Biosensors and Bioelectronics 122 (2018) 140–146

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Construction and application of amperometric sarcosine biosensor based on SOxNPs/AuE for determination of prostate cancer

Parveen Kumar[a], Vinay Narwal[b], Ranjana Jaiwal[a], C.S. Pundir[b,*]

[a] Department of Zoology, M.D. University, Rohtak 124001, India
[b] Department of Biochemistry, M.D. University, Rohtak 124001, India

ARTICLE INFO

Keywords:
Sarcosine
Sarcosine oxidase nanoparticles
Sarcosine biosensor
Prostate cancer
Serum

ABSTRACT

An improved amperometric sarcosine biosensor was constructed based on covalent immobilization of sarcosine oxidase nanoparticles (SOxNPs) onto gold electrode (AuE). The SOxNPs/AuE was characterized by scanning electron microscopy (SEM), fourier transform infrared (FTIR) spectroscopy and electrochemical impedance spectroscopy (EIS) at different stages of its construction. The biosensor worked optimally within 2 s at a potential of 1.0 V, against Ag/AgCl, pH 6.5 and 35 °C. A linear relationship was observed between sarcosine concentration range, 0.1–100 μM and the biosensor response i.e. current in mA under optimum conditions. The biosensor offered a low detection limit of 0.01 μM and gratifying storage stability. The SOxNPs/AuE was unaffected by a number of serum substances at their physiological concentrations. The biosensor measured sarcosine level in sera collected from persons suffering from prostate cancer (mean13.5 μM, n = 8), which was significantly higher ($p < 0.01$) than those in apparently healthy persons (mean 2.2 μM, n = 8). The SOxNPs/Au electrode was reused 300- times during the span of 180 days, with only 10% loss in its initial activity while being stored dry at 4 °C.

1. Introduction

Sarcosine ($C_3H_7NO_2$), N-methylglycine, is a natural amino acid found in muscles and other body tissues, as a byproduct of glycine synthesis and degradation (Narwal et al., 2018a). It is metabolized to glycine by sarcosine dehydrogenase and sarcosine oxidase. It is found naturally, as an intermediate in the metabolism of choline to glycine. Normally, the concentration of sarcosine in blood ranges as 1.4 ± 0.6 μM in healthy people and 2–10 μM in prostate cancer patients. Its supplementation can be used to alleviate symptoms of depression and schizophrenia. Sarcosine is also used for detection of prostate cancer. It implies that if sarcosine levels in the blood are higher than ordinary, it could be a biomarker of prostate cancer (Sreekumar et al., 2009). But sarcosine itself does not cause cancer. Sarcosine level expands likewise in invasive prostate cancer cell lines with respect to favorable prostate epithelial cells (Couzin, 2009).

Various methods are available for determination of sarcosine, such as enzymic colorimetric method (Mazzu-Nascimento et al., 2016), enzymatic method (Burton et al., 2012), spectrometric and electrochemical analysis method (Cernei et al., 2012) and liquid chromatography with tandem mass spectroscopy (Jiang et al., 2010). All these methods have one or the other drawbacks e.g. low sensitivity, poor specificity, time-consuming sample preparation and requirement of a skilled person to operate. Biosensing methods overcome these difficulties, as these are simple, sensitive, specific, fast and simple to work (Turner, 2014).

A few sarcosine biosensors based on sarcosine oxidase (SOx)/screen printed electrode (SPE) (Rebelo et al., 2014), 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, paramagnetic nanoparticles based immuno-sensor (Heger et al., 2015), SOx/graphene/chitosan/silver nanoparticles (AgNPs)/glassy carbon electrode (GCE) (Zhou et al., 2012) and MIP/AuNPs/screen printed carbon electrode (SPCE) (Nguy et al., 2017), SOx/ chitosan/CuNPs/carboxylated multiwalled carbon nanotubes(c-MWCNT)/Au electrode have been reported. In these biosensors, SOx is required to be immobilized onto the working electrode. However, the direct immobilization of enzyme onto the support prompts to its denaturation, which leads to decreased activity and stability of the enzyme. These drawbacks were overcome by using nanoparticles of the enzyme(s) (ENPs) in place of native/free enzyme molecules (Pundir, 2015). The applications of ENPs has not only improved the analytic performance of biosensors but also simplified the construction of nanocomposite based enzyme electrodes

* Corresponding author.
E-mail address: chandraspundir@gmail.com (C.S. Pundir).

https://doi.org/10.1016/j.bios.2018.09.003
Received 24 May 2018; Received in revised form 29 August 2018; Accepted 1 September 2018
Available online 05 September 2018
0956-5663/

