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Fabrication and application of an amperometric lysine biosensor based on covalently immobilized lysine oxidase nanoparticles onto Au electrode

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Short running title: Lysine biosensor based on LOxNPs/AuE

Keywords: Lysine, Lysine oxidase nanoparticles (LOxNPs), Lysine biosensor, Gold electrode (AuE), Serum

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Abstract

The fabrication of an amperometric lysine biosensor is described in this study, wherein nanoparticles (NPs) of lysine oxidase (LOx) are covalently immobilized onto gold electrode (AuE). The LOxNPs were prepared by desolvation method and characterized by UV Vis spectroscopy, Fourier transform infra red (FTIR) spectroscopy and transmission electron microscopy (TEM). The LOxNPs/AuE modified working electrode was studied by scanning electron microscope (SEM) and cyclic voltammetric (CV) techniques. The electrode exhibited optimum current within 3.5 s at applied potential, 0.8 V, pH 6.5 and temperature, 35°C. The sensor displayed a linear relationship between lysine concentration and current in the range 10-800 μM with a limit of detection of 10 μM. Within assay and between batch coefficients of variation were 0.0751% and 0.0637% respectively. The analytical recoveries of added lysine at 10 μM and 20 μM in sera were 98.39% and 98.23% respectively. There was a good correlation between level of lysine in sera and milk samples (R^2=0.999 and R^2=0.98 respectively) as determined by the standard spectrophotometric method and the present method. The biosensor measured lysine levels in milk, pharmaceutical tablet and sera of healthy individuals and cancer patients. The biosensor showed slight interference by common interferents found in serum.

Keywords: Lysine, Lysine oxidase nanoparticles (LOxNPs), Lysine biosensor, Gold electrode (AuE), Serum

1. Introduction

Lysine ($C_6H_{14}N_2O_2$) or 2, 6-diaminohexanoic acid is an essential amino acid not synthesized by the body. So, it needs to be administered through diet. Its concentration in food products and body fluids may recognize the nutritional quality and diagnosis of certain diseases. The normal lysine levels in healthy human sera are 150-250 μmol/L [1]. It has been observed that the cancerous tissues like those of normal tissues require lysine for their growth [2]. So, decreased lysine levels in serum could be a biomarker for early detection of cancer.

Various approaches are possible for detecting lysine levels, like chemical assays depending on the ninhydrin test, capillary electrophoresis, gas phase chromatography [3], reverse phase liquid chromatography [4], HPLC [5] and liquid chromatography [6]. These approaches, however, need costly instruments and reagents, long pre-treatment/purification stages and highly skilled operators [7]. These drawbacks warrant the development of a new, rapid, sensitive and selective method for lysine determination. Thus, there is an increasing demand for inexpensive, rapid and reliable methods for lysine determination, which has been fulfilled by biosensing methods.

A number of amperometric lysine biosensors have been reported based on lysine oxidase immobilized on platinum electrode by glutaraldehyde co-crosslinking with bovine serum albumin; diamond paste; Au nanoparticles/c-MWCNT/PANI/poly -DAB/Au electrodes; platinum electrode previously modified by an overoxidized polypyrrole film; gold nanoparticles (AuNPs) and platinum nanoparticles (PtNPs)/Au electrode using 3-aminopropyltriethoxy silane (3-APTES) and glutaraldehyde cross linking; polyvinylferrocenium (PVF^+) and platinum deposited PVF^+; and Platinum (Pt) electrodes modified by overoxidized polypyrrole [8,9,1,10,11,12,13]. These sensors are based on immobilization of native lysine oxidase onto different matrices and electrodes. The direct immobilization of enzyme at modified electrode could degrade the enzyme thus affecting its stability and activity, which in turn leads to the reduced performance of the biosensor. To overcome these pitfalls, the aggregated form of enzymes i.e. enzyme nanoaprticles are used instead of native enzyme. Enzyme nanoparticles have improved the working of modified electrodes due to their increased surface area to volume ratio and distinctive mechanical, electronic, electrical, optical and thermal properties [14]. Various biosensors have been fabricated based on enzyme nanoparticles and showed better analytical properties, such as sarcosine oxidase nanoparticles onto Au electrode [15], lactate

dehydrogenase nanoparticles onto Au electrode [16] and pyruvate dehydrogenase nanoparticles onto Au electrode [17]. Due to little background current, easy availability, larger cathodic potential range and good conductivity; gold electrode serves as better choice over other electrodes like, platinum and carbon based electrode [18]. We report herein the fabrication of an upgraded amperometric lysine biosensor depending on covalent immobilization of lysine oxidase nanoparticles onto the surface of Au electrode for determination of lysine levels in sera of cancer patients for early detection of cancer, in pharmaceutical tablet and in food samples.

2. Materials and methods

2.1 Chemicals and reagents

Lysine oxidase (native enzyme) from *Trichoderma viride* (≥20 U/mg), horseradish peroxidase (HRP), 4-aminoantipyrine and 25% glutaraldehyde were bought from Sigma Aldrich, Co., (St. Louis, MO, USA). L-Lysine, ethanol, sodium phosphate monobasic and sodium phosphate dibasic were purchased from Sisco Research Laboratory, Mumbai, India and Populus Multi Vitamin capsules, manufactured by N.V.Oryx food & pharma (India) were used. Gold wire with 25 mm height and 2 mm uniform diameter was bought from local market. Sera samples of apparently healthy persons and cancer patients were collected from PGIMS, Rohtak which already has ethical clearance. All other chemicals of analytical reagent grade were used. Throughout the experiment, double distilled water (DW) was used.

2.2 Equipment used

Potentiostat/Galvanostat (Make: autolab, model: AUT83785, manufactured by: Eco Chemie, The Netherlands) with NOVA 1.4 software integrating a three electrode system consisting of LOxNPs/AuE as the working electrode, Ag/AgCl as a reference electrode and Pt wire as an auxiliary electrode. Transmission electron microscope (TEM) (JEOL JEM-1400), Scanning electron microscope (SEM) (Zeiss EVO 18 Special**),** Fourier transform infrared (FTIR) spectrophotometer (Make: Bruker, Alpha II), UV-vis spectrophotometer (Lab India Analytical, UV 3092), were used.

2.3 Assay of lysine oxidase

Lysine oxidase assay elucidated by Pundir et al., 2010 [19] with some changes depending on the following reactions was performed:

L-Lysine + O_2 + Lysine oxidase → α-keto-ε-aminocaproate + H_2O_2 + NH_3

2 H_2O_2+4-aminoantipyrine+phenol peroxidase → Quinoneimine dye (Pink) +4 H_2O_2

The assay was performed in test tubes covered with black paper and the whole assay was carried out in a dark room. The reaction mixture, containing 1.8 mL of 0.1 M sodium phosphate buffer (PBS, pH 7.5), 0.1 mL LOx (1 mg/mL) and 0.1 mL Lysine (1mM) was incubated at 37°C for 15 min, followed by addition of 1.0 mL color reagent (50 mg 4-aminophenazone, 100 mg phenol and 1.0 mg HRP in 100 ml of 0.4 M sodium phosphate buffer, pH 7.0 and stored at 4°C), maintaining the same temperature for 30 min to develop the color. At the same time, the blank was run, in which lysine oxidase suspension was used in place of the reaction mixture. The optical density of colour was recorded at 520 nm counter to blank and the H_2O_2 concentration produced in the assay was figured out from the standard curve of H_2O_2.

