International Journal of Biological Macromolecules 186 (2021) 445–461

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Review

A comprehensive review of methods for determination of L-lysine with detailed description of biosensors

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ARTICLE INFO

Keywords:
L-lysine
L-lysine biosensor
Amperometric

ABSTRACT

L-lysine being one of the essential amino acids is not produced by the body, but is obtained through diet. L-lysine determination is important in the food and pharmaceutical industries as well as have medical and diagnostic applications. The normal L-lysine levels in a healthy human serum sample is 150 to 250 μmol/l. There is imbalance in L-lysine levels in certain diseased conditions. So, it could be a biomarker for diagnosis. Various basic methods are available for the determination of L-lysine such as colorimetric, radioisotope dilution, chromatographic, fluorometric and voltammetric methods. These methods have certain disadvantages like sample pretreatment, costly, time consuming and requirement of skilled personnel. These drawbacks are overcome by the use of biosensors due to their high sensitivity, stability and specificity. The present review article discusses about the principles, merits and demerits of the various analytic methods for determination of L-lysine with special emphasis on biosensors. L-lysine biosensors work ideally under the optimum pH 5 to 10, potential range −0.05 to 1.5 V, temperature 25 to 40 °C, with linear range 0.01 to 5500 μM, detection limit 0.000004 to 650 μM and response time 2 to 300 s. The sensor had storage stability between 14 and 200 days.

1. Introduction

L-lysine (2, 6-diaminohexanoic acid) is one of the essential amino acids that is not synthesized by the body, hence obtained through the diet. It is used as a supplement in human nutrition and considered as an index of nutritional quality of foodstuff and pharmaceutical formulations [1]. As compared to animal proteins, plant proteins have lower levels of L-lysine, which makes it a limiting amino acid in various proteins [2]. It is commonly used in feed supplement for poultry, swine and other livestock in order to accomplish more quick and greater weight gain and thus enhanced productivity [3,2]. Athletes administer L-lysine orally or intravenously to increase the release of growth hormones that subsequently leads to gain of muscle mass [4]. It helps the body in calcium absorption and renal conservation and increases the cross-linking process of bone collagen [5]. L-lysine acts as a precursor for synthesis of carnitine, which is required in the transport of long chain fatty acids to mitochondria for energy production and metabolic functions [6]. It is also the precursor for the biosynthesis of neurotransmitters, acetylcholine and glutamate and thus, gamma amino butyric acid (GABA) in central nervous system [7,8]. Low L-lysine levels lead to mental and physical retardation [9].

People, who don't get enough L-lysine from diet, might develop certain signs and symptoms of L-lysine deficiency, which can lead to additional problems, if not treated appropriately e.g., appetite loss or poor growth, fatigue or mood changes, nausea, dizziness, agitation, slow growth, anemia, hair loss and disorders of reproductive system. Thus, the L-lysine content of foods can be used as an index of nutritional quality or the evaluation of food processing techniques [10]. The deficiency of lysine-α-ketoglutarate reductase leads to the accumulation of L-lysine causing hyperlysinemia, asymptomatic to several neurological disorders including psychomotor impairment, epilepsia, spasticity and ataxia [11,12]. Blood L-lysine levels are related to anemia [13], ageing progression [14], HIV infections [15] and leukemic cell proliferation/tumor growth [16,17].

Thus, determination of L-lysine has great importance in clinical diagnosis, food and feed industry and fermentation technology. The present review deals with the various methods for determination of L-lysine with special emphasis on biosensing methods (Fig. 1). In conclusion, we have also included the outlook of challenges and future opportunities for L-lysine biosensors.

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https://doi.org/10.1016/j.ijbiomac.2021.07.010
Received 11 April 2021; Received in revised form 30 June 2021; Accepted 1 July 2021
Available online 3 July 2021

2. Conventional methods of L-lysine determination

The importance of L-lysine levels in foodstuffs and body fluids has led to the development of various methods for its quantitative determination [18–22]. A number of analytic methods such as colorimetric method, enzymic colorimetric method [23], enzymic spectrophotometric method, high performance liquid chromatography (HPLC), reverse phase high performance liquid chromatography (RP-HPLC) [24], hydrophilic interaction chromatography (HILIC) [25], spectrophotometric method [26], flow injection techniques combined with spectrophotometry [27], fluorometric method, microbial method, nuclear magnetic resonance spectroscopy (NMR) [28], liquid chromatography [29], gas phase chromatography, reverse phase liquid chromatography [30] and voltammetric method [31] are available for determination of L-lysine. Some of these methods are described below along with their merits and demerits.