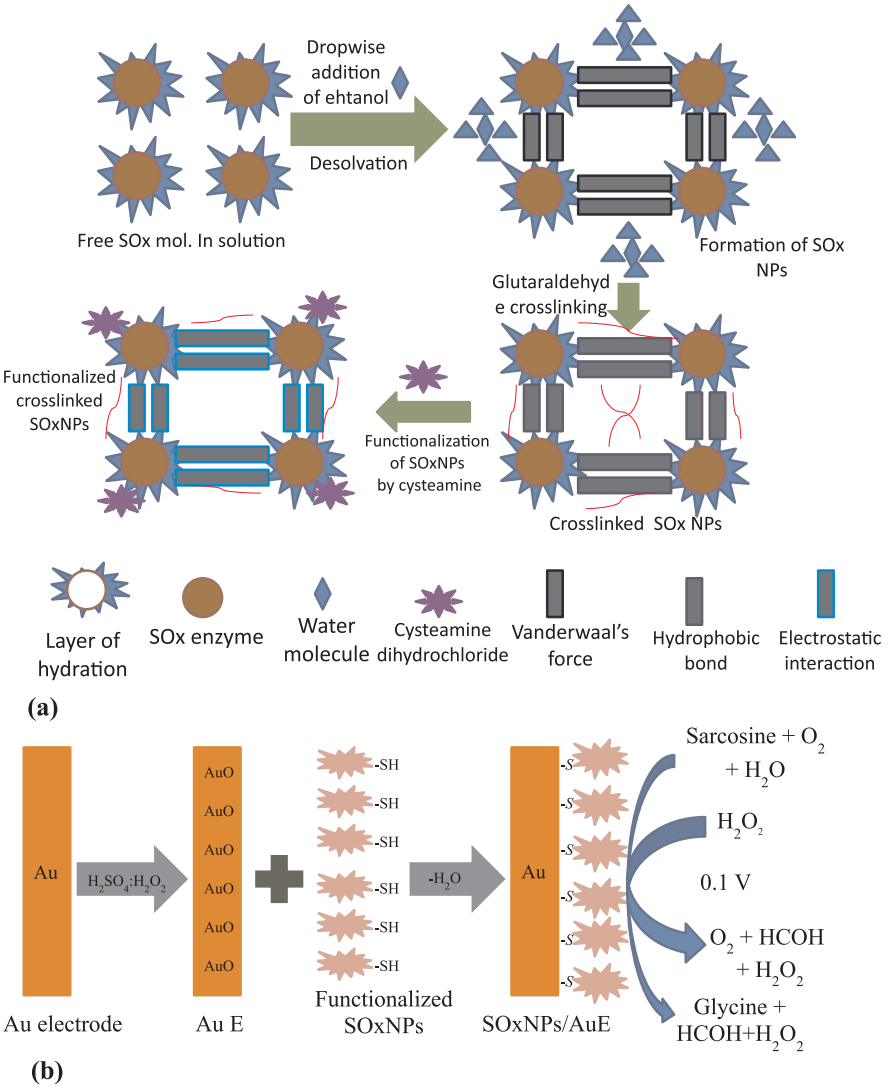


Fig. 1. (a) Schematic representation of the preparation of nanoparticles of SOx, **(b)** Schematic representation of chemical reactions involved in the fabrication of SOxNPs/AuE.

(Narwal et al., 2018b). Due to their unique electronic, optical, mechanical, electrical thermal and catalytic (ability to facilitate the transfer of electron) properties, besides increased surface area, the ENPs have exhibited great promises in improving enzyme electrodes (Pundir, 2015). The ENPs have an exclusive ability to boost the quick electron exchange between the working electrode and enzyme (Pandey et al., 2008). Au electrode has many attractive features such as a good conductor of electricity, low cost, low background current and easy availability, which renders it a better option over Pt and glassy carbon (GC) electrodes (Narwal and Pundir, 2018). The present work describes the simplified construction of an improved amperometric sarcosine biosensor based on covalent immobilization of SOxNPs onto Au electrode for detection of blood sarcosine to diagnose prostate cancer at an early stage.

2. Materials and methods

2.1. Chemicals and reagents

Sarcosine oxidase (SOx) from *Bacillus* species (25 U/mg), 4-aminophenazone and glutaraldehyde (25%) were purchased from Sigma Aldrich Co., St. Louis, USA. Horseradish peroxidase (HRP), sarcosine, and ethanol were purchased from SISCO Research Lab. Mumbai India. Gold (Au) wire (2 mm x 20 mm, diameter x height) was purchased from local jeweler's shop. The blood sera samples of apparently healthy subjects and persons suffering from prostate cancer were collected from the hospital of local Pt. BDS Post Graduate Institute of Medical Science, and stored at −20 °C until use. All the chemicals were used as analytical reagent grade (AR), without further purification. Double distilled water (DW) was used for all the experiment.

2.2. Instruments used

Potentiostat/Galvanostat (Make: Autolab, model: AUT83785, manufactured by Eco Chemie, The Netherland), (interfaced to a computer with GPES software, for CV & EIS) with a three-electrode system consisting of a platinum (Pt) wire as a auxiliary electrode, an Ag/AgCl electrode as a reference electrode and SOxNPs modified gold electrode (SOxNPs/AuE) as a working/enzyme electrode, transmission electron microscope (TEM) (JEOL 2100 F, Japan), scanning electron microscope (SEM) (Zeiss EV040, USA) and Fourier transform infrared spectrometer (FTIR) (Thermo Scientific, USA), were used.

2.3. Assay of sarcosine oxidase (SOx)

The assay of sarcosine oxidase was based on the following chemical

reactions:

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

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

The assay was carried out in 15 ml test tube wrapped with a black carbon paper. The reaction mixture contained 1.8 ml of 0.1 M sodium phosphate buffer (PBS) (pH 7.8), 0.1 ml SOx (2 mg/ml PBS, 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 it was kept at the similar temp for 30 min to develop the color. A blank was run simultaneously in the similar way in which enzyme solution was replaced by reaction buffer. The absorbance at 520 nm was measured against blank and concentration of H_2O_2 created in the assay was extrapolated from the standard curve of H_2O_2. The color reagent comprised 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 each week. The protein content dissolved in SOx was measured by the Lowry method. One unit of enzyme is characterized as an amount of enzyme required to generate 1 nmol H_2O_2/min/ml (Pundir et al., 2010).