2.4 Preparation of LOxNPs

The enzyme nanoparticles (ENPs) were prepared by aggregation of indigeneous enzyme molecules. The LOxNPs were synthesized by desolvation method described by Kundu et al., 2012 [20]. 2.0 mL solution of LOx was prepared by dissolving in 0.1 M sodium phosphate buffer, pH 7.0 (1.0 mg/mL). To this solution, 4.0 mL of absolute ethanol was added dropwise at the rate of 0.1-0.2 mL/min under constant stirring at 500 rpm in cold conditions i.e. 4-8°C until the ENPs are formed. Then, 1.8 mL of 2.5% of glutaraldehyde was added to the LOxNPs solution under the same stirring for 24 hrs. This was followed by thiol functionalization of LOxNPs after adding 0.12 g of cysteamine dihydrochloride and stirring under same conditions for 5-6 hours. Lastly, to separate LOxNPs from indigenous enzyme solution, it was centrifuged at 1200 rpm for 10 min at 4°C. The LOxNPs were then redispersed into 4 ml of 0.1M sodium phosphate buffer (pH 7.0). Later, it was sonicated for 6 min and then stored in refrigerator at 4°C before further use (Figure 1a).

2.5 Characterization of LOxNPs

The UV-Visible spectra of LOxNPs suspension was recorded in the range 200-500 nm to confirm formation of LOxNPs. The size and morphology of LOxNPs were studied by taking their image in transmission electron microscope (TEM). The functional groups of LOxNPs were determined by recording FTIR spectra (4000-400 cm^{-1}).

2.6 Preparation of working electrode

The bare gold electrode's surface was cleaned manually by alumina/silica slurry (diameter 0.05 μm) with the help of tissue paper, accompanied by proper cleaning by the DW. Thereafter, the electrode was dipped in piranha etch (3 ml H_2SO_4: 1 ml H_2O_2) for 20 min, polished with alumina/silica slurry and then put into ethanol for 5-6 hours, followed by sonication so as to remove adsorbed debris/particles. For the covalent immobilization of ENPs, the polycrystalline gold electrode was then put into cysteamine functionalized LOxNPs suspension for 24-72 hrs at 4°C (Figure 1b). Thereafter, LOxNPs/Au electrode was washed with 0.1 M sodium phosphate buffer, pH 7.0 and stored in the same buffer until further use at 4°C. To analyze the morphology of LOxNPs/Au electrode, SEM images were taken before and after the immobilization of ENPs.

2.7 Construction and response measurement

An improved amperometric lysine biosensor was fabricated by connecting LOxNPs modified Au electrode as working electrode, Ag/AgCl as reference electrode and Pt wire as counter electrode, through potentiostat-galvanostat. The graphical representation of the fabrication of LOxNPs modified Au electrode is given in figure 1 (b). The biosensor response was studied by recording the cyclic voltammetric (CV) response of the present biosensor in potentiostat-galvanostat using 25 ml of 0.1 M sodium phosphate buffer (pH 7.0) as the electrolyte and 0.1 ml of 0.1 M lysine as the substrate, both added in the electrochemical cell.

2.8 Optimization of lysine biosensor

To obtain the optimum conditions for the working of the lysine biosensor, effects of different parameters on biosensor response was studied. To determine optimum pH, different buffers with a pH range from 5.0 to 9.0 at interim of 0.5 pH i.e. sodium acetate buffers (pH 5.0, 5.5), sodium phosphate buffers (pH 6.0-8.0) and glycine-NaOH buffers (pH 8.5, 9.0); each at final

concentration of 0.1 M were used. Incubation temperature was varied between 1 to 70 °C at interim of 5 °C, so as to determine optimum temperature. The effect of lysine concentration was studied by recording the current (in A) at various lysine concentrations ranging from 0 μM to 1000 μM. A standard curve was plotted between lysine concentrations and current (Figure 4c).

2.9 Evaluation of lysine biosensor

To evaluate performance of the LOxNPs biosensor, different analytical parameters were studied i.e. limit of detection (LOD), analytical recovery, coefficient of variation (CV), correlation coefficient (R^2) and storage stability. The effects of different interferents found in blood such as sarcosine, glucose, urea, citric acid, creatinine and other amino acids was also studied at their physiological concentrations.

2.10 Application of lysine biosensor

The present biosensor was used for determination of lysine in milk samples, pharmaceutical tablet and serum samples collected from apparently healthy and diseased persons. The fresh blood samples were collected and allowed to coagulate for 1 h at room temperature. Further, serum collection was done from blood sample by centrifuging for 11 min at 1200 rpm. CV of the LOxNPs/Au electrode was measured in the potentiostat under the standard conditions to determine the lysine levels and the potential range was found to be 0-0.8 V. The lysine levels in serum were inferred using the standard curve obtained under optimum conditions between lysine concentration and current (in A). To obtain the free amino acid ends of milk samples, they were hydrolyzed in 6N HCl in sealed tubes for 24 hours, followed by evaporation of acid under vacuum. Using 1M KOH, the pH of the milk samples was set to 7.0 and then were used to determine the lysine levels. To determine the lysine content in pharmaceutical tablet (Populus multivitamin capsules), the tablet was added in the sodium phosphate buffer (0.1 M, pH 6.5), vortexed to mix content thoroughly and then analysed for lysine content, using the current LOxNPs/Au electrode.

3. Results and discussion

3.1 Characterization of LOxNPs by TEM, UV-Vis Spectroscopy and FTIR

The average size of the LOxNPs as determined by TEM was found to be 22.14±0.025 nm (Figure 3a). UV-visible spectra of LOx and LOxNPs exhibited broad absorbance peak at 250 nm. Increase in absorbance at 250 nm demonstrates the small size of nanoparticles obtained due to the aggregation of free enzyme particles (Figure 3b). The FTIR study displayed the spectrum of indigenous LOx and LOxNPs, where the indigenous LOx exhibited distinctive peaks at 3328.70 cm^{-1}, 2112.18 cm^{-1}, 1635.35 cm^{-1}, 1076.51 cm^{-1} and the LOxNPs displayed peaks at 3330.31 $cm^{-1,}$ 2116.18 cm^{-1}, 1632.31 cm^{-1} and 1076.51 cm^{-1} (Figure 3c). The basic FTIR mode of LOx and LOxNPs displayed similar pattern, thus inferring that the formation of LOxNPs did not change the operational as well as structural characteristics of the native enzyme LOx. The peaks at 3300 cm^{-1}, 2100 cm^{-1}, 1630 cm^{-1} and 1070 cm^{-1} could be assigned to O-H stretching (alcohols and phenols), C≡C stretching (alkynes), C=C stretching (alkenes) and –C=O stretch (1° alcohol) respectively.

3.2 Characterization of working electrode by SEM

SEM image of the bare gold electrode displayed smooth, homogeneous and featureless morphology. Due to the covalent immobilization of LOxNPs onto the Au electrode, the modified/working electrode exhibited globular and granular structural morphology with bead like structures (Figure 3d).

