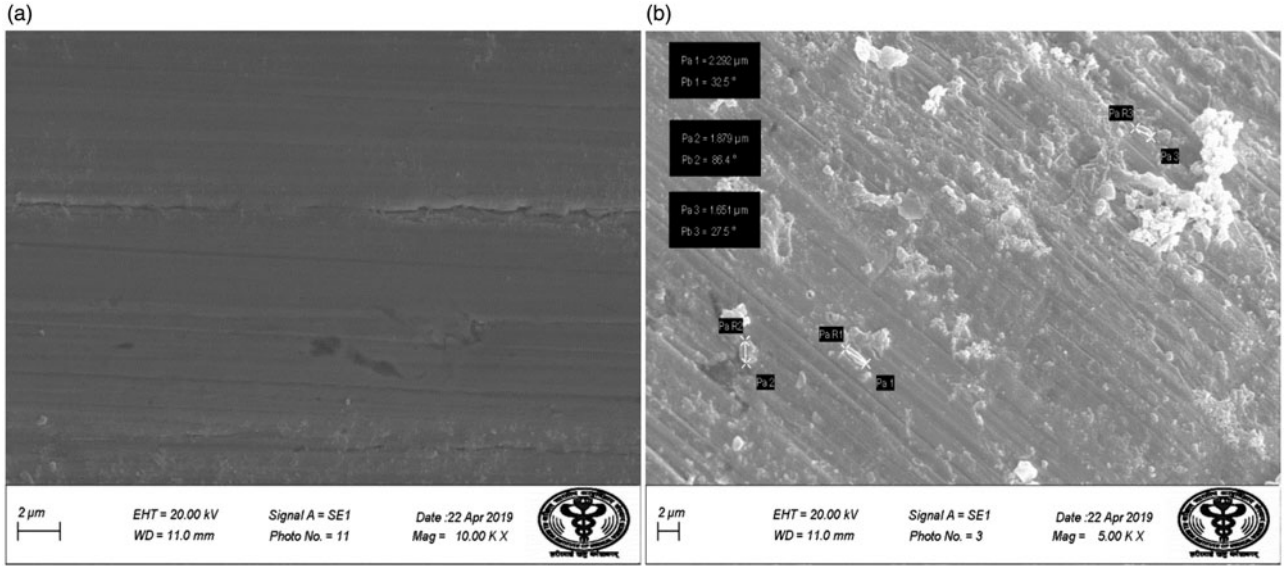

Figure 5. Scanning electron microscopic (SEM) images of (a) bare Au electrode and (b) CANPs/CINPs/SOxNPs modified Au electrode (Au: gold electrode; CA: Creatininase; CI: Creatinase; SOx: Sarcosine Oxidase; NPs: Nanoparticles,).