2.4. Preparation of SOxNPs

The SOxNPs were prepared by desolvation method (Kundu et al., 2012). To 2.0 ml of dissolved SOx, in 0.1 M sodium phosphate buffer pH 7.0 (1.0 mg/ml), 4.0 ml of absolute ethanol was added dropwise at the rate of rate of 0.1–0.2 ml/min under constant stirring at 500 rpm in cold (4–8 °C). The SOxNPs were formed by aggregation of native/free enzyme molecules. After 24 h, 1.8 ml of 2.5% of glutaraldehyde was added to this ENPs suspension under constant stirring at 500 rpm in cold. After 5–6 h, 0.12 g of cysteamine dihydrochloride was added to this suspension under the same stirring. Finally, SOxNPs were separated from native/free enzyme solution by centrifugation at 1200 rpm at 4 °C for 10 min and then dispersing in 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) and sonication for 5 min. All the ENPs were stored at 4 °C, until use (Fig. 1a).

2.5. Characterization of SOxNPs

The size, shape and functional groups of SOxNPs were measured by taking their image in **a** transmission electron microscope (TEM) and recording their UV and FTIR spectra respectively. The TEM image measured the size of SOxNPs in the range 2–100 nm. The UV spectra showed optical properties and dispersion of SOxNPs. FTIR spectra of SOxNPs exhibited functional groups of the C=C stretching, O–H stretching, C-C stretching, C–O (epoxy/ether), COOH stretching and N-containing bio-ligand vibrations.

2.6. Preparation of working electrode

The surface of bare gold (Au) electrode was polished by alumina slurry with tissue/polishing paper, followed by washing in DW. Thereafter, it was dipped into piranha solution (3:1, H_2SO_4:H_2O_2) for 20 min, it was cleaned by alumina slurry and then placed into ethanol for 5–6 h and thereafter sonicated to remove adsorbed particles. Finally, the polycrystalline Au electrode was placed into cysteamine functionalized SOxNPs solution for 48 h at 4 °C, for covalent immobilization of SOxNPs aggregates onto Au electrode (Fig. 1b). The SOxNPs modified Au electrode was washed with 0.1 M sodium phosphate buffer, pH 7.0 and stored in the same buffer at 4 °C, when not in use. The following chemical reactions occurred during the covalent immobilization of enzyme nanoparticles (ENPs) i.e. SOxNPs onto Au electrode through thiolate bond (Narwal et al., 2018b):

$$2Au \xrightarrow{H_2SO_4:H_2O_2} Au_n{-}AuO$$

$$\underset{\text{Polycrystalline AuE}}{Au_n{-}AuO} + \underset{\text{Functionalized ENPs mixture}}{2(ENPs){-}SH} \rightarrow \underset{\text{ENPs–AuE}}{Au_n(Au{-}S{-}ENPs)_2} + H_2O$$

2.7. Characterization of SOxNPs/AuE by SEM, FTIR, and EIS

The morphology of working electrode (SOxNPs/AuE) was characterized before and after immobilization of SOxNPs onto AuE by SEM, FTIR, and EIS in potentiostat. The EIS measurement was carried out in 5 mM $K_3Fe(CN)_6$/$K_4Fe(CN)_6$ at the polarization potential of 1.0 V. To record FTIR spectra, SOxNPs were scrapped off from the Au electrode and their KBr pellet was kept into the assembly of the FTIR spectrometer and its spectrum was recorded.

2.8. Construction and response measurement of sarcosine biosensor

To construct the sarcosine biosensor, SOxNPs modified Au electrode as working electrode, Ag/AgCl as a reference electrode and Pt wire as a counter electrode were connected through potentiostat-galvanostat. Fig. 1b gives the schematic representation of fabrication of SOxNPs/AuE electrode. To measure biosensor response, cyclic voltammetry (CV) of working/enzyme electrode was recorded in potentiostat in the potential range, 0.3–1.0 V in 25 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1 ml of 10 μM sarcosine.

2.9. Optimization of sarcosine biosensor

To optimize working conditions of the sarcosine biosensor, effects of pH, incubation temperature, response time and substrate (sarcosine) concentrations on biosensor response were examined. To decide optimum pH, the pH was changed between pH 4.0–8.0 at an interim of pH 0.5 utilizing the following buffers, each at a final concentration of 0.1 M, sodium acetate buffers (pH 4.0–5.0) and sodium phosphate buffer (pH 5.5–8.0). To decide optimum temp, the reaction mixture was incubated at various temperatures (5–80 °C at an interval of 5 °C). The impact of sarcosine concentration on biosensor response was measured in the range 0.01–100 μM. All experiments were repeated five times.

2.10. Evaluation of sarcosine biosensor

The SOxNPs biosensor has been evaluated in terms of different analytical parameters like detection limit, linear range, analytical recovery, response time and storage stability. In addition correlation of the present method was also done with the already available method i.e Immuno chromatography kit method which is based on the principle that two kinds of specific antibodies against antigen are used. One of the antibodies is immobilized on the chromatographic paper, and the other is labeled with colloidal gold and infiltrated into the sample pad. An immunochromatographic unit is completed by attaching the sample pad at the end of the membrane. When the liquid sample is dropped on the sample pad, the antigen in the sample forms an immunocomplex with the antibody labeled with colloidal gold. Its complex moves along with the liquid sample and makes a contact with the antibody immobilized on the membrane, followed by forming an immunocomplex with the immobilized antibody it results in generating a colored red-purple line. The appearance of red-purple line on the membrane indicates the presence of the antigen of interest in the sample. Since the liquid of the sample migrates through the membrane very fast, it makes it possible to detect the presence or absence of antigen within 15 min.