3.3 Optimization of lysine biosensor

An upgraded novel lysine biosensor was fabricated by immobilizing LOxNPs covalently onto Au electrode. The present working electrode showed the optimum current within 3.5 s at 0.8 V across Ag/AgCl electrode. So, for further electrochemical examination, the current was noted at same voltage within 3.5 s. For cyclic voltammetry, the current LOxNPs/Au electrode exhibited the anodic as well as cathodic peaks having equal slopes using the substrate lysine, interfaced to computer with NOVA 1.4 software (Figure 2). At pH 6.5, the maximum current response was observed, that is near the physiological pH of native enzyme (Figure 4a). The optimum pH of this biosensor is closer to those of earlier biosensors, pH 7.0 based on

AuNPs/MWCNT/PANI/Au and AuNPs/MWCNT/DAB/Au electrode [1], Yttria/Titania/CNT [21], Graphite electrode/Os polymer [22]; pH 7.4 based on PVF and PVF/Pt electrode [12]; pH 7.5 based on overoxidized polypyrrole bilayer [10] and 3-APTES/AuNPs-PtNPs/Au electrode [11]; pH 7.6 based on immunodyne ABC nylon membrane [23], but higher than those showing optimum pH 5.0 and based on overoxidized polypyrrole/Pt electrode [13,27] and lower than those exhibiting optimum pH 10.0 and based on PVF/MWCNTs-GEL, PVF/MWCNTs-GEL/GR, c-MWCNT/GR/SnO_2/Chitosan and Graphene/Poly(vinylferrocene) composite [24,25,26]. The optimum temperature was recorded at 35°C (Figure 4b), which is higher than those biosensors based on AuNPs/MWCNT/DAB/Au electrode (optimum temperature, 30°C) and 3-APTES/AuNPs-PtNPs/Au electrode [1,11] and lower than biosensors based on PVF and PVF/Pt electrode (optimum temperature, 40°C) [12]. The biosensor showed optimum response at 3.5s, which is better than earlier biosensors i.e. 4s based on AuNPs/MWCNT/DAB/Au electrode [1] and based on 3-APTES/AuNPs-PtNPs/Au electrode [11]; <6s based on overoxidized polypyrrole bilayer [10] and <30s based on PVF and PVF/Pt electrode [12]. The current response (in amperes, Amp.) increased in a linear fashion upto 800 μM and then became constant after 800 μM (hyperbolically) (Figure 4c). The full form of all the abbreviations used in this paragraph are given in the foot note of Table 6.

3.4 Evaluation of biosensor

Under the optimum conditions, a linear relationship was observed between the current(in Amp.) and lysine concentration in the range, 10-800 μM. A comparison of these results with those of previous biosensors revealed that the present biosensor had better linearity compared to previously stated biosensors. The biosensor showed wider linear range than those of earlier biosensor,5-600 μM for that based on AuNPs/MWCNT/PANI/Au electrode [1]; 1-600 μM based on 3-APTES/AuNPs-PtNPs/Au electrode [11]; 5-110 μM based on MWCNT/TiO_2NPs/GC electrode [28]; 5-500 μM based on Graphite electrode/Os polymer [22]; 10-250 μM based on Immunodyne ABC nylon membrane [23] but higher than few earlier reported biosensors,0.01-0.1 μM based on diamond paste [9] and 0.99-700 μM based on PVF/MWCNTs-GEL and PVF/MWCNTs-GEL/GR [24]. The minimum detection limit of the current LOxNPs/AuE was 10 μM, which is better/ lower than 20 μM for biosensors based on AuNPs/MWCNT/DAB/Au electrode [1] but similar to 10 μM based on immunodyne ABC nylon membrane [23] and higher

than 4 μM for biosensor based on overoxidized polypyrrole bilayer and overoxidized polypyrrole/Pt electrode [10,13]; 5 μM based on AuNPs/MWCNT/PANI/Au electrode [1]; 2 μM based on Overoxidized polypyrrole/Pt electrode [27] (Table 6). By adding lysine in sera at concentrations of 10 μM and 20 μM , the analytical recoveries of added lysine were found as 98.39% and 98.23 % respectively (Supplementary Table 1). Within assay and between batch coefficients of variations (CV) of the present biosensor were 0.0751% and 0.0637% respectively (Supplementary Table 2). Better reproducibility, reliability and consistency of the biosensor were observed due to the above highly precise observations, which could be due to the use of covalently immobilized LOxNPs onto the surface of Au electrode through thiolate bond.

3.5 Applications and correlation of lysine biosensor

The lysine levels as determined by current LOxNPs/AuE were in the range 205 to 257.3 μM in the milk samples (Supplementary Table 4) and 49.4 mg in the amino acid tablet (Supplementary Table 5). The serum lysine content of apparently healthy individuals as determined using the current biosensor were between 201.6 μM ±0.57 to 266.4 μM ± 0.57 that lies in normal range. The serum lysine content of cancer patients were in the range, 80.4 μM ± 0.89 to145.6 μM ±0.54, which is lower than the healthy individuals (Supplementary Table 3). Thus, lower lysine levels could be an early biomarker for detection of cancer and the disease progression. The present biosensor was used to measure the lysine content in milk samples, sera of apparently healthy persons and 4 different types of cancer patients. These values were matched with those using standard spectrophotometric procedure. A good correlation was found between both the methods (R^2=0.98 for milk and R^2=0.999 for sera samples) (Figure 4d and 4e).

3.6 Interference study and selectivity

Interferents such as isoleucine, alanine, arginine, leucine, serine, glutamine, glycine, glutamic acid, tryptophan, sarcosine, creatinine, citric acid, glucose and urea showed interference of 2.75%, 1.5%, 1.75%, 1.6%, 2%, 3.05%, 2.1%, 1.35%, 1.25%, 1.35%, 1.45%, 1.55% and 1.85% respectively at their physiological concentrations. This shows that under normal assay conditions, the above side products found in blood have approximately no influence on the

biosensor performance. This confirms that the present lysine biosensor is very specific for the substrate lysine.

3.7 Storage stability of LOxNPs/Au electrode

The current LOxNPs/AuE lost 14% of its original performance after 290 continuous usage over time period of 200 days, stored at 4°C (Supplementary figure 5). These results suggest the exceptional durability of the LOxNPs/AuE than the previous biosensors, 120 days and 90 days [1]; 120 days [11]; 40 days [10]; 22 days and 30 days [12] and 30 days [24].

4. Conclusion

In the current research work, the employment of the LOxNPs instead of native/free enzyme i.e. LOx led to the improved analytical performance of the lysine biosensor, concerning working range (10 μM to 800 μM), lessened response time (3.5 s), lower detection limit (10 μM), heightened storage stability (200 days) and low interference by other blood interferents compared to the earlier biosensors. Enzyme nanoparticles because of their high surface area to volume ratio provided large electrosurface area to AuE. Also, it was simpler than the complex fabrication process involved in nanocomposites based biosensors. In this sensor, the LOxNPs were directly immobilized onto Au electrode. Other types of electrodes like pencil graphite electrode and graphene nanoparticles modified pencil graphite electrode could also be used in future to make cost effective biosensor. The concept of miniature biosensors is not fulfilled by the present biosensor. So, the future research could also aim at fabrication of miniaturized lysine biosensor that can be used for the point-of-care diagnosis of the patients.

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List of Figures

Figure: 1 (a) Schematic representation of preparation of nanoparticles of LOx, **(b)** Schematic representation of chemical reactions involved in the fabrication of LOxNPs/AuE.

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

Figure: 3 (a) Transmission electron microscopic (TEM) images of LOxNPs, **(b)** UV spectra of native LOx and LOxNPs, **(c)** FTIR spectra of (I) Native LOx and (II)LOxNPs, **(d)** Scanning electron microscopic (SEM) images of (I) Bare Au electrode and (II) LOxNPs/AuE.