2.1. Titrimetric method

A titrimetric method for L-lysine determination was introduced by Barbosa et al., 1992 [32]. Both L-lysine and the basicity of L-lysine were estimated in the low colored proteinaceous samples by the visual titration instead of the potentiometric titration. The proteinaceous sample was prepared by Kjeldahl method using soybean meal and milk powder and their experimental as well as theoretical titration curve was prepared. The method involved the titration of L-lysine samples contrary to brilliant green and solid red as indicator (ad hoc screened indicator).

Advantages: It is a cheap method not needing costly equipment and highly skilled operator.

Disadvantages: It is a non-specific, non-sensitive, time consuming method with no accuracy and has interference by other amino acids.

2.2. Colorimetric method

One of the colorimetric methods involves the use of gold nanorods and 11-mercaptoundecanoic acid (MUA). The combination of gold nanorods with MUA, $Eu^{3+}$and carboxyl groups of MUA with the amino groups of L-lysine leads to the change of color of gold nanorods from red to blue. This is followed by spectrophotometric determination of blue color [22].

Advantages: It is a simple method with no costly equipment.

Disadvantages: It is a non-sensitive, non-specific and time-consuming method.

2.3. Enzymic colorimetric method

This method is based on the catalytic activity of lysine decarboxylase that react with L-lysine to form the product cadaverine and CO_2 is released.

$$\text{L} - \text{lysine} \rightarrow \text{Cadaverine} + CO_2$$

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$

An increase in pH is observed due to cadaverine leading to change in color of bromocresol purple from pale yellow to bright blue, which absorb at 595 nm [33]. In one of the methods, lysine-ε-oxidase isolated from *Marinomonas mediterranea* was used to determine L-lysine concentration in plasma and serum samples. This method involves the use of lysine-ε-oxidase rather than lysine-α-oxidase due to its improved sensitive and selective behavior. Due to higher affinity of lysine-ε-oxidase to the L-lysine, this method proved to be an ideal method for L-lysine determination. The results obtained by this method were comparable to ultra-performance liquid chromatography (UPLC). A linearity was observed in the range 0–0.2 mM [34].