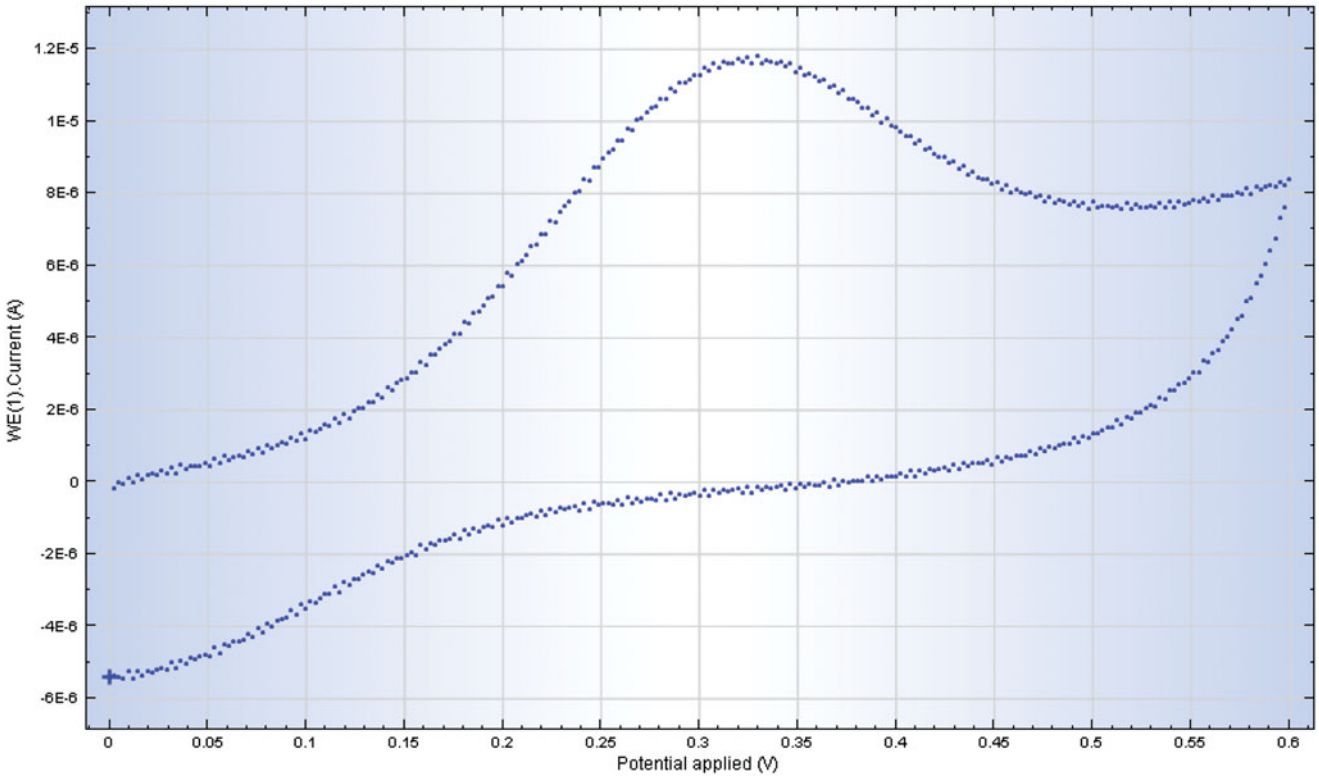

Figure 6. Cyclic voltammogram (CV) of CANPs/CINPs/SOxNPs/Au electrode in 25 mL 0.1 M sodium phosphate buffer (pH = 6.5) containing 0.1 mL 0.1 M creatinine solution at a scan rate of 20 V/s, (CA: Creatininase; CI: Creatinase; SOx: Sarcosine Oxidase; NPs: Nanoparticles; Au: gold electrode).

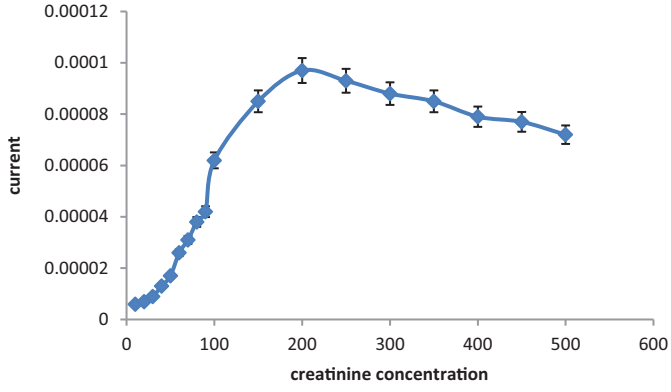

Figure 7. Standard curve of creatinine concentration, by creatinine biosensor based on CANPs/CINPs/SOxNPs/Au gold electrode, prepared in 25 mL 0.1 M sodium phosphate buffer (pH = 6.5) containing 0.1 mL (0.1 M) creatinine solution under standard conditions (CA: Creatininase; CI: Creatinase; SOx: Sarcosine Oxidase; NPs: Nanoparticles; Au: gold electrode).

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

Creatinine added (mM)	Creatinine found (mM)	% Recovery
–	**0.032**	–
0.5	0.124	92.0 ± 0.1
1.0	0.520	79.2 ± 0.4

3.6 Correlation

The creatinine level measured by present biosensor in sera of apparently healthy persons and persons suffering from kidney disorders were compared with other enzymatic methods. A good correlation ($R^2 = 0.99$) was found between these two methods or techniques as shown in Figure 8.