Figure: 4 (a) Influence of applied pH on the current response of LOxNPs/AuE, **(b)** Influence of applied incubation temperature on the current response of LOxNPs/AuE, **(c)** Standard curve of lysine concentration by lysine biosensor based on LOxNPs/AuE, **(d)** Correlation between serum lysine level measured by standard method (x axis) and the present method (y axis) using the lysine biosensor based on LOxNPs/AuE, **(e)** Correlation between lysine level in milk samples measured by standard method (x axis) and the present method (y axis) using the lysine biosensor based on LOxNPs/AuE.

Supplementary Figure: 5 Storage stability of LOxNPs/AuE at 4°C

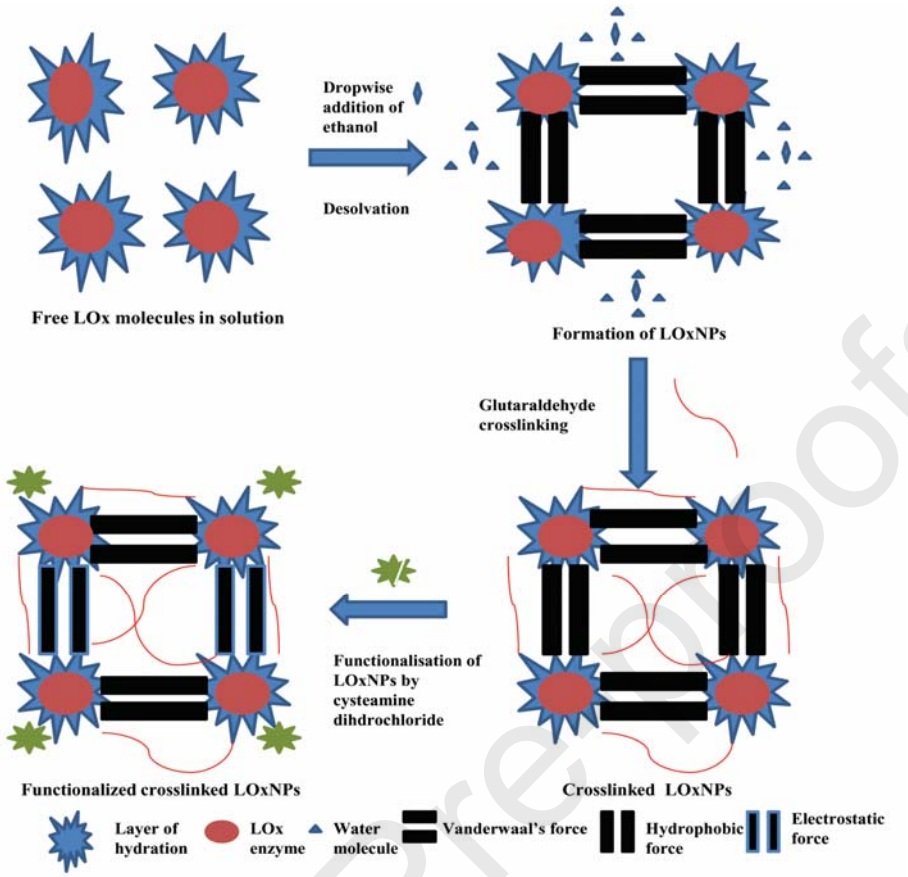


Fig. 1 (a)

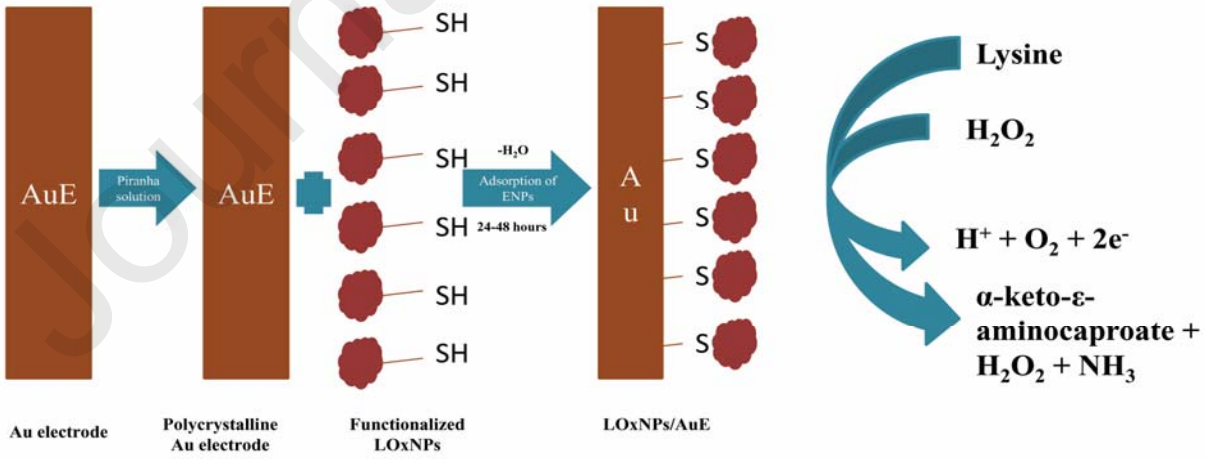


Fig.1 (b)