2.11. Application of SOx biosensor

The blood samples (1 ml each) were drawn from apparently healthy male and persons suffering from prostate cancer disorder at Pt. BDS PGIMS, Rohtak using sterilized needle and syringe (2 ml), transferred it

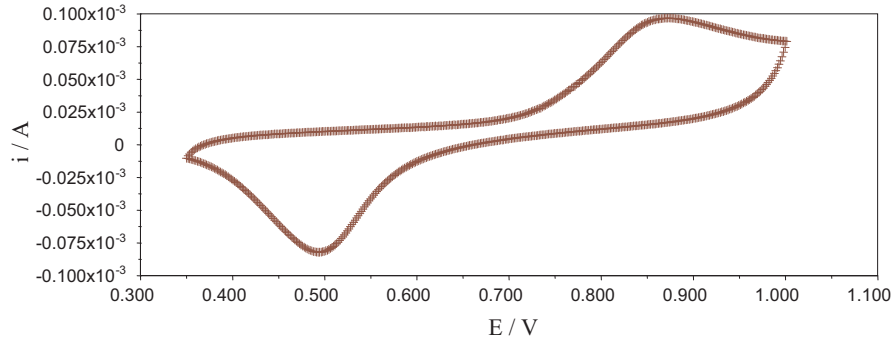


Fig. 2. Cyclic voltammogram for SOxNPs/AuE in 25 ml 0.1 M sodium phosphate buffer (pH= 6.5) containing 2 mM (0.1 ml) sarcosine at a scan rate of 20 mVs^{-1}.

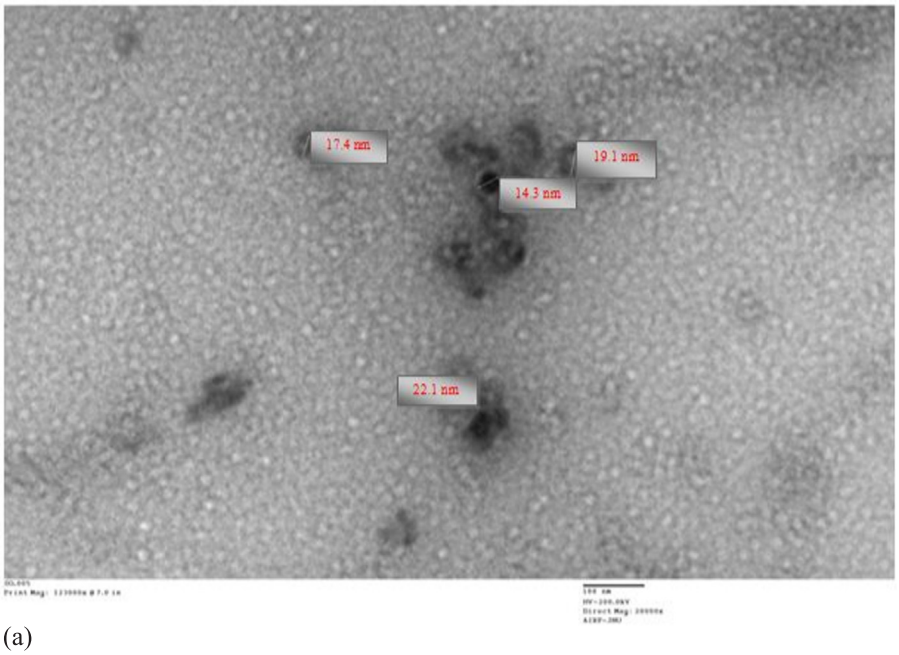


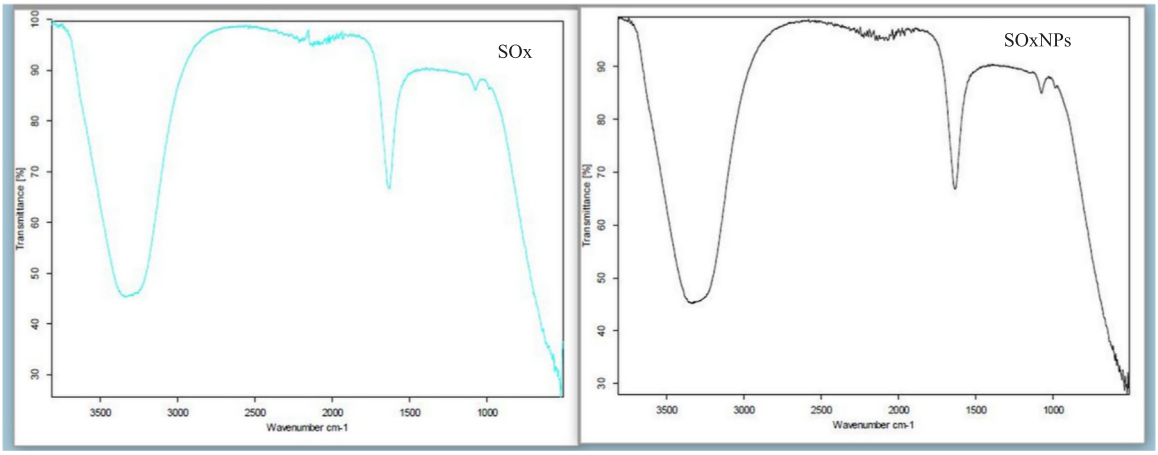
Fig. 3. (a) Transmission electron microscopic (TEM) images of SOxNPs, **(b)** UV spectra of native SOx and SOxNPs, **(c)** FTIR spectra of native SOx and SOxNPs, **(d)** Scanning electron microscopic (SEM) images of bare Au electrode and SOxNPs/AuE, **(e)** Electrochemical Impedance Spectra (EIS) of bare electrode and SOxNPs/AuE.

to a centrifuge tube and kept at room temp for 30 min for coagulation. After that, blood was centrifuged at 1200 rpm for 10–12 min and its supernatant (serum) was collected and analyzed for sarcosine content by measuring CV of working/enzyme electrode in potentiostat in the potential range, 0.3–1.0 V in 25 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1 ml serum samples under optimum condition. The sarcosine content in serum was interpolated from a standard curve between sarcosine concentrations vs current in mA prepared under optimal assay conditions of the biosensor (Fig. 4c).