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/Au electrode (CA: Creatininase; CI: Creatinase; SOx: Sarcosine Oxidase; NPs: Nanoparticles; Au: gold electrode).

N	Creatinine (μm)	CV %
Within assay (5)	86.4	2.04
86		
87		
84		
88		
87		
Between assay (5)	85.6	3.06
85		
86		
84		
86		
87		

Bold value show the initial concentration of creatinine in urine.

3.7 Interference study and selectivity

Compounds which show interference such as glutamic acid, glucose, uric acid, ascorbic acid, glycine and urea caused obstruction of 2.60%, 2.20%, 3.10%, 3.20% and 1.40% individually at their physiological concentrations (1.0, 5000, 240, 200, 210, 4600 μM, respectively), which showed practically no effect on present biosensor response under the standard conditions. The effect of ascorbic acid, uric acid was studied on the biosensor performance. Because of substrate specificity of present creatinine biosensor, these side products did not influence the performance of biosensor.

3.8 Storage stability of ENPs/Au electrode

The present ENPs/Au electrode lost 10% of its initial activity after measuring creatinine in 200 sera sample during the

Table 3. A comparison of various analytic parameters of creatinine biosensors.

Sr. No.	Support for immobilisation	Enzyme(s) used	Electrode	Method of immobilisation	Optimum pH	Optimum temperature (°C)	Potential applied (V)	Response time (s)	Detection limit (μM)	Linear range (μM)	Storage ability (days)	Ref.
1	AgNPs	Polyoxometalate (POM)	Glassy carbon	Electrostatic interactions	6.0	4	0.6–1.5	NR	1.51×10^{-17}	50–1500	10	Zhang et al. 2018
2	Microfluidic cartridge	Creatininase, creatinase and creatinine	NR	Rapid prototyping	NR	37	NR	300	67.1–1768	0–199.7	NR	Dosso et al. 2018
3	Nafion-nanostructured polyaniline (nsPANi) composite film	CD	Au	Drop-casting	NR	30	(−0.2)–(−0.3)	NR	1300	5×10^{-6} -4×10^{-4}	NR	Do et al. 2018
4	Nano laminared SPR	Creatininase	Gold film	NR	NR	NR	NR	30	0.01	0.01–0.2	NR	Menon et al. 2018
5	Microfluidic paper-based	Sodium hydroxide ,picric acid	Heating module	NR	NR	37	NR	300	8.92	16.7–675.3	100	Tseng et al. 2018
6	Graphene oxide	TMSPMA-GO-co-HEMA/MMA	Glassy carbon	Drop-casting	7.4	NR	0.02	120	16.6	44.2–265.2	NR	Anirudhan et al. 2019
7	Graphene oxides	Formic acid	Chromatography Paper	NR	NR	275	3000	120	1502.8	884–300.5	NR	Fernandes et al. 2019
8	Pd	Ethylenediamine+ 1,8-naphthalimide (NCP)	NR	NR	7.2	37	NR	1800	0.16	1–70	NR	Du et al. 2019
9	Membrane cocktail	PVC, 4′,5′-Dibromofluorescein octadecyl ester, o-nitrophenyloctylether, potassium tetrakis, tetrahydrofuran	Polyester sheet	Drop-casting	3.8	37	NR	180	0.15	$1 \times 10^{-5} - 1 \times 10^{-11}$	NR	Erenas et al. 2019
10	Polyethylene Tetephthalate (PET)	CA, CI, SOx	Screen Printed Carbon Electrode	Adsorption	6.7	25	0.1	10	2.4	5–1000	180	Ping et al. 2013
11	AuNP/CHIT/c-MWCNT	CA, CI, SOx	Glassy Carbon	Cross-linking	7.5	25	0.2	4	0.5	0.5–1000	180	Jain et al. 2015
12	Ag NPs	CI	NR	NR	NR	NR	NR	300	0.003	0.01–1.0	0.003	Mohammadi and Khayatian 2015
13	Nafion/Pd	CI	Carbon Electro De/Pd-Ni/sin W	Adsorption	7.0	25	0.4	NR	NR	10–1700	NR	Yang et al. 2015
14	AuNP/ Calix arene	CI, SOx	Nafion Coated Copper plating	Covalent	4.0	NR	NR	60	0.01	0.01–0.1	35	Marchenko et al. 2015
15	AuNP	CI	NR	Cross-linking (CI & AuNP)	6.0	25	NR	144	80	100–20,000	NR	He et al. 2015
16	PANi	CA, CI, SOx	Carbon	Adsorption	7.25	34	0.4	10	50	50–1000	30	Busono 2015
17	pH Sensitive Fieldeffect Transistors (pH-FET)	CA	Silicalite	Adsorption	7.4	NR	NR	240	5	5–2000	390	Marchenko et al. 2016
18	Poly(ethyleneimine	CA	Phosphotungstic	NR	NR	NR	NR	NR	0.06	0.125–62.5	NR	Han et al. 2016
19	Molecular Imprinting Polymers (MIP)	CI	Epoxy Graphite Matrix	Adsorption	NR	NR	NR	NR	7	9–100	28	Kamel 2016
20		CA	Au	Covalent	NR	NR	NR	NR	0.005	0.01–120	NR	Ellairaja et al. 2017

(*continued*)

Table 3. Continued.

Sr. No.	Support for immobilisation	Enzyme(s) used	Electrode	Method of immobilisation	Optimum pH	Optimum temperature (°C)	Potential applied (V)	Response time (s)	Detection limit (µM)	Linear range (µM)	Storage ability (days)	Ref.
	Rhodamine B dye											
21	Nano-gold Nps	CA	Zeolite	Cross-linking	NR	NR	NR	240	10	NR	NR	Kasap et al. 2017
22		CA	NR	NR	NR	NR	NR	NR	10	1–10	NR	Guinovart et al. 2017
23		CA, Cl, SOx	Glassy carbon	Adsorption	6.0	25	0.1	2	0.001	0.01–1000	240	Kumar et al. 2017
24		CA, Cl, SOx	Au	Covalent bonding	6.5	30	0.0-0.6	2	0.1	0.1–200	180	Present Biosensor

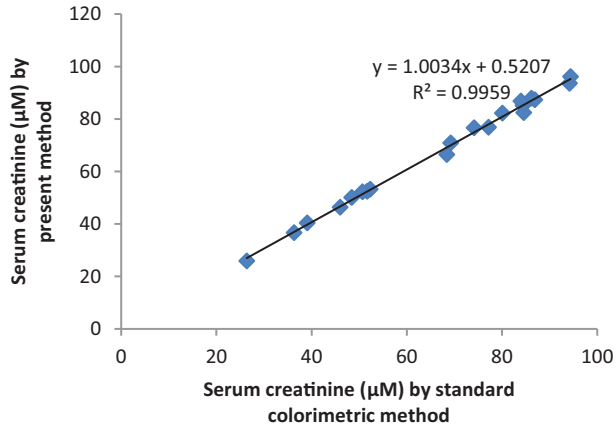

Figure 8. Correlation curve between sera creatinine values as measured by standard colorimetric (x axis) and the current biosensor (*y* axis) based on CANPs/CINPs/SOxNPs/Au electrode (CA: Creatininase; Cl: Creatinase; SOx: Sarcosine Oxidase; NPs: Nanoparticles; Au: gold electrode).

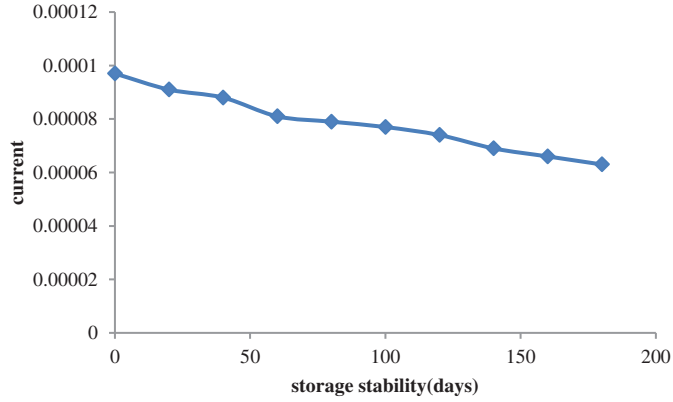

Figure 9. Storage stability of enzyme nanoparticles modified gold (Au) electrode in 0.1 M sodium phosphate buffer (pH = 6.5) at 4 °C.