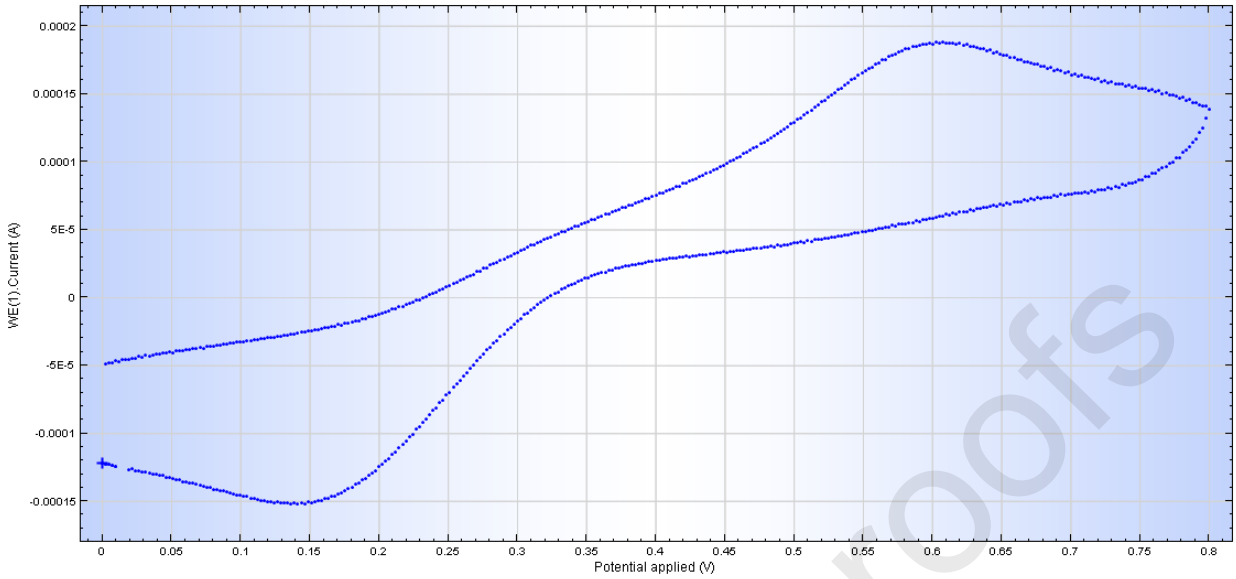


Fig.2

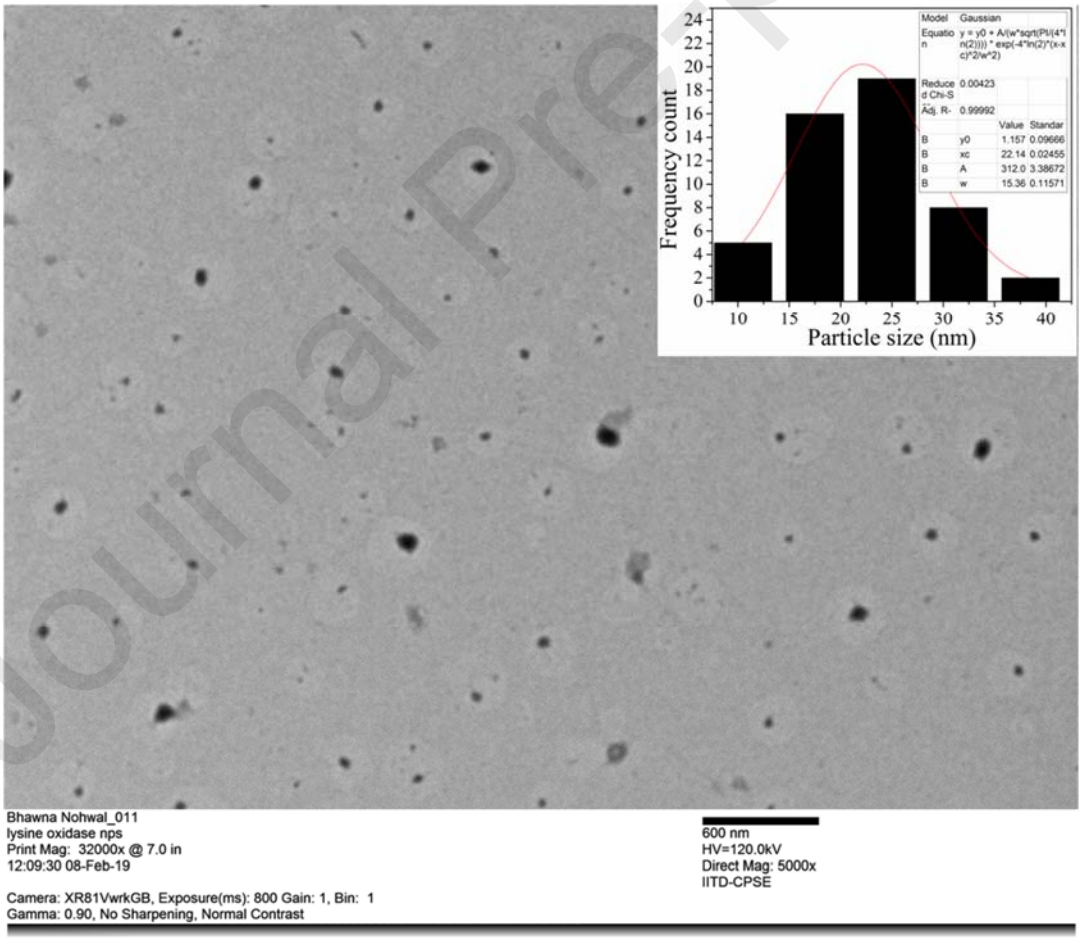


Fig. 3 (a)

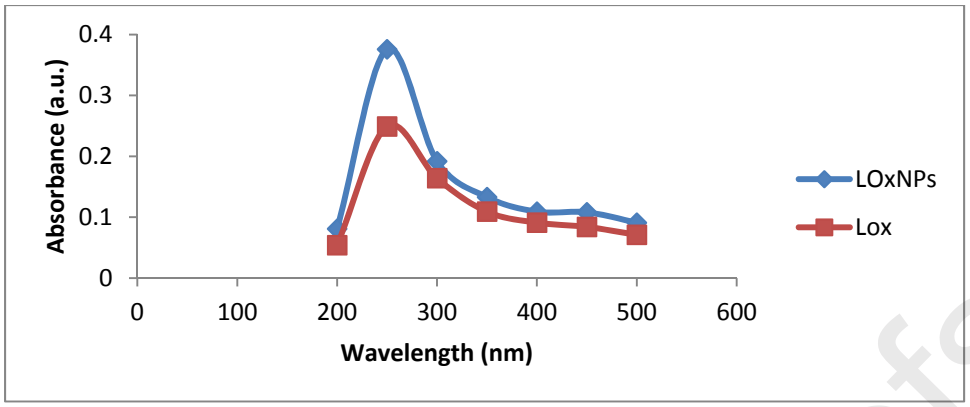


Fig. 3b

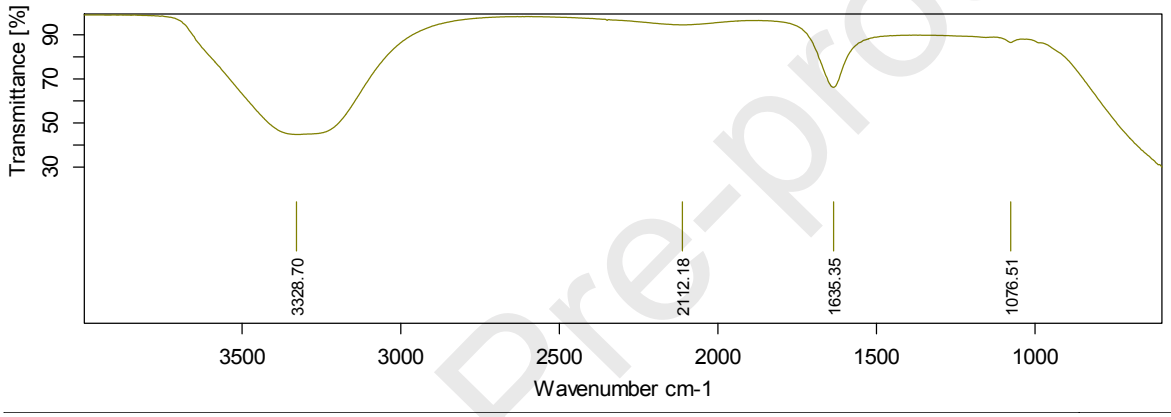


Fig. 3c(I)

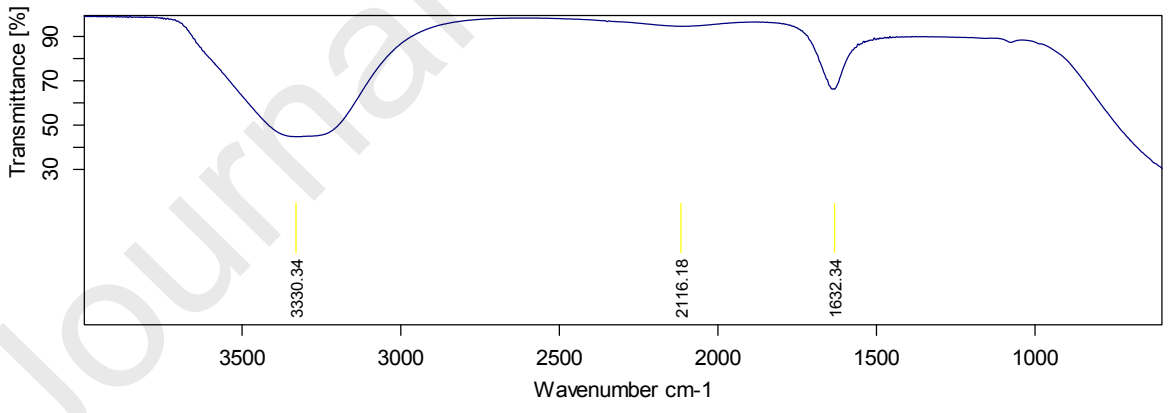


Fig. 3c(II)