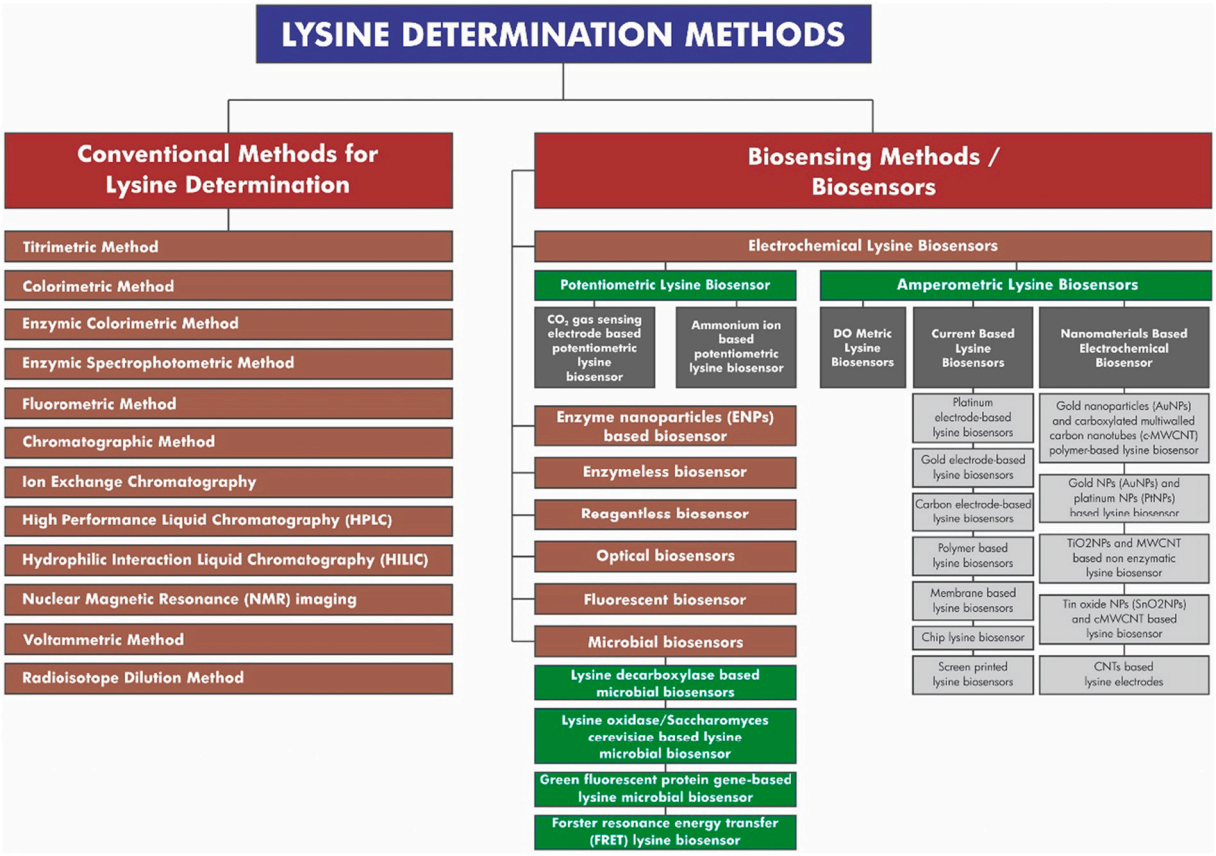

Fig. 1. Lysine determination methods.

Advantages: This method is not costly and involves the use of simple instrument.

Disadvantages: It is comparatively less sensitive method and requires long time for sample preparation.

2.4. Enzymic spectrophotometric method

L-lysine-ε-dehydrogenase catalyzes the conversion of L-lysine and NAD^+ to formazan $+NADH+H^+$ which is determined spectrophotometrically [23].

Advantages: It is an easy method with better accuracy.

Disadvantages: It is time consuming method and requires costly equipment. The external factors may interfere with the results.

2.5. Fluorometric method

A fluorometric assay was proposed by Ferrer et al., 2003 [35] involving the use of ortho-pthaldialdehyde (OPA). This method was used to analyze the content of available L-lysine in ultra-high temperature milk samples and various other milk samples. This method was comparable to the carpenter technique involving the use of 1-fluoro-2,4-dinitrobenzene and showed linearity with correlation coefficient 0.99 and a limit of detection 6.6 μg/ml. One of the methods involves the catalytic conversion of L-lysine with the peroxidase, lysine oxidase and homovanillic acid to form a fluorescence product, which is determined using a fluorimeter [36].

Advantages: It shows higher specificity, better sensitivity and is a fast analysis method.

Disadvantages: The method shows the problems of interference susceptibility, toxicity and suffers from less lifetime of fluorescent compound.

2.6. Chromatographic method

2.6.1. Ion exchange chromatography

An ion exchange column chromatographic method was used to determine amino acids in the protein samples. Anionic exchangers were used to separate various amino acids in a mixture and measure amino acids individually by ninhydrin reaction. These anion exchangers are available commercially in the form of automated amino acid analyzer. The new advanced technique involved the prior elimination of carbohydrates from the samples by the integration of offline sample preparation with the amperometric detection [37].

Advantages: This method has higher specificity for amino acid analysis and the contaminants are discarded rapidly prior to the amino acid's separation.

Disadvantages: The biological samples are hydrolyzed and deproteinized leading to the partial decomposition of some of amino acids.

2.6.2. High performance liquid chromatography (HPLC)

In high performance liquid chromatography, L-lysine from the food samples is separated from other amino acids by hydrolysis followed by derivatization with ophthaldialdehyde and 9-fluorenylmethyl chloroformic acid. These are separated on column (Zorbax Eclipse-AAA) and then detected using diode detector [38].

2.6.3. Hydrophilic interaction chromatography (HILIC)

Hydrophilic interaction chromatography (HILIC) is used for separation of L-lysine followed by fluorescence detection. Isolation of L-lysine is performed using different HILIC columns for isocratic elution. Fluorometric detection was done at excitation wavelength 345 nm and emission wavelength 450 nm. This method is the simpler one and didn't need any pretreatment step [25].

Advantages: It is a fast, automated method giving accurate results with small sample requirement and greater reproducibility.

Disadvantages: It requires trained personnel and expensive equipment.

2.7. Nuclear magnetic resonance (NMR) imaging

L-lysine is determined using triple resonance NMR on the basis of non-exchanged protons of side chain and the changes in the hetero-nuclear electron patterns. Due to deprotonation, changes occur in hetero-nuclear electron distribution leading to the chemical shifts in the side chains ^{15}N for L-lysine and ^{15}N or ^{13}C for arginine. The method was used to determine pKa (site specific) of individual L-lysine residues present in the proteins [28].