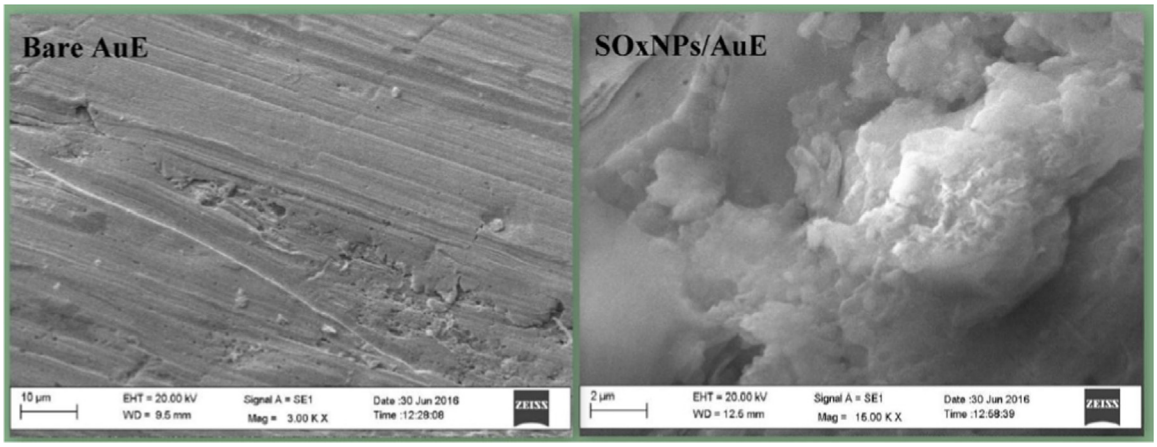
3. Results and discussion

3.1. Characterization of SOxNPs by TEM, UV spectroscopy, and FTIR

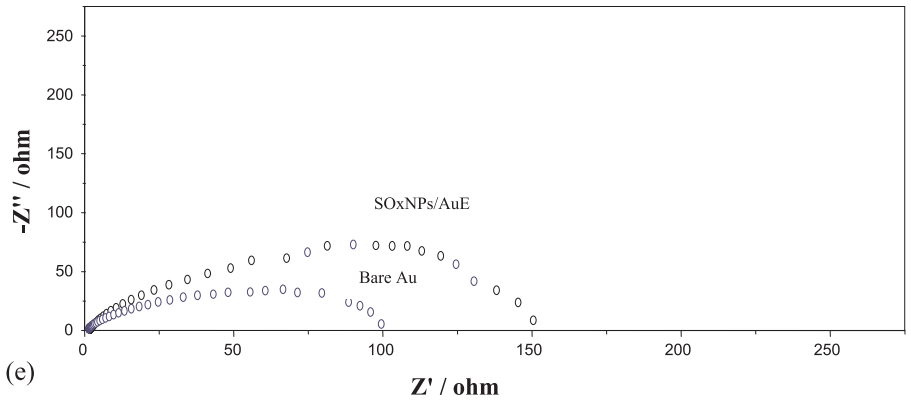
The TEM image provides the average size of SOxNPs as 18.22 nm (Fig. 3a). UV spectra of SOx and SOxNPs showed broad absorbance peak at the optical range, 250 nm, while SOx showed a small peak at 400 nm due to its yellowish color in its dissolved form. High absorbance in case of SOxNPs was demonstrating the small size and high degree of



(c)



(d)



(e)

Fig. 3. (*continued*)

optical conductivity of ENPs. The higher absorption peak of SOxNPs at 250 nm as compared to that of soluble SOx, was due to aggregation of free/soluble enzyme molecules to form enzyme nanoparticles. The presence of the single absorption peak implied that the ENPs were nearly spherical attributed to the coherent oscillation of the conduction electrons (Fig. 3b). The fundamental FTIR mode of SOx and SOxNPs showed a similar pattern, which depicted that the synthesis of SOxNPs didn't affect the structural and functional properties of enzyme SOx. In both cases, the FTIR showed peaks at 3400 cm^{-1}, 2300 cm^{-1}, 1580 cm^{-1}, 900 cm^{-1} due to O-H stretching, C–O bond, C=O stretching, CO stretching respectively (Fig. 3c).

3.2. Characterization of working gold (Au) electrode during construction

3.2.1. By SEM

The SEM images of the surface of bare Au electrode and SOxNPs/Au electrode have appeared in Fig. 3d. The modification of bare Au electrode onto immobilization of ENPs (working electrode) was seen clearly from these SEM images. The SEM image of the bare Au electrode showed a smooth and featureless morphology (Fig. 3d). The SOxNPs/Au electrode showed granular, globular structural morphology, which was due to immobilization of SOxNPs of onto Au electrode (Fig. 3d).

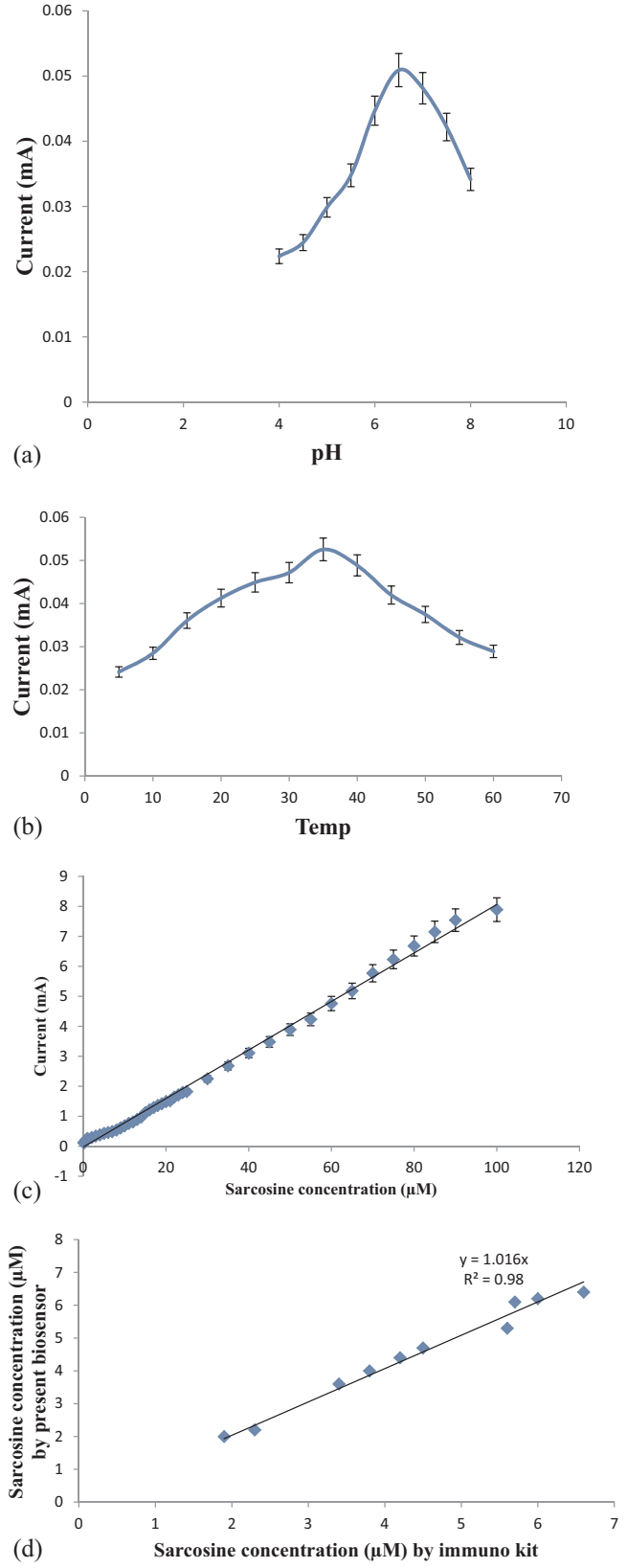


Fig. 4. (a) Influence of applied pH on the current response of SOxNPs/AuE, **(b)** Influence of applied incubation temperature on the current response of SOxNPs/AuE, **(c)** Standard curve of sarcosine concentration by sarcosine biosensor based on SOxNPs/AuE, **(d)** Correlation between serum sarcosine level measured by immuno kit method (x-axis) and the present method (y-axis) employing the sarcosine biosensor based on SOxNPs/AuE, **(e)** Effect of metabolites (citric acid, glutamic acid, uric acid, ascorbic acid and urea) on the sarcosine biosensor response. All readings are mean of five observations (n =

3.2.2. By EIS

The EIS gives the valuable information on impedance changes of the electrode surface during the fabrication process. It was carried out to investigate immobilization of SOx/NPs onto the Au electrode. The diameter of the semicircle at higher frequencies corresponds to the electron transfer resistance (R_{CT}), which controls the electron exchange kinetics of the redox probe at the electrode interface, while the straight part at lower frequencies corresponds to Warburg diffusion process. The Nyquist plot (Fig. 3e) shows the EIS of bare Au electrode, immobilized Au electrode in 5 mM K3Fe(CN)6/K4Fe(CN)6. The R_{CT} value for the SOxNPs/Au electrode was obtained as 150 Ω, which was higher compared to the bare electrode (R_{CT} = 100 Ω). These outcomes demonstrated that the increased R_{CT} value of SOxNPs/Au electrode was because of the immobilization of SOxNPs onto Au electrode surface. This increase in R_{CT} is attributed to the fact that most of the biological particles, including enzyme nanoparticles, are poor electrical conductors at low frequencies and cause hindrance to the electron transfer.

3.3. Construction and optimization of sarcosine biosensor

An improved amperometric sarcosine biosensor was constructed based on covalent immobilization of SOxNPs onto Au electrode. In this biosensor, maximum current was observed within 2 s at 1.0 V against Ag/AgCl electrode. The biosensor indicated both anodic and cathodic peaks with the equal slope in presence of substrate, interfaced to a computer with GPES software, for CV (Fig. 2). Thus, in this subsequent electrochemical observation, the current was recorded within 2 s at 1.0 V, at a scan rate of 20 V/s. The biosensor showed optimum current at pH 6.5 at 35 °C in 0.1 M sodium phosphate buffer, which is closer to the physiological pH (Fig. 4a, b). This optimum pH and temperature of the present biosensor is comparable to earlier biosensors, optimum pH 7.2, temp 37 °C (Rebelo et al., 2014); optimum pH 7.0, temp 37 °C (Heger et al., 2015); optimum pH 7.0, temp 35 °C (Narwal et al., 2018b); (Table 1). Thus, the subsequent electrochemical experiments were carried out in 0.1 M sodium phosphate buffer, pH 6.5 at 35 °C. The biosensor offered an optimum response of 2 s, which was better than earlier reported sarcosine biosensors i.e < 120 s (Özkütük et al., 2016); 2 s (Narwal et al., 2018b), which was due to nanoparticles of SOx. There was a linear relationship between biosensor response and sarcosine concentration in the range 0.01 µM–100 µM and showed a hyperbolic relationship after 100 µM (Fig. 4c).