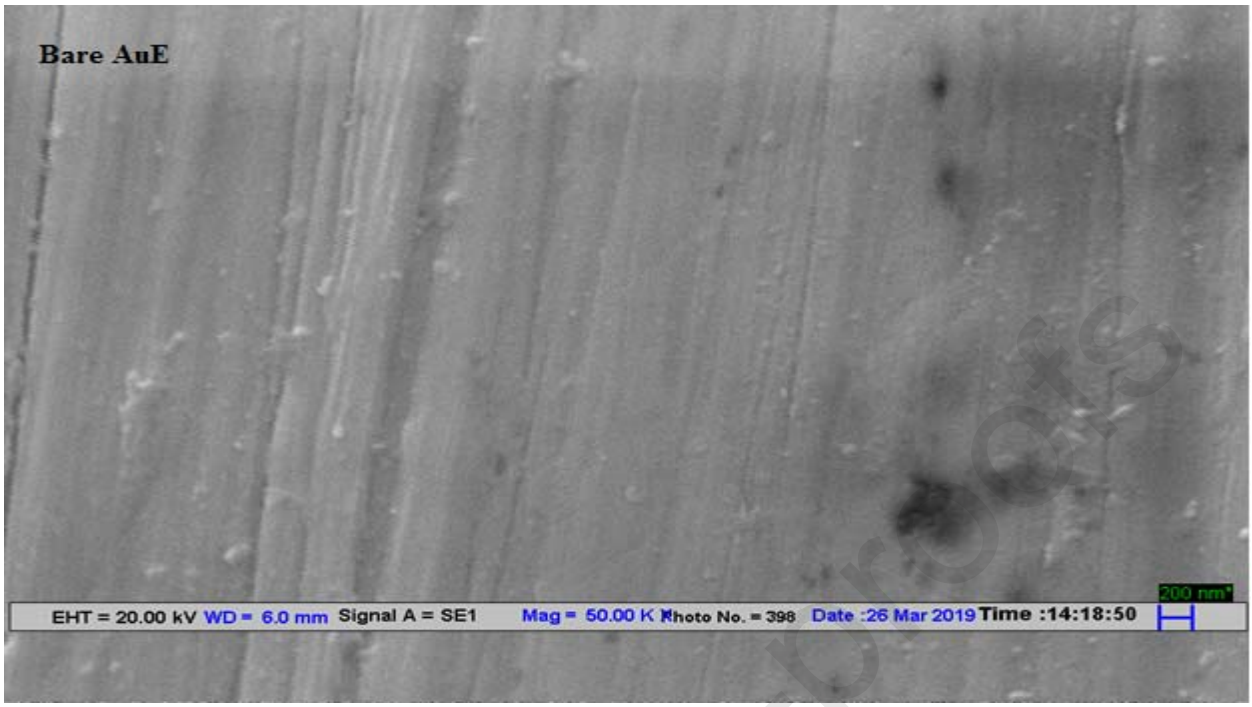


Fig. 3d (I)

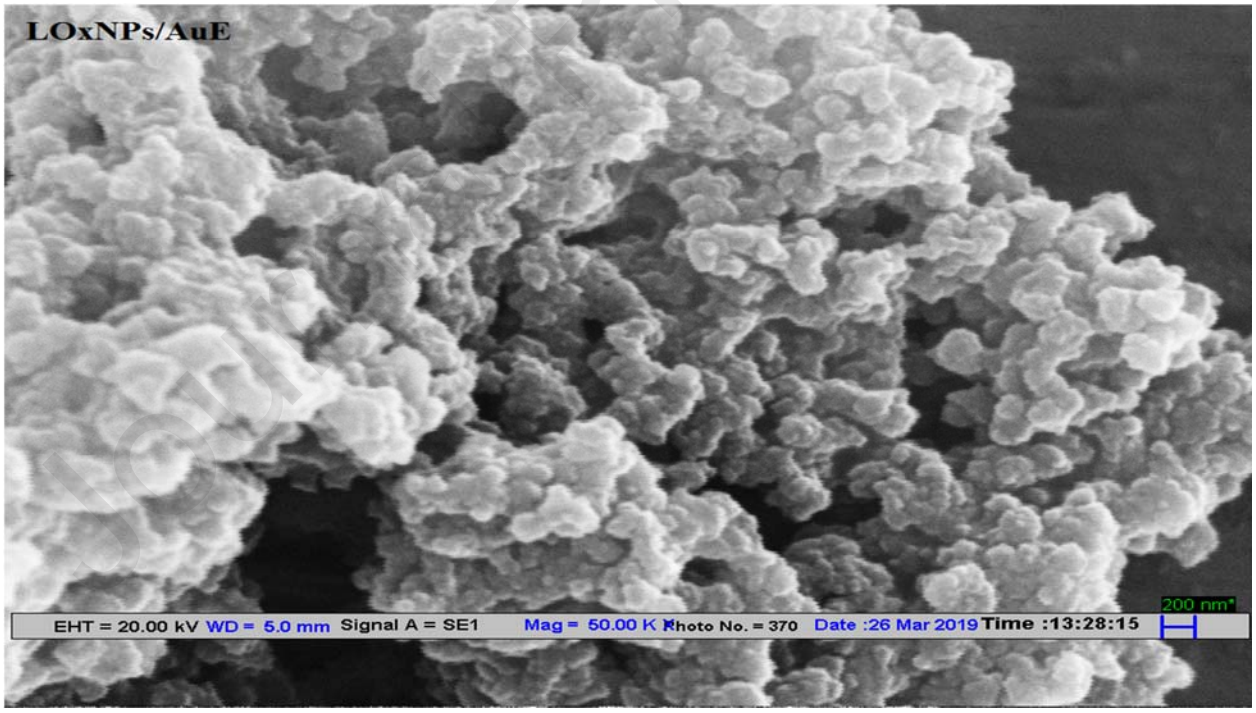


Fig. 3d (II)

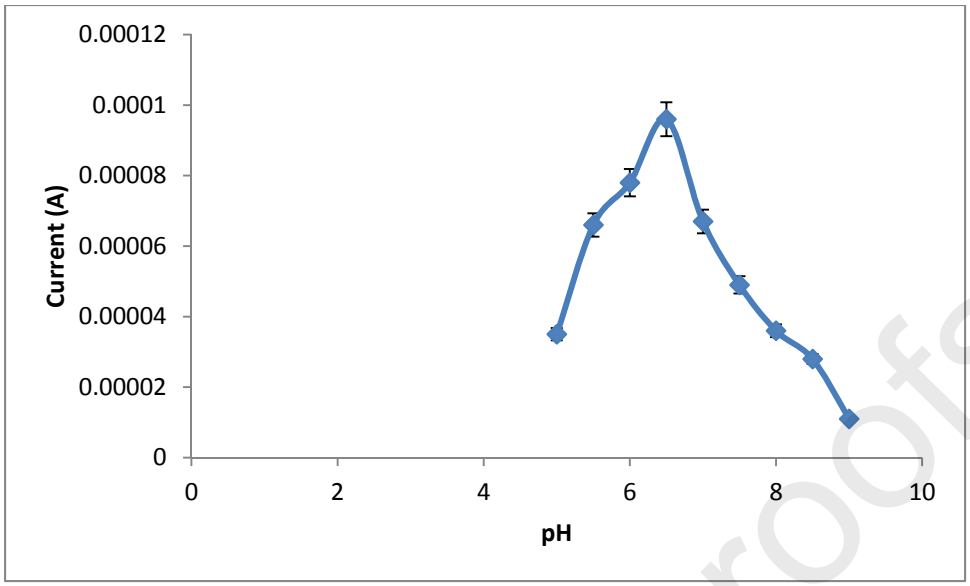


Fig.4 (a)