Advantages: This method shows better selectivity, accuracy and sensitivity than ^1H-based NMR.

Disadvantages: It requires comparatively higher concentrations of the protein in soluble form.

2.8. Voltammetric method

The voltammetric method involves the application of potential and the measurement of current. L-lysine is determined by adsorption voltammetry on derivatization with sodium hydroxide and acetaldehyde buffer. The height of the peak obtained determines the L-lysine concentration [31].

Advantages: It has better sensitivity and is not time consuming.

Disadvantages: The substance needs to be oxidizable or reducible in the specific range and the sample is required in the dissolved form.

2.9. Radioisotope dilution method

The radioisotopic method involves the addition of isotopic substance to sample. This mixing dilutes isotopic enrichment and is the basis of the method. A L-lysine requirement determination method was proposed by Bergner et al., 1978 [39] involving the 14-C and 15-N labelling of L-lysine. After the administration of 14-C labeled L-lysine into the male wistar rats, the released $CO_2$was measured and 15-N frequency was also determined.

Advantages: This is highly sensitive and specific method.

Disadvantages: It requires use of costly radioactive material, expensive equipment and specific guidelines for use of radioisotopes.

Table 1 outlines the principles and analytical parameters of the traditional methods for quantitative analysis of L-lysine in biological samples.

3. Advanced methods: biosensors

A biosensor is an analytical device combining a biological sensing element, transducer and electronic system to convert response of substance being measured into measurable signal [40]. The biological sensing elements can be micro-organisms, enzymes/ligand, nucleic acids, antibodies/antigen, biological tissues, organelles, etc. The recent definition of a biosensor according to IUPAC recommendations is, “A biosensor is a self-contained integrated device, which is capable of providing specific quantitative and semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is in direct spatial contact with a transducer element” [41]. The biosensors are used widely in diagnostic, medical, quality control, agriculture industry, veterinary, dairy sciences, bacterial and viral diagnostics, drug production, control of industrial waste water, mining, military and defense [42]. However, this technique requires expensive equipment and trained person to operate. During the last decade, great attention has been given to various biosensors for determination of L-lysine in different biological samples. These L-lysine biosensors can be classified into many classes such as electrochemical biosensors, optical biosensors, microbial biosensors, screen printed sensors, enzymeless sensors, reagentless sensors and nanomaterials based electrochemical biosensors as given below:

Table 1
A comparison of various traditional methods for determination of L-lysine.

S. no.	Methods	Principle/based on	Limit of detection (mM)	Working range (mM)	Samples	Reference
1	Titrimetric	Detection of L-lysine based on titration of samples against the indicators brilliant green and solid red	NR	NR	Soyabean meal and milk samples	[32]
2	Colorimetric	Detection of color change of gold nanorods from red to blue due to their reaction with L-lysine, 11-mercaptoundecanoic acid and Eu^{3+}	$1.6*10^{-3}$	$5*10^{-3}$-1	Food samples (cookies, milk and bread)	[22]
3	Enzymic colorimetric	Detection of hydrogen peroxide produced due to the reaction of L-lysine with lysine-ε-oxidase.	NR	0–0.02	Human plasma	[34]
4	Enzymic spectrophotometric	Detection of NADH or formazan produced due to the reaction of L-lysine with lysine-ε-dehydrogenase	NR	$5–10^{-4}$	Rabbit serum	[23]
5	Fluorometric	Reaction between L-lysine and ortho-phthaldialdehyde	0.00004	NR	Milk samples	[35]
6	Chromatographic	Detection of L-lysine using reverse phase C18 column and diode array detector	NR	0.03–0.325	Rice samples	[100]
7	Voltammetric	Detection of L-lysine based on derivatized product formed with acetaldehyde	$4*10^{-4}$	$6*10^{-4}$-$1*10^{-2}$	Nutrition samples	[31]

3.1. Electrochemical L-lysine biosensors

Electrochemical biosensors have been considered as the most sensitive device for monitoring L-lysine levels in biological materials. A number of methods were designed to amplify the electronic signals so as to detect the trace amount of analyte. Predominantly, electrochemical sensors are of three types: potentiometric, amperometric and conductometric. The principles of various types of electrochemical biosensors are shown in Fig. 2. Various enzyme-based L-lysine biosensors have been discussed in the present review. The enzymes used in L-lysine biosensors are lysine decarboxylase, lysine dehydrogenase and lysine oxidase. Lysine decarboxylase catalyzes the decarboxylation of L-lysine, resulting in the liberation of carbon dioxide, which in turn is detected by carbon dioxide specific electrode. Lysine dehydrogenase requires NAD^+ as cofactor along with redox mediators, in which NAD^+ is reduced into $NADH^+$, which is sensed amperometrically. The most widely used enzyme for analysis of L-lysine is lysine oxidase. The technique involved in lysine oxidase biosensors is based on the measurement of oxygen with clark type oxygen electrode or the hydrogen peroxide formation.