3.4. Evaluation of biosensor

There was a linear relationship between the biosensor response i.e. current in mA and the sarcosine concentration in the range, 0.1–100 µM, under optimized conditions (Fig. 4c). This linearity is better than earlier biosensors 0.01–0.1 µM (Rebelo et al., 2014); 0.005–0.05 µM (Heger et al., 2015) (Table 1). The limit of detection (LOD) of the present biosensor was 0.01 µM, which is comparable with earlier sensors 50 p.M. (Heger et al., 2015), 16,000 p.M. (Rebelo et al., 2014), 1350 p.M. (Özkütük et al., 2016); 1000 p.M. (Nguy et al., 2017) (Table 1). The analytical recoveries of added sarcosine in sera at levels of 0.5 µM and 1.0 µM were 94.22% and 97.81% respectively (Supplementary Table 1). In addition, within and between batch coefficients of variations (CVs) for the working electrode were detected as 0.083% and 0.067%, respectively (Supplementary Table 2). These high precisions highlighted the good reproducibility and consistency of the present biosensor as compared to earlier reported biosensors. This can be attributed to the covalent immobilization of SOxNPs onto the Au electrode.

3.5. Determination of sarcosine in sera

The biosensor measured sarcosine level in sera was collected from suffering from prostate cancer (mean13.5 µM, n = 8), which was

Table 1
A comparison of different analytic parameters of present sarcosine biosensor with those of earlier biosensors.

Sr no	Working electrode	Optimum pH	Temp (°C)	LOD (pM)	Linear range (μM)	Response time (sec)	Potential	Storage stability (days)	Reference
1	Screen printed	7.2	37	16,000 p.M.	0.01–0.1 μM		0.6 V	60	Rebelo et al. (2014)
2	Molecular imprinted polymer (MIP)			1350 p.M.	100–0.01 μM	< 120		165	Ozkutuk et al. (2016)
3	Au	7.0	37	50 p.M.	0.005–0.05 μM				Heger et al. (2015)
4	Glassy carbon	7.0		1,000,000 p.M.	1.0–177 μM		0.49 V		Zhou et al. (2012)
5	Au			1000 p.M.					Nguy et al. (2017)
6	Au	7.0	35	0.10 p.M.	0.1–100 μM	2	0.2 V	180	Narwal et al. (2018b)
7	Au	6.5	35	0.10 p.M.	0.1–100 μM	2	1.0 V	180	Present work

significantly higher ($p < 0.01$) than those in apparently healthy persons (mean 2.2 μM, n = 8) (Supplementary Table 3).

3.6. Correlation

The sarcosine level in sera of apparently healthy subjects and persons suffering from prostate cancer was measured by the present biosensor and compared with those obtained by standard immuno kit method. There was a good correlation ($R^2 = 0.98$) between these two methods (Fig. 4d).

3.7. Interference study and selectivity

Interfering compounds like citric acid, glutamic acid, uric acid, ascorbic acid, and urea showed interference of 4.20%, 2.70%, 1.50%, 3.80%, and 2.80% respectively at their physiological concentrations which showed practically no impact on biosensor response under the standard assay conditions. As these side products (ascorbic acid, uric acid etc) are found in blood, their effect on biosensor performance was studied. These products did not affect the performance of the biosensor, due to the substrate specificity of the present sarcosine biosensor. The biosensor optimum working conditions are not suitable for the other metabolites so, it works specifically on sarcosine (Kumar et al., 2017).

3.8. Storage stability of SOxNPs/Au electrode

The present SOxNPs/Au working electrode lost 10% of its initial activity after 300 regular uses during the span of 180 days, when being stored dry at 4 °C (Supplementary Fig. 5). This storage stability of the present biosensor is better than earlier biosensors i.e 165 days (Özkütük et al., 2016); 180 days (Narwal et al., 2018b); and 60 days (Rebelo et al., 2014), Nguy et al. (2017) but equal to that reported by Narwal et al., 2018b. The enhanced storage stability of the biosensor was due to using of enzyme nanoparticles, which were immobilized directly onto the Au electrode through a covalent bond (Narwal et al., 2018b). A comparison of analytical properties of this sarcosine biosensor with earlier biosensors is summarized in Table 1. These observations indicate the better analytical performance of present biosensors over earlier biosensors.

4. Conclusion

The use of SOxNPs in place of native/free enzyme (SOx) gave better analytic performance of sarcosine biosensor, in terms of quicker response (2 s), lower detection limit (0.01 μM), wider working range (0.1 μM to 100 μM) and longer storage stability (180 days) compared to earlier biosensors employing SOx. Further, it has simplified by the fabrication process of a nano-composite based enzyme electrode, as SOxNPs were immobilized directly onto the Au electrode in the present case. The other enzyme nanoparticles could also be immobilized onto Au electrodes to improve the analytical performance of native enzyme-based sensors. The current work lacks the miniaturization of the biosensor. Therefore, the future research could also be focused on the miniaturization of sarcosine biosensor, which can be specially used at the bedside of the patients for real-time monitoring of sarcosine (Kumar et al., 2017).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.09.003.