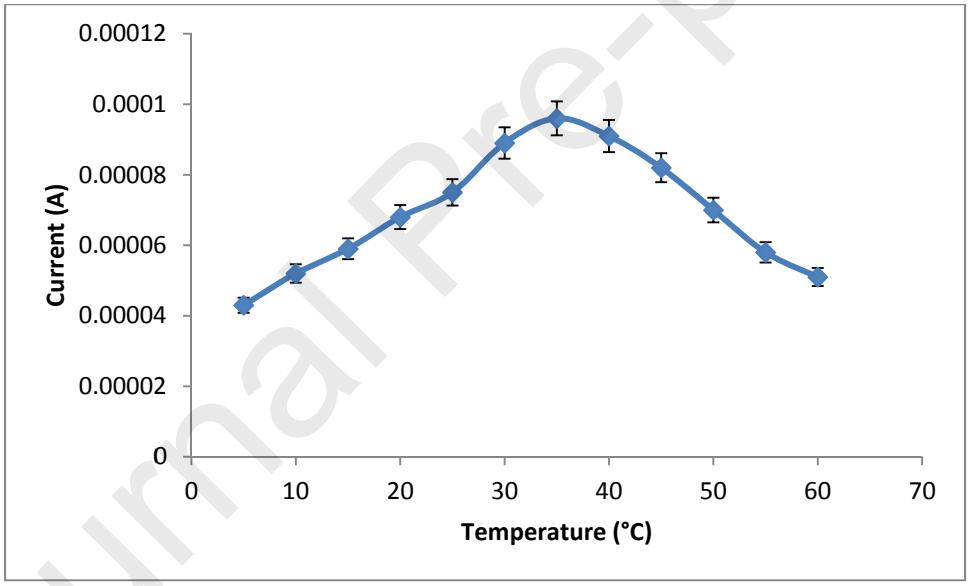


Fig.4 (b)

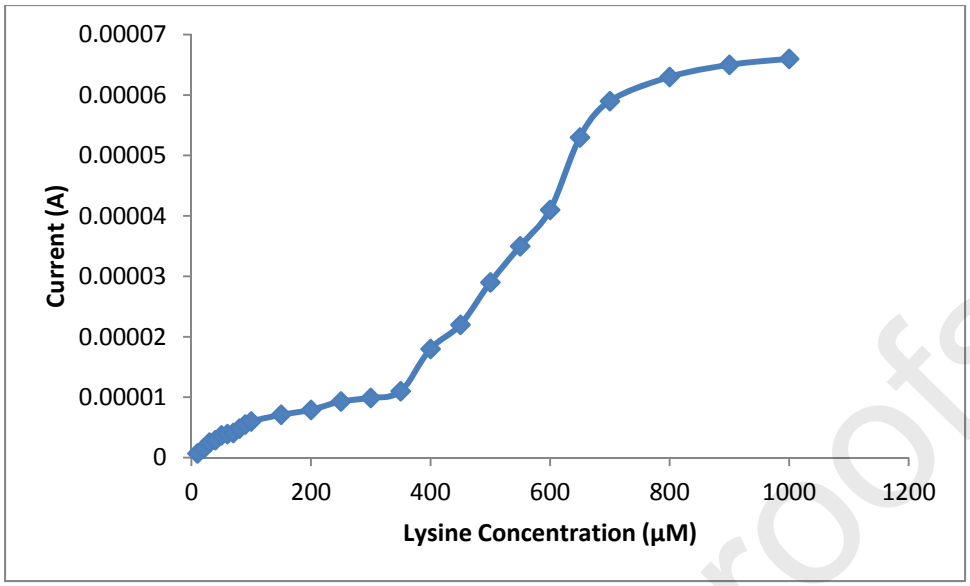


Fig.4 (c)

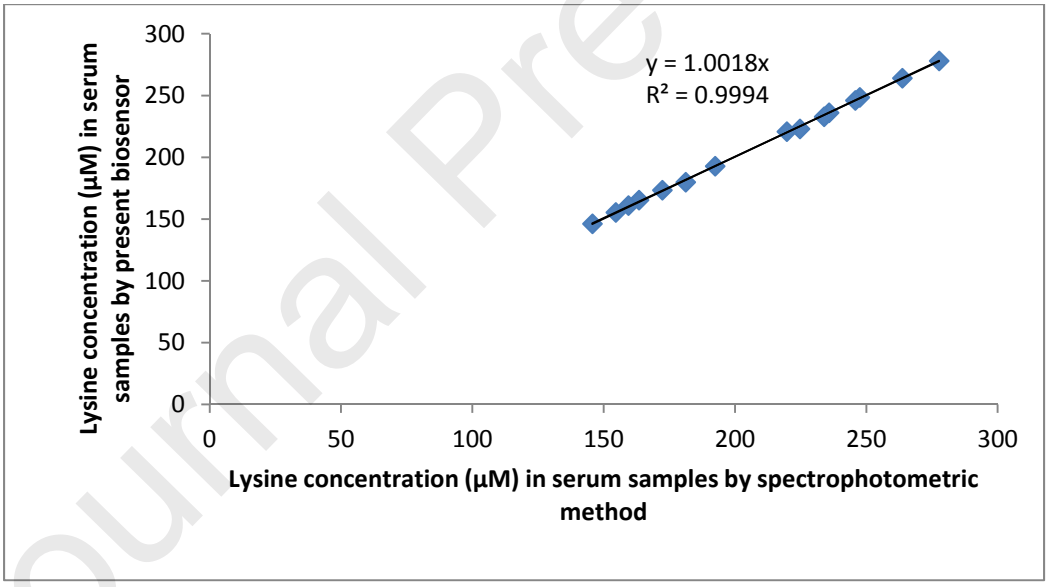


Fig. 4(d)