3.1.1. Potentiometric L-lysine biosensor

Potentiometric biosensors quantify the potential difference between reference electrode and working electrode in an electrochemical cell, when zero or no current flows between them. These are based on ion-selective electrodes and ion-sensitive field effect transistors. The output signal is due to accumulation of ions at the ion selective chitosan interface.

These potentiometric L-lysine biosensors can be classified into following sub- sub classes based on use of lysine oxidase, lysine dehydrogenase, lysine decarboxylase and monooxygenase:

3.1.1.1. CO_2 gas sensing electrode based potentiometric L-lysine biosensor.

The first potentiometric L-lysine biosensor was developed by White and Guilbault, 1978 [43] based on the CO_2 sensing electrode employing a teflon membrane. The biosensor was constructed on the basis of enzymatic decarboxylation of L-lysine by L-lysine decarboxylase:

$$\text{L} - \text{lysine} \rightarrow \text{Cadaverine} + CO_2$$

The CO_2 thus liberated was detected by the electrode. L-lysine decarboxylase was isolated from *Escherichia coli* B. It showed linearity in the concentration range 5×10^{-5} to 10^{-1} M and a response time 5 to 10 min. The biosensor was applied for L-lysine determination in grains, flour and corn meal samples. It did not show response to other amino acids.

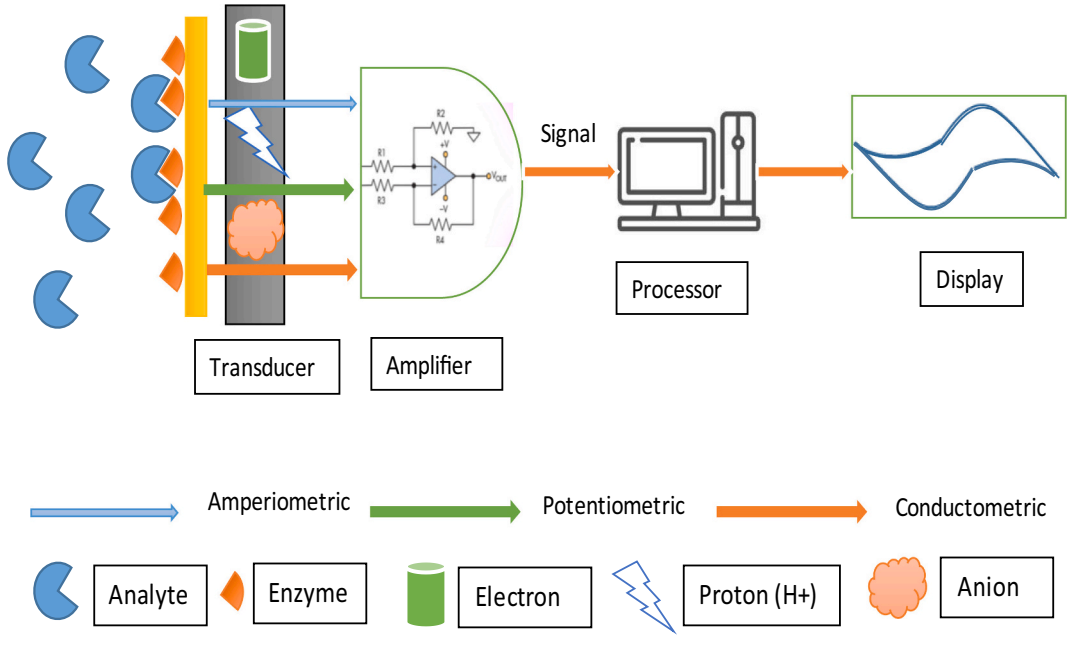

Fig. 2. Schematic representation of principle of an electrochemical biosensor.