time period of 180 days on regular basis, when kept dry at 4˚C (Figure 9). The biosensor showed more valuable result comparable to earlier biosensor (after 180 days, loss in 15% of its initial response) (Yadav *et al.* 2011); (after 20 times utilisation in 60 days, loss in 58% of its initial response) (Busono *et al.* 2015) and (after used for 100 times in 180 days, 50% loss in its initial response (Zhang *et al.* 2018). A correlation of analytical properties of present biosensor with prior biosensors is outlined in Table 3. These preceptions demonstrate the better analyte performance of present biosensors or working Au electrode over prior biosensors (Table 3).

4. Conclusions

The use of CANPs, CINPs and SOxNPs (bound to Au electrode) being development of amperometric creatinine biosensor led to its better diagnostic performance such as quicker response time (2 s) (Figure 10(c)), lower detection limit (LOD) (0.1 µM), wide working range (0.1–200 µM) and higher storage stability (180 days) in contrast to earlier creatinine biosensors. The measurement could be reached out to advance other amperometric biosensors, utilising ENPs and Au electrode. The most extreme sensitivities for

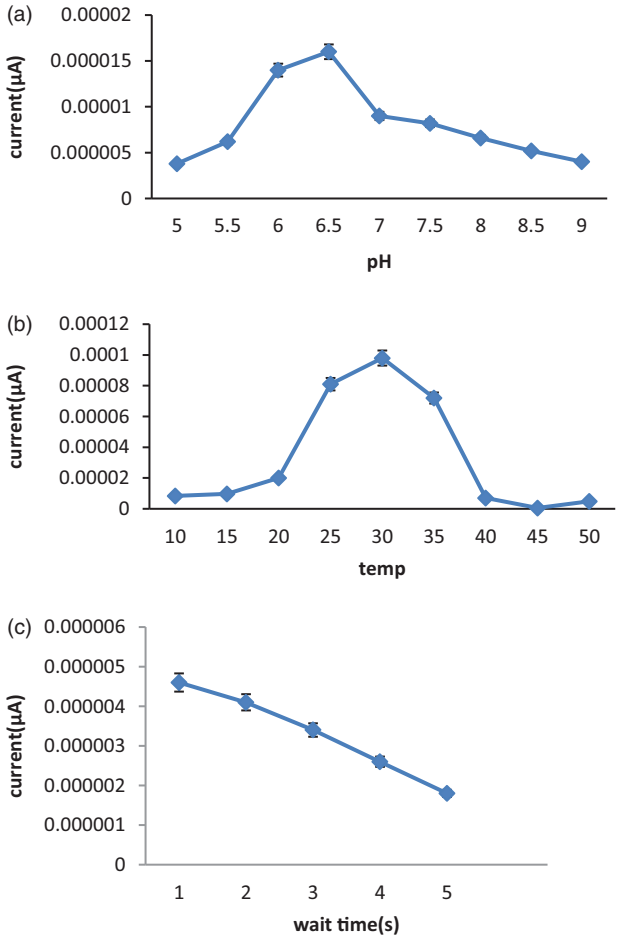

Figure 10. Influence of (a) pH, (b) incubation temperature and (c) time on the current response of CANPs/CINPs/SOxNPs/Au electrode, in 25 mL 0.1 M sodium phosphate buffer (pH = 6.5) containing 0.1 mL 0.1 M creatinine solution (CA: Creatininase; CI: Creatinase; SOx: Sarcosine Oxidase; NPs: Nanoparticles; Au: gold electrode).

detecting creatinine were obtained for the ENPs/Au electrode arranged by CV method at 30 °C in 0.1 M PBS buffer. Accordingly ENPs/Au electrode could be utilised to improve the diagnostic presentation of other biosensors.

Disclosure statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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