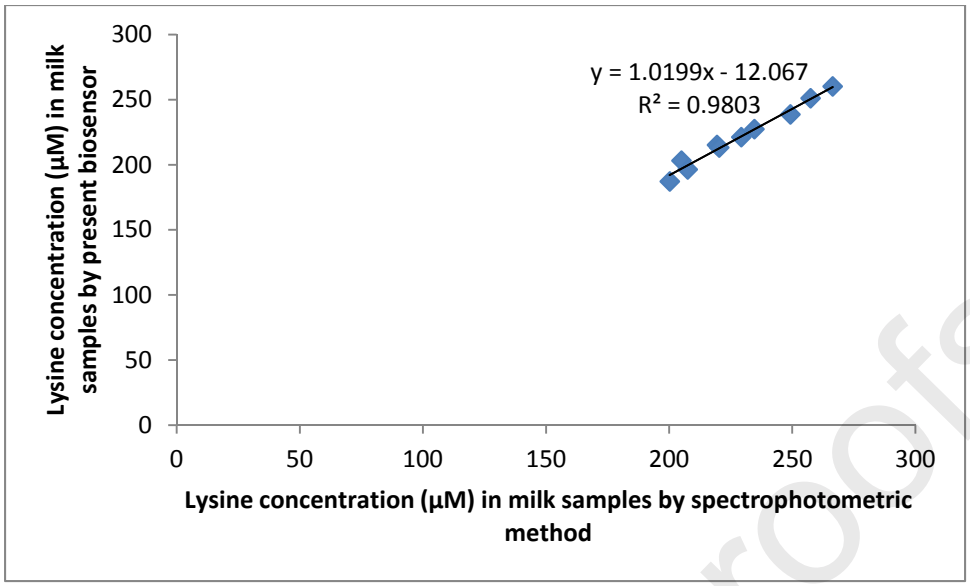
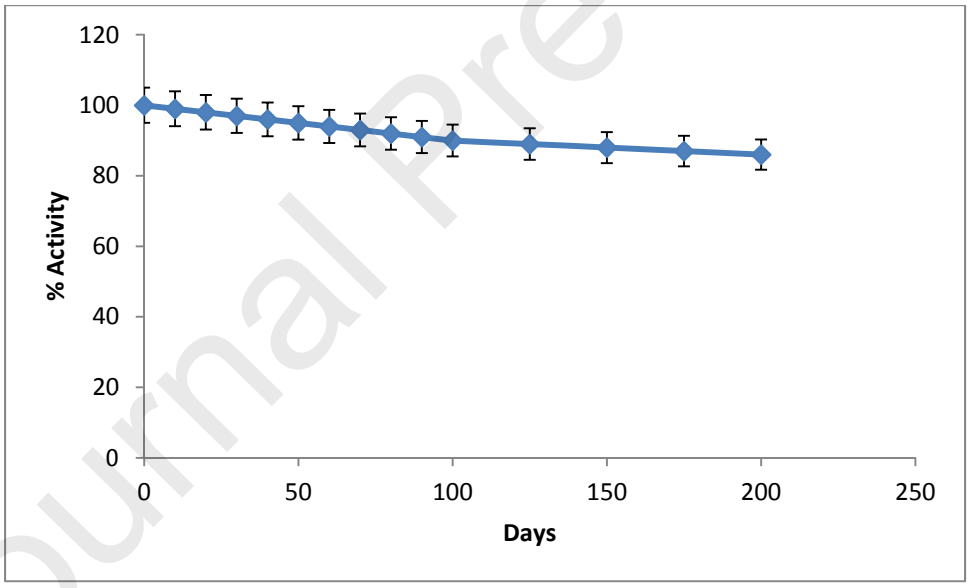


Fig.4 (e)



Supplementary Fig.5

List of Tables

Supplementary Table 1

Lysine added (µM)	Lysine found (µM)	% Recovery
-	195.2	-
10	201.9	98.39±0.2
20	211.4	98.23±0.4

Supplementary Table 2

Lysine (µM)	Mean	CV (%)
Within assay	190.4	0.0751
210		
197		
191		
181		
173		
Between assay	187.2	0.0637
201		
196		
187		
181		
171		

Supplementary Table 3

Sex	Age (Year)	Apparently healthy persons (µM)	Sex	Age (Year)	Cancer patients (µM)
M	80	215.1±0.73	F	60	114.6±0.53
F	32	217.7±0.47	M	62	106.4±0.85
M	31	246.7±0.46	M	70	119.3±0.62
M	40	235.6±0.82	M	65	84.3±0.65
F	28	220.7±0.93	M	75	127.9±0.37
F	60	214.8±0.86	M	50	99.5±0.61
F	29	232±1.02	F	38	90.5±0.77
F	70	266.4±0.57	F	41	140.1±0.86
M	42	243.4±0.60	F	63	118.7±0.69
M	47	251.7±0.77	F	58	107.5±0.53
F	26	201.6±0.57	M	74	97.5±0.49
M	43	209.5±0.72	F	44	102.4±0.41
M	37	203.3±0.98	F	38	87.3±0.42
F	39	235.4±0.95	F	29	138.4±0.73
F	44	249.6±1.75	M	45	125.8±0.77
M	62	215.5±0.45	M	38	135.5±0.49
F	40	246.8±0.58	F	33	145.6±0.54
M	37	219.4±0.76	M	39	130.3±0.67
M	48	212.5±0.88	M	47	112.5±0.49
F	35	243.7±0.65	F	40	80.4±0.89

Supplementary Table 4

Milk Samples	Lysine concentration (µM)
Raw milk	257.3±1.25
Sterilized milk	234.6±1.7
Double toned sterilized milk	205±1.63

Supplementary Table 5

Tablet	Lysine concentration (mg/tablet) (reported value)	Lysine concentration by LOxNPs/Au electrode (mg/tablet)
Populous Multi Vitamin Capsules	50	49.4±0.33

Table 6

S. No.	Working electrode	Optimum pH	Temp (°C)	LOD (μM)	Linear range (μM)	Response time (sec)	Potential (V)	Storage stability (days)	Reference
1	(a) AuNPs/MWCNT/PANI/Au electrode (b) AuNPs/MWCNT/DAB/Au electrode	7.0 7.0	25 30	5 20	5-600 20-600	2 4	1.5 1.2	120 90	[1]
2	Diamond paste	NR	NR	0.000004	0.001-0.1	NR	0.65	NR	[9]
3	Yttria/Titania/CNT	7.0	NR	5000	5000-25000	NR	NR	NR	[21]
4	3-APTES/AuNPs-PtNPs/Au electrode	7.5	30	1	1-600	4	0.4	120	[11]
5	Overoxidized polypyrrole bilayer	7.5	NR	4	20-2000	<6	NR	40	[10]
6	(a) $LOx^{-}PVF^{+}$ (b) $LOx^{-}PVF^{+}$/Pt	7.4 7.4	40 50	500 650	500-1300 650-3000	<30	0.6	22 30	[12]
7	MWCNT/TiO_2NPs/GC electrode	NR	NR	4	5-110	NR	NR	NR	[28]
8	Overoxidized polypyrrole/Pt electrode	5.0	NR	4	20-2000	NR	0.7	NR	[13]
9	Graphite electrode/Os polymer	7.0	NR	5	5-500	NR	-0.05	NR	[22]
10	Immunodyne ABC nylon membrane	7.6	NR	10	10-250	5	NR	NR	[23]
11	(a) PVF/MWCNTs-GEL (b) PVF/MWCNTs-GEL/GR	10.0	NR	0.18 0.0092	0.99-700 0.99-700	<5	NR	NR	[24]
12	c-MWCNTs/GR/SnO_2/Chitosan	10.0	NR	0.15	0.99-160	<25	0.7	NR	[25]
13	Graphene/Poly(vinylferrocene) composite	10.0	RT	0.23	0.99-310	<5	0.7	30	[26]
14	Overoxidized polypyrrole/Pt electrode	5.0	NR	2	2-4000	NR	NR	NR	[27]
15	LOxNPs/Au electrode	6.5	35	10	10-800	3.5	0.8	200	Present work

Abbreviations: NR: Not Reported, AuNPs: Gold nanoparticles, AuE: Gold electrode, LOxNPs: Lysine oxidase nanoparticles, Pt: Platinum, cMWCNT: carboxylated multi walled carbon nanotube, DAB: poly-1,2-diaminobenzene, PANI:Polyaniline, PVF: Poly(vinylferrocene), GEL: Gelatin, GR: Graphene

Highlights:

- Synthesized and characterized lysine oxidase nanoparticles (LOxNPs).
- Fabricated an amperometric lysine biosensor based on LOxNPs/Au electrode.
- Response time and LOD of biosensor was 3.5 s and 10μM respectively.
- Biosensor was applied for determination of lysine in serum samples, milk samples and pharmaceutical tablet.
- Working electrode lost 14% of original activity after 200 days stored at 4°C.