References

Burton, C., Gamagedara, S., Ma, Y., 2012. A novel enzymatic technique for determination of sarcosine in urine samples. Anal. Methods 4, 141–146. https://doi.org/10.1039/c1ay05541k.

Cernei, N., Zitka, O., Ryvolova, M., et al., 2012. Spectrometric and electrochemical analysis of sarcosine as a potential prostate carcinoma marker. Int. J. Electrochem. Sci. 7, 4286–4301.

Couzin, J., 2009. Biomarkers: metabolite in urine may point to high-risk prostate cancer. Science 323. https://doi.org/10.1126/science.323.5916.865a.

Heger, Z., Cernei, N., Krizkova, S., et al., 2015. Paramagnetic nanoparticles as a platform for FRET-based sarcosine picomolar detection. Sci. Rep. 5, 8868. https://doi.org/10.1038/srep08868.

Jiang, Y., Cheng, X., Wang, C., Ma, Y., 2010. Quantitative determination of sarcosine and related compounds in urinary samples by liquid chromatography with tandem mass spectrometry. Anal. Chem. 82, 9022–9027. https://doi.org/10.1021/ac1019914.

Kumar, P., Jaiwal, R., Pundir, C.S., 2017. An improved amperometric creatinine biosensor based on nanoparticles of creatininase, creatinase and sarcosine oxidase. Anal. Biochem. 15, 41–49. https://doi.org/10.1016/j.ab.2017.08.022.

Kundu, N., Yadav, S., Pundir, C.S., 2012. Preparation and characterization of glucose oxidase nanoparticles and their application in dissolved oxygen metric determination of serum glucose. J. Nanosci. Nanotechnol. 13, 1710–1716. https://doi.org/10.1166/jnn.2013.7102.

Mazzu-Nascimento, T., Leão, P.A.G.C., Catai, J.R., Morbioli, G.G., Carrilho, E., 2016. Towards low-cost bioanalytical tools for sarcosine assays for cancer diagnostics. Anal. Methods 8, 7312–7318. https://doi.org/10.1039/c6ay01848c.

Narwal, V., Kumar, P., Joon, P., Pundir, C.S., 2018a. Fabrication of an amperometric sarcosine biosensor based on sarcosine oxidase/chitosan/CuNPs/c-MWCNT/Au electrode for detection of prostate cancer. Enzyme Microb. Technol. 113, 44–51.

Narwal, V., Pundir, C.S., 2018. Fabrication of glycerol biosensor based on co-immobilization of enzyme nanoparticles onto pencil graphite electrode. Anal. Biochem. 555, 94–103. https://doi.org/10.1016/j.ab.2018.06.005.

Narwal, V., Sharma, M., Rani, S., Pundir, C., 2018b. An ultrasensitive amperometric determination of lactate by lactate dehydrogenase nanoparticles immobilized onto Au electrode. Int. J. Biol. Macromol. 115, 767–775. https://doi.org/10.1016/j.ijbiomac.2018.04.114.

Nguy, T.P., Phi, T.V., Tram, D.T., Eersels, K., Wagner, P., Lien, T.T., 2017. Development of an impedimetric sensor for the label-free detection of the amino acid sarcosine with molecularly imprinted polymer receptors. Sens. Actuators B: Chem. 246, 461–470. https://doi.org/10.1016/j.snb.2017.02.101.

Özkütük, E.B., Diltemiz, S.E., Avc, S., Uğurağ, D., Aykanat, R.B., Ersöz, A., 2016. Potentiometric sensor fabrication having 2D sarcosine memories and analytical features. Mater. Sci. Eng. 69, 231–235. https://doi.org/10.1016/j.msec. 2016.06.057.

Pandey, P., Datta, M., Malhotra, B.D., 2008. Prospects of nanomaterials in biosensors. Anal. Lett. 41, 159–209. https://doi.org/10.1080/00032710701792620.

Pundir, C.S., 2015. Enzyme Nanoparticles: Preparation, Characterization And Application. Elsevier, London. https://doi.org/10.1016/b978-0-323-38913-6.00002-7.

Pundir, C.S., Chauhan, N., Kumari, G., Vandana, 2010. Immobilization of arthrobacter sarcosine oxidase onto alkylamine and arylamine glass and its application in serum sarcosine determination. Indian J. Biotechnol. 219–223.

Rebelo, T.C.A.S., Pereira, C.M., Sales, M.F., Noronha, J.P., Costa-Rodrigues, J., Silva, F., 2014. Sarcosine oxidase composite screen-printed electrode for sarcosine determination in biological samples. Anal. Chim. Acta 850, 26–32. https://doi.org/10.1016/j.aca.2014.08.005.

Sreekumar, A., Poisson, L.M., Rajendiran, T.M., Khan, A.P., Cao, Q., Yu, J.D., Laxman, B., Mehra, R., Lonigro, R.J., Li, Y., 2009. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature 457, 910–914.

Turner, A.P., 2014. Biosensors: fundamentals and applications. Biosens. Bioelectron. 65C, A1.

Zhou, Y., Yin, H., Meng, X., Xu, Z., Fu, Y., Ai, S., 2012. Direct electrochemistry of sarcosine oxidase on graphene, chitosan and silver nanoparticles modified glassy carbon electrode and its biosensing for hydrogen peroxide. Electrochim. Acta 71, 294–301. https://doi.org/10.1016/j.electacta.2012.04.014.