One more potentiometric lysine decarboxylase-based L-lysine biosensor (enzyme electrode) was fabricated based on CO_2 electrode [44]. The enzymic solution was sandwiched amidst a dialysis and gas-based plastic membrane. To decrease the measuring time, two different types of measuring principles i.e., quasi steady state and kinetic measurement were used. The measuring cycle reduced by half using these principles. The linearity was observed in the range 5×10^{-4} to 10^{-2} M with response time 3 to 5 min and stability for 3 to 6 weeks. This electrode was used for process control of L-lysine fermentations.

Advantages: The electrode provided a rapid and simple procedure. During sample analysis, removal of the insoluble material was not required. The electrode was highly specific for L-lysine and cheaper than earlier electrodes.

Disadvantages: The long response time was one of the drawbacks of this electrode. The CO_2 in the sample may interfere with L-lysine detection.

3.1.1.2. Ammonium ion based potentiometric L-lysine biosensor. An ammonium electrode based on the detection of ammonium ions was designed by Saurina and the group. The electrode was developed by chemical immobilization of lysine oxidase onto the ammonium electrode. The sensor worked on the basis of detection of ammonia being generated in the following reaction catalyzed by lysine oxidase:

$$L-\text{lysine} + O_2 \rightarrow 2-\text{ketoglutarate} + H_2O_2 + NH_3$$

Chemometric analysis of data was done followed by mathematical treatment of the calibrated data in order to overcome the issue of poor selectivity. The resulting biosensor was used for determination of L-lysine in pharmaceutical samples [45].

In another study, a potentiometric L-lysine biosensor array consisting of 7 different ion selective electrodes sensitive for K^+, Mg^{2+}, NH^{4+}, H^+, Na^+, Li^+ and Ca^{2+} was developed. Due to the low selectivity of the lysine oxidase to other electrodes, the lysine oxidase membrane was immobilized onto the NH^{4+} electrode. Partial least square regression was one of the strategies used for multi-variate calibration to quantify L-lysine in the feed samples [46].

Garcia et al., 2003 [47] reported a L-lysine biosensor combining the flow injection technique with potentiometry. This biosensor was also based on the chemical immobilization of lysine oxidase onto nylon membrane anchored to NH^{4+} electrode. But this newer biosensor was advanced version due to involvement of different strategies including kinetic study, multivariate calibration and differential potentiometry utilizing two electrodes. The use of combination of these methods led to the improved selectivity, sensitivity, accuracy and robustness of the biosensor for quantification of L-lysine in food samples.

Yarar and Karakus (2014) [48] fabricated a potentiometric L-lysine biosensor based on ammonium ion electrode and lysine oxidase. The ammonium ion selective electrode was prepared using polyvinyl chloride, palmitic acid, non-actin and bis(2-ethylhexyl) sebacate in the tetrahydrofuran. The sensor worked under the optimum conditions of pH 7.5 (10 mM Tris buffer), temperature of 25 °C with response time 0.5 to 1 min. It was stable for upto 45 days with good reproducibility and linearity in the concentration range 1×10^{-5} to 1×10^{-1} M. The resulting biosensor was used to determine L-lysine content in pharmaceutical tablets and capsules.

Advantages: The use of mathematical approach led to the avoidance of removal of endogenous ammonium from the samples.

Disadvantages: The applications of the biosensor are limited due to lower selectivity.

A comparison of analytical parameters of potentiometric L-lysine biosensors is summarized in Table 2.

3.1.2. Amperometric L-lysine biosensors

The general principle of the working of an amperometric L-lysine biosensors is formation of hydrogen peroxide due to the reaction of L-

Table 2
A comparison of analytical properties of potentiometric L-lysine biosensors.

S. no.	Support for immobilization	Enzyme	Working electrode	Method of immobilization	Optimum pH	Optimum temperature (°C)	Linear Range (μM)	Detection limit (μM)	Response time (*sec*)	Interference by	Storage stability (days)	Samples used	Reference
1	Teflon membrane	L-Lysine decarboxylase	CO_2 electrode	Crosslinking	~6.0	NR	$50–1*10^5$	~60	300–600 *s*	NR	50	Grains and foodstuffs	[43]
2	Gelatin and polypropylene membrane	L-Lysine-α-oxidase	O_2 electrode	NR	9.0	NR	$2*10^3$	NR	NR	L-Arginine, L-Phenylalanine, L-Ornithine	180	Fermentation broth	[101]
3	Dialysis membrane and gas selective plastic membrane (Teflon and silicon)	L-Lysine decarboxylase	CO_2 electrode	NR	NR	NR	$5*10^2–1*10^4$	NR	180–300 *s*	NR	≥21	NR	[44]
4	Nylon membrane	Lysine oxidase	Ammonium electrode	NