www.amlett.com, www.vbripress.com/aml, DOI: 10.5185/amlett.2015.5735 Published online by the VBRI press in 2015

Development of lysine biosensor based on core shell magnetic nanoparticle and multiwalled carbon nanotube composite

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Received: 15 November 2014, Revised: 20 January 2015 and Accepted: 22 January 2015

ABSTRACT

An amperometric lysine biosensor was fabricated by immobilizing lysine oxidase onto core shell magnetic nanoparticles (Core–shell MNPs)/multiwalled carbon nanotube (MWCNT) layer deposited on Au electrode via carbodiimide linkage. Transmission electron microscopy (TEM) for core–shell MNPs, scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and Fourier transform impedance spectroscopy (FTIR) studies were used to characterize the modified electrode. Sensor showed optimal response within 2s at 30ºC in 0.05 M sodium phosphate buffer pH 6.0 when polarized at +0.2 V vs. Ag/AgCl. Linear working range of the biosensor was determined by 0.05 -700 µM with a detection limit of 0.05 µM. A good correlation (r = 0.98) was obtained between serum lysine levels measured by the standard HPLC method (y) and the present method (x). A number of serum substances had practically no interference. The sensor was used in 150 assays and had a storage life of 180 days at 4 ^{o}C. This nanohybrid biosensor will be useful for detection of lysine in food and pharmaceutical industries. Copyright © 2015 VBRI press.

Keywords: Lysine oxidase; lysine; core–shell MNPs; multiwalled carbon nanotube.

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Introduction

Lysine is an essential amino acid for both humans and animals; and it is usually the first or second limiting amino acid in most formulated diets **[1]**. Therefore it is considered as an index in the nutrition and pharmaceutical fields. In order to correct possible deficiencies of this amino acid in humans, lysine is often added as a dietary supplement to food and drugs. Improved methods for lysine involving the amino acid analyzer **[2]** and gas chromatography **[3]** were developed, however these sophisticated tools are expensive. Colorimetric **[4]** and enzymatic **[5]** methods, which are widely used for lysine measurement in grains, are time-consuming and require considerable technical skills. In routine laboratory work, amino acid analysis by ion exchange chromatography using automated analyzers and ninhydrin detection are well-established. In addition, HPLC methods based on spectrophotometric and fluorimetric determination of derivatized amino acids are used **[6]**. Both these methods are time consuming and require expensive equipments. The determination of lysine and other amino acids are usually carried out by liquid chromatography **[7]** and capillary electrophoresis **[8]**. Unfortunately, chromatographic methods are unsuitable for analysis of large sets of samples because the separation step is often tedious and time consuming. However, biosensors can be used to overcome these limitations. The use of electrochemical enzyme sensors has extensively reduced the sampling and testing times in food monitoring with high sensitivity, specificity, long term stability, fast analysis, low cost and easy detection principles **[9-11]**.

Nanomaterials (NPs) are known to have several unique properties which can be used to improve sensitivity and detection limits of electrochemical biosensors. NPs have been employed in biosensors as effective catalyst supports due to their large surface areas and unique structural and electromechanical properties, good biocompatibility, easy

preparation and renewal of their surface **[12,13]**. Recently, magnetic nanoparticles have aroused great interest in research as special biomolecules immobilizing carriers **[14,15]**. Magnetic nanoparticles have a large surface area which can be easily -oxidized and form aggregates, leading to change in their original structure and unique properties. To prevail over this difficulty, the surface of NPs was coated with a protective layer. The inner iron oxide core with outer shell of silica not only stabilizes the nanoparticles but also facilitated the dispersion of nanoparticles in the solution. In the present study, magnetic nanoparticles (MNPs) were synthesized and silanized to form core–shell (MNPs–SiO_2) structure.

Multi-walled carbon nanotubes (MWCNTs) consist of several concentric tubes of graphite inside one another **[16]**. These have been employed in biosensors as an effective catalyst support due to their large surface areas and unique structural and electromechanical properties, good biocompatibility, easy preparation and renewal of their surface **[17-20]**. The pooled presence of core–shell MNPs and c-MWCNT (carboxylated multi-walled carbon nanotubes) in the LyOx/core–shell MNPs/c-MWCNT modified Au electrode is expected to provide an electron conductive complex and thus helps to improve electronic competence.

The present study describes a novel approach to immobilize covalently a commercial LyOx onto core–shell MNPs/c-MWCNT modified Au electrode and facilitates the detection of lysine in food-stuffs, serum and pharmaceutical tablets.

Experimental

Materials

LyOx (E.C. 1.4.3.14 from *Trichoderma viride*; 20-60 units/mg protein, tetraethylorthosilicate (TEOS; purity 98%) and aniline (PANI monomer; purity ≥99.5%), were purchased from Sigma Aldrich Chemical Co St. Louis USA. Polyethylene glycol (PEG) (PEG 6000, Carbowax 6000; flakes/powder 99%), N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC; purity 99% and N-hydroxysuccinimide (NHS; purity 97%) were purchased from Sisco Research Laboratory, Mumbai, India. Carboxylated multi-walled carbon nanotubes (c-MWCNT) (Functionalized c-MWCNT) (12 walls, length 15–30mm, purity 90%, metal content: nil) from Intelligent Materials Pvt. Ltd., Panchkula (Haryana), India were used. Alamin M Forte (Amino acids with minerals capsule) manufactured by M/S Albert David Limited, B-12/13, Meerut Road Industrial Area, Ghaziabad, India and Au wire (1.5 ×0.05 cm^2, 23 carat) were purchased from local market. All other chemicals were of analytical reagent grade. Double distilled water (DW) was used throughout the experiments.

Preparation of MNPs

The synthesis of MNPs was conducted by following a typical procedure. Before mixing, 0.4 M hydrochloric acid and 0.7 M ammonia solution were bubbled by N_2 for 10 min. 8.5 g $FeCl_3 \cdot 6H_2O$ and 3 g $FeCl_2 \cdot 4H_2O$ were then dissolved in 38 ml of 0.4 M hydrochloric acid. This mixture was then added quickly to 375 ml ammonia solution at room temperature under vigorous stirring (non-magnetic). After half an-hour stirring, the precipitates were isolated by magnetic force. The precipitates were then washed three times with DW diluted with 150 ml DW. Additionally, the silica was coated on the magnetic core **[21]**. MNPs suspension (20 ml) was added to 200 ml of 2-propanol and sonicated for 20 min. PEG (5.36 g), 20 ml water, 10 ml ammonia solution (28 wt.%) and 1.2 ml TEOS were added respectively into the suspension and allowed to react for 24 h at room temperature under continuous stirring **[22]**. After the reaction was completed, the products were collected through centrifugation at 4000 rpm for 5 min, sonicated with ethanol and washed by DW twice and then lyophilized to obtain the core–shell MNPs.

Preparation of modified Au electrode
Core–shell MNPs/c-MWCNT modified Au electrode

Firstly, a polycrystalline Au electrode (1.5×0.05 cm^2) was polished with alumina slurry and immersed in piranha solution (a hot mixed solution of conc. H_2SO_4 and 30% H_2O_2, 3:1 ratio (v/v)) for 15 min followed by ultrasonic cleaning with DW. The electrode was then immersed in an ethanol solution (10 ml) containing 1 mM cysteamine for 10 h to give a cysteamine self-assembled monolayer (SAM). On the other hand, 1.0 gm c-MWCNT was suspended in a mixture of concentrated H_2SO_4 and HNO_3 in 3:1 ratio and ultrasonicated for 2 h to get a finely dispersed black colored solution of c-MWCNT. A mixture of 100 mM EDC and 100 mM NHS was prepared by mixing 1.0 ml each of 200 mM EDC and 200 mM NHS and the pH was adjusted to 6.0. The 0.1 ml of this mixture was then added to a 0.1 ml dispersed solution of c-MWCNT and kept further for 1 h at room temperature to convert the carboxyl groups of the shortened c-MWCNT into active carbodiimide esters. The cysteamine-modified Au electrode was placed in the above active c-MWCNT solution (adjusting the pH at 8.5 with NaOH) for 10 h. During this time, the amines at the termini of the SAM formed amide bonds with active carbodiimide esters of c-MWCNT **[23]**. Furthermore, the electrode was washed thoroughly with DW and then immersed into core–shell MNPs solution for 12 h at 4 ^oC. The core–shell MNPs were simply adsorbed on the nanotubes surface. The electrode was washed again with DW to remove any unbound matter and kept in a dry petri plate at 4 °C, until use **[24]**.

Immobilisation of LyOx on the modified electrode

The enzyme electrode was prepared by smearing 50 µl (6 Units) of LyOx solution onto the surface of each modified Au electrode and keeping them at 4 ^oC for 24 h. The enzyme electrode was rinsed with DW, dried at room temperature and stored at 4 ^oC until use.

SEM study of LyOx/core–shell MNPs/c-MWCNT modified Au electrode

In order to confirm immobilization of LyOx enzyme, Scanning electron microscopy (SEM) studies of bare Au electrode, core–shell MNPs/c-MWCNT/Au electrode and LyOx/core–shell MNPs/c-MWCNT/Au electrode were conducted at Department of Chemistry, M. D. University, Rohtak. In this process, the electrodes were cut into small

pieces and placed on a copper disc of 2 cm diameter. Then Au particles were deposited on the surface of electrodes using a spray gun and their electron micrographs were taken.

Electrochemical characterization and response measurement of modified Au electrode

In order to measure the lysine concentration, an amperometric biosensor was constructed by LyOx/core–shell MNPs/c-MWCNT/Au electrode as working electrode, Ag/AgCl as reference and platinum (Pt) wire as auxiliary electrodes connected through galvanostat/potentiostat. The working principle of this biosensor is summarized below:

$$\text{L-lysine} + O_2 + H_2O \xrightarrow{\text{Lysine oxidase}} \alpha\text{-Keto-}\varepsilon\text{-aminocaproate} + NH_3 + H_2O_2$$

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$

Cyclic voltammetric studies were carried out for individual biosensor. The applicability of the biosensor was tested by the three electrode system connected through potentiostat immersed into reaction cell containing 15 ml 0.1 M sodium phosphate buffer pH 6.5 containing 0.1 M KCl. The reaction was started by adding appropriate amount of lysine and the current (mA) was generated at different voltages. Cyclic voltammograms of modified electrodes were recorded in suitable buffers containing optimized quantity of lysine substrate after applying a suitable voltage.

Optimization of biosensor

Lysine measurements with modified Au electrode was optimized by testing biosensor response in phosphate buffers at 0.05 M final concentration containing 0.1 M potassium chloride on various pH (pH 6.0, 6.5, 7.0 and 7.5). To determine optimum temperature, the reaction mixture was incubated between 20 ºC to 55 ºC with an change in temperature of 5 ºC. The optimum response time was determined by incubating the reaction mixture for 2 – 10s. The effect of substrate concentration was tested by cyclic voltammetry from 0.0 to + 0.8 V at a rate of 20 mVs^{-1}. The tested lysine concentrations were considered which were ranging from 0.05 to 800 µM. The amperometric response was also measured in presence of potential interfering substances at the concentration of 1 mM including methionine, phenylalanine, proline, serine, uric acid, ascorbic acid, histidine, cysteine, tyrosine, arginine, tryptophan, glutamic acid and ornithine.

Amperometric determination of lysine in milk, serum and pharmaceutical samples

The present biosensor was employed for determination of lysine in real samples e.g. milk, pharmaceutical tablets and serum. Prior to this assay, the milk samples were digested in 6 N HCl for 15 min to hydrolyse proteins. These samples were brought to pH 6.0 with 1 M KOH and then assayed for lysine content.

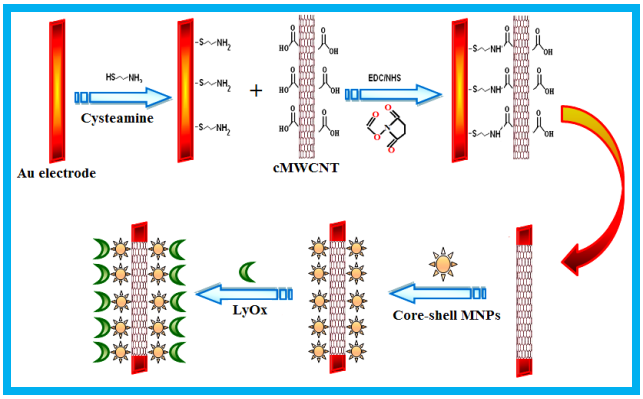
Storage stability and reusability of modified Au electrode

In order to reuse, the working electrode was washed by dipping in a test tube containing 2 ml of reaction buffer. The long-term storage and stability of the biosensor was investigated over the period of 4-months. When the electrodes were not in use, they were stored dry in a refrigerator at 4 ^oC. The sensitivity of the modified Au electrode was measured once in a week.

Results and discussion

Construction of lysine biosensor

The construction of an amperometric lysine biosensor by immobilizing LyOx onto core–shell MNPs/c-MWCNT-modified Au electrode is summarized in **Scheme 1A** and **1B**. An amine-functionalized SAM was formed on Au electrode surface with cysteamine. c-MWCNTs were solubilized after refluxing and sonication in concentrated sulfuric acid and nitric acid. Carboxylic acids moieties located at the sidewalls of the c-MWCNTs were converted to carbodiimide esters after treatment with EDC and NHS. The CNTs were then immobilized onto the electrode surface by forming amide bonds with amines at the terminus of the SAM.



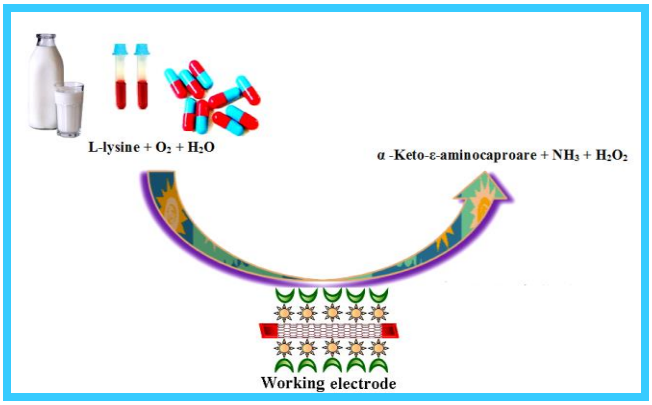
Scheme 1A. Schematic representation for the fabrication of the LyOx/core–shell MNPs/c-MWCNT modified Au electrode.

TEM, SEM and EIS studies of modified Au electrode

The typical image showing the core–shell MNPs synthesized in this study was very fine and monodispersed with diameter of ca. 20-30 nm (**Fig.1**). The cores appear darker and shells lighter in the images due to the large difference in electron penetration efficiency on Fe and oxides.

The immobilization of LyOx onto core–shell MNPs/c-MWCNT modified Au electrode was confirmed by SEM images showing surfaces of the bare electrode and LyOx immobilized onto core–shell MNPs/c-MWCNT/Au electrode. **Fig. 2** showed the SEM image of the bare Au electrode (image A); a smooth and featureless morphology. **Fig. 2B** shows the presence of core–shell MNPs/c-MWCNT on the surface of Au electrode as tubular and granular structures. Twisted MWCNTs distribute among the nanoparticles to form a three dimensional structure (**Fig. 2B**). While, some of them are flat and embedded into the nanoparticles, which may be due to the pressure during the screening process. High-resolution SEM images was

observed that the core–shell MNPs/c-MWCNT/Au surface with immobilized enzyme had folds and clusters along some beaded structures (image C) that were not observed in the electrode without immobilized enzyme. After the immobilization process, a change in surface morphology was observed.



Scheme 1B. Schematic representation of the electron flow and current generation in the lysine biosensor employing LyOx/core–shell MNPs/c-MWCNT/Au as the working electrode.

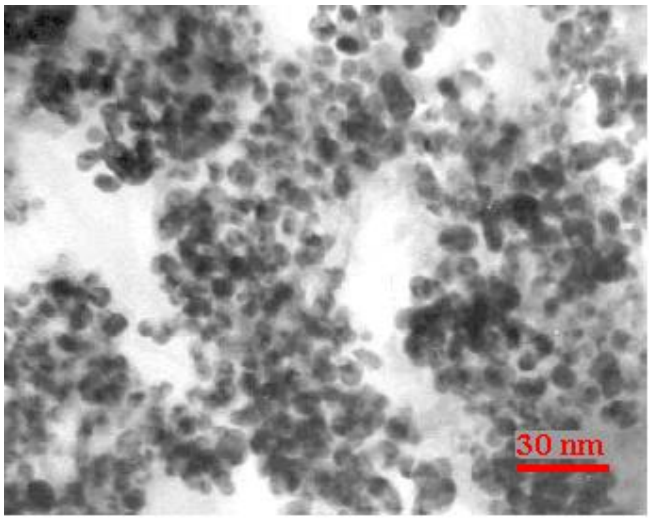


Fig. 1. Transmission electron microscope (TEM) image of core–shell MNPs.

Fig. 3 shows electrochemical impedance spectra (EIS) of (a) c-MWCNT/Au electrode (b) core–shell MNPs/c-MWCNT/Au electrode and (c) LyOx/core–shell MNPs/c-MWCNT/Au electrode. The resistance charge transfer (RCT) values for the c-MWCNT/Au, core–shell MNPs/c-MWCNT/Au and LyOx/core–shell MNPs/c-MWCNT/Au electrodes were 16.68×10^2, 7.47×10^2 and 22.38×10^2 Ω, respectively. The electron transfer via redox couple was hindered by the presence of enzymes on electrode surface. The increased RCT value of LyOx/core–shell MNPs/c-MWCNT/Au electrode was due to the immobilization of enzyme onto core–shell MNPs/c-MWCNT/Au electrode. This increase in RCT is attributed to the fact that most biological molecules, including enzymes, are poor electrical conductors at low frequencies (at least <10 KHz), which cause hindrance to the electron transfer.

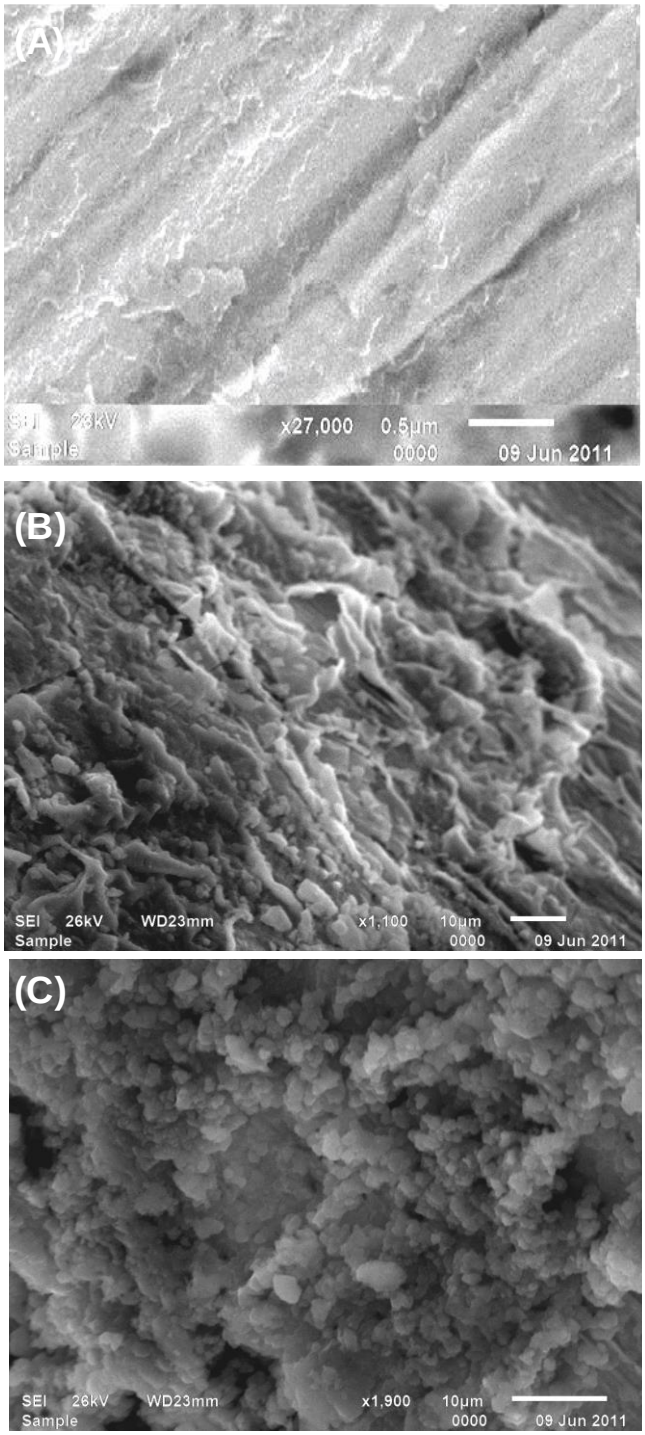


Fig. 2. Scanning electron micrographs of the (a) bare Au electrode, (b) core–shell MNPs /cMWCNT modified Au electrode and (c) LyOx/core–shell MNPs/cMWCNT modified Au electrode.

FTIR characterization of the modified Au electrode

FTIR spectrum for c-MWCNT/Au electrode showing the attachment of c-MWCNTs to the bare Au electrode through cysteamine was observed as shown by the peak at 1650.45 cm^{-1}. This peak was observed due to the amide bond between SAM of cysteamine-modified Au electrode and the active carbodiimide esters of the MWCNTs (**Fig. 4**).

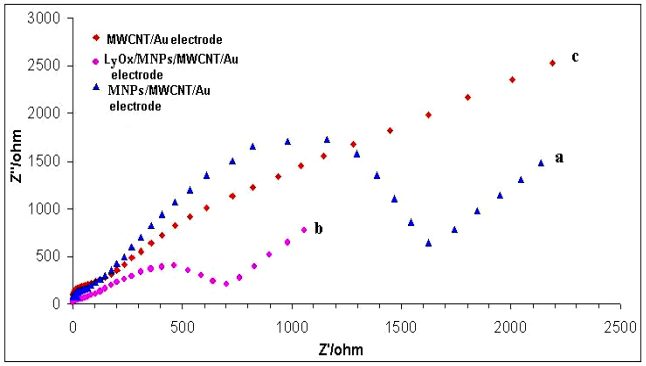


Fig. 3. Nyquist plots of the sensing electrode response at different stages in the electrode assembly process: c-MWCNT modified Au electrode **(a)**; core–shell MNPs/c-MWCNT modified Au electrode **(b)** and LyOx/core–shell MNPs/c-MWCNT modified Au electrode **(c)**. All spectra were recorded in the presence of 10 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1M KCl as a redox-active indicator. Frequency range: 0.01 Hz to 10 kHz.

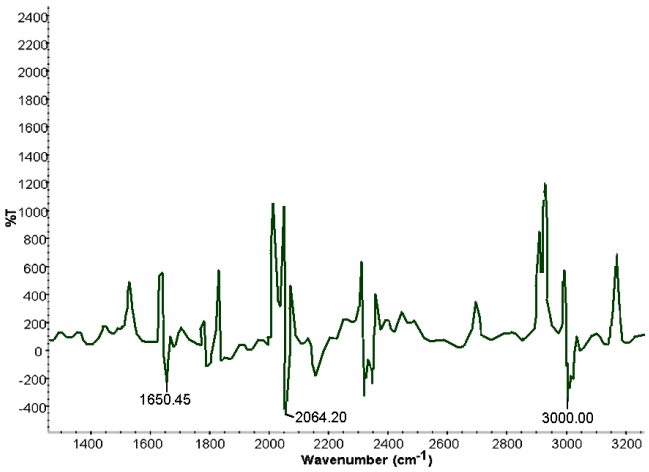


Fig. 4. FTIR spectra of c-MWCNT modified Au electrode.

Electrochemical characterization of LyOx/core–shell MNPs/c-MWCNT/Au electrode by cyclic voltammetry study

The electrochemical response of LyOx/core–shell MNPs/c-MWCNT/Au electrode was studied by using cyclic voltammetry. Cyclic voltammetric studies were carried out using the three electrode system composed of modified Au electrodes as working electrode, Ag/AgCl as reference electrode and Pt wire as auxiliary electrode. To discern the role of individual components, cyclic voltammograms of bare Au electrode, c-MWCNT/Au electrode and core–shell MNPs/c-MWCNT/Au electrode were recorded in 50 mM phosphate buffer (pH 7.5) containing 0.1 mM H_2O_2 at a scan rate of 0.0 to +1.0 V s^{-1} at an interval of 50 mV s^{-1}. **Fig. 5A** shows current responses of Au electrode after the addition of 0.1 mM H_2O_2 at different stages of construction. The bare electrode was measured by a very small current response to H_2O_2 (trace a) that was not detectable. This response was occurred because H_2O_2 was not electro-oxidized at the bare electrode surface once a potential of -0.2 to +0.6 V was applied. The small current measurement indicated that the direct oxidation of H_2O_2 at the Au electrode was inefficient. After the addition of H_2O_2 onto the c-MWCNT/Au electrode (trace b) a slight increase in current response was observed. This increase of current may be ascribed to the fact that the relative surface area of the electrode was increased after immobilization of c-MWCNT on it. Furthermore, the introduction of core–shell MNPs into the c-MWCNT/Au electrode dramatically improved the current response (trace c). The significant increase in the current response after the addition of core–shell MNPs to the electrode attributes to the good catalytic activity of core–shell MNPs to the electro-oxidation of H_2O_2. An increased anodic current was measured from 0.079 to 0.150 mA after the addition of c-MWCNT/core–shell MNPs. The amplified H_2O_2 current was measured by two times compared with c-MWCNT/Au electrode and by four times compared with bare Au electrode. This phenomenon is attributed to the increase in effective electrode surface due to immobilization of c-MWCNT and core–shell MNPs on the Au electrode.

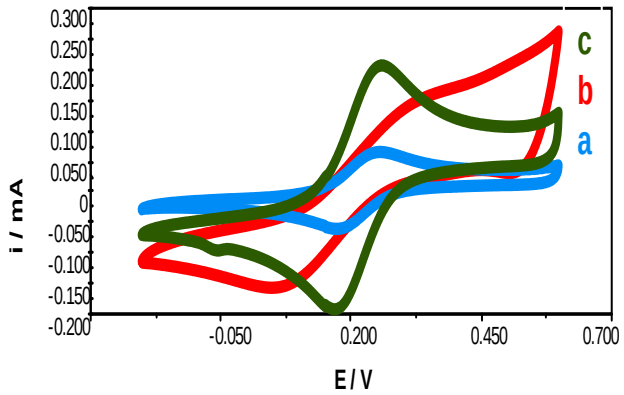


Fig. 5A. Cyclic voltammograms of (a) bare Au electrode, (b) c-MWCNT modified Au electrode and (c) core–shell MNPs/c-MWCNT modified Au electrode in 50 mM phosphate buffer (pH 7.5) containing 0.1 mM H_2O_2 at a scan rate of 0.0 to +1.0 V s^{-1}.

Optimization of biosensor

The experimental conditions affecting the biosensor response were studied in terms of the effects of pH, incubation temperature, time and substrate (lysine) concentration. The flow of electron (i.e., current) was measured in milliamperes (mA) at +0.2 V. The current response resulting from the LyOx/Core–shell MNPs/c-MWCNT/Au electrode was achieved by a maximum value at pH 6.0, which is more or less similar to that of free enzyme. The optimum temperature for this biosensor was 30 ºC. The decrease in the optimum temperature in comparison to free enzyme temperature (37 ºC) might be occurred due to the improvement in the enzyme rigidity upon immobilization through covalent binding. The biosensor showed optimum response within 2s, which is lower than 180s **[25]**, 42s **[26]** and 13s **[27]** reported previously.

Effect of lysine concentration on response of modified electrode

In order to measure the response of working electrode/sensor, the three-electrode system was immersed into 15 ml of 50 mM phosphate buffer and the reaction was started by adding 0.1 ml of lysine, which was oxidized and produced an electroactive H_2O_2. Formation of H_2O_2 was detected by its oxidation to generate electrons (i.e., current at the electrode). **Fig. 5B** shows the cyclic voltammograms of modified electrodes response at different lysine

concentrations (0.05 -800 µM). There was a linear increase in oxidation current with increase in lysine concentration in the range 0.05 -700 µM, thereafter it was constant. The detection limit (LOD) of the LyOx/core–shell MNPs/ c-MWCNT modified Au electrode was 0.05 µM (S/N=3). The LOD was lower than reported earlier (**Table 1**).

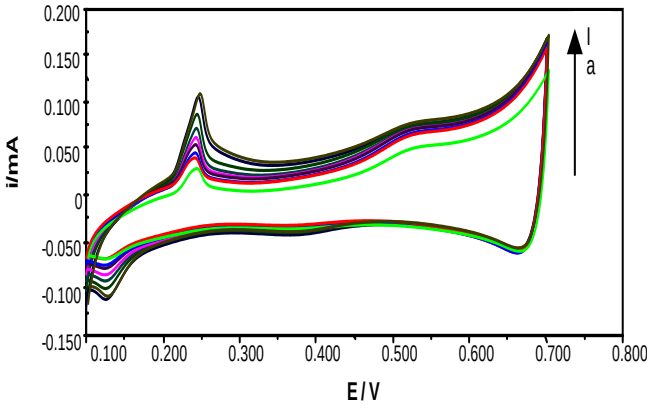


Fig. 5B. Cyclic voltammograms of LyOx/core–shell MNPs/c-MWCNT modified Au electrode in phosphate buffer (50 mM, pH 7.5) at a scan rate of 50 mV s^{-1} at different concentrations of lysine (µM).

Table 1. Comparison of analytical characteristics of LyOx based biosensors with the present biosensor.

Matrix for immobilization	Type of electrode	Immobilization method	Applied voltage (V)	Response time (s)	Detection limit (µM)	Linearity (µM)	Storage stability (Months)	Reference
Imidodiacetic acid chelating beads	Glassy carbon foil	Non-covalent	+0.850	180	1.0	1.0 to 500	NR	[25]
Nylon membrane	Graphitemeth acrylate electrode	Glutaraldehyde crosslinking	+1.0	42	0.82	0.82 to 100	1	[26]
DAB	Pt electrode	Passive adsorption	NR	13	0.2	10 to 100	NR	[27]
LyOx/AuNPs/c-MWCNT/PANI	Au electrode	Covalent	+0.4	2	5.0	5.0 to 600	4	[10]
LyOx/AuNPs/c-MWCNT/DAB	Au electrode	Covalent	+0.4	4	20	20 to 600	3	[10]
LyOx/3-APTES/AuNPs –PtNPs	Au electrode	Covalent	+0.2	4	1.0	1.0–600	4	[11]
Core–shell MNPs/c-MWCNT	Au electrode	Covalent	+0.2	2	0.05	0.05–700	6	Present biosensor

Analytic recovery and precision study

The analytic recoveries of added lysine into spiked pretreated milk samples at concentrations of 50 µM and 100 µM were 86.0 and 87.0 % respectively. Lysine content in six serum samples was determined on single day (within batch) five times and again after storage at -20ºC for one week (between batch). The batch precisions were less than 3.5 % and 4.2 % respectively. These results demonstrated that the described method was reliable and reproducible.

Accuracy

In order to study the correlation of the developed biosensors, lysine level in 15 serum samples as determined by the present method (x) were compared with those obtained by standard HPLC method (y). All tests were carried out in triplicate and results were presented as mean value of three tests. The correlation value obtained by LyOx/core–shell MNPs/c-MWCNT/Au electrode was 0.98 (**Fig. 6**) showing the high accuracy of the method.

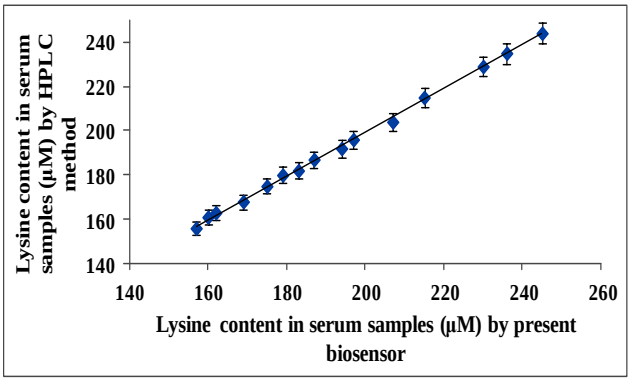


Fig. 6. Correlation between values of serum lysine contents as determined by LyOx/core–shell MNPs/c-MWCNT modified Au electrode and HPLC method.

Interference study

No significant interference was detected with methionine, phenylalanine, proline, serine, uric acid, ascorbic acid, histidine, cysteine, tyrosine, arginine, tryptophan, glutamic acid and ornithine, each at 1 mM concentration.

Table 2. Determination of lysine in pharmaceutical amino acid tablets using LyOx/core-shell MNPs/c-MWCNT modified Au electrode.

Tablet	L-Lysine conc. (mg/tablet) (reported value)	Lysine conc. by biosensor (mg/tablet) Mean±S.D	RSD%
Alamin M Forte	25	23.6± 0.52	2.2

Application

In order to test the more applicability of this proposed biosensor to real life samples other than milk, lysine content was also determined in pharmaceutical tablets and serum samples. The mean value of serum lysine in apparently healthy persons was 248 µM (n=10) (**Table 2**), which is very close to normal established range (150 – 250 µM). Lysine in amino tablets, as measured by this sensor, was 23.6 mg/tablet, which were almost same as reported by the manufacturing company (25 mg) (**Table 3**).

Table 3. Lysine levels in serum samples of apparently healthy adults, as determined by biosensor based on LyOx/core-shell MNPs/c-MWCNT modified Au electrode.

Serum Sample	Sex	Lysine (µM) Mean±S.D.
S1	M	219.0±0.50
S2	M	234.0±0.60
S3	M	236.8±0.46
S4	M	245.8±0.88
S5	M	250.0±0.60
S6	F	251.3±0.10
S7	F	224.5±0.54
S8	F	264.7±0.35
S9	F	278.8±1.00
S10	F	220.8±0.30

Stability and reusability

The LyOx/core–shell MNPs/c-MWCNT/Au electrode showed practically no loss of its initial activity after 150 uses over a period of 180 days, when stored dried at 4 °C. Thereafter, there was a gradual but low decrease in its response, which amounted to 50% after 6 months. It can thus be concluded that the LyOx/core–shell MNPs/c-MWCNT/Au electrode can be used many times without significant decrease of response signal. A comparison of different analytical parameters of present LyOx biosensor with those of earlier biosensors is summarized in **Table 1**.

Conclusion

A more stable and reproducible deposition of c-MWCNT film on Au electrode was constructed using the covalent linkage method. The immobilization of LyOx on Core–shell MNPs decorated c-MWCNT modified Au electrode showed an improved performance for lysine biosensor in terms of:

- Response time (3 s),
- Broader linear range (0.05–700 µM),
- Lower detection limit (0.05 µM),
- Good reproducibility
- and longer stability (6 months).

In addition to its improved performance, this nanocomposite hybrid can also be used for construction of other biosensors for detection of various metabolites. Furthermore, it is certainly a potential nanohybrid technology which may develop future advanced biosensors for detection of amino acids with easy fabrication, low cost having high affinity and superior stability.

Acknowledgements
Financial assistance by Science and Engineering Research Board (SERB), New Delhi, in the form of Young Scientist Scheme (Fast Track) to Dr. Nidhi Chauhan and Dr. Jagriti Narang is greatly acknowledged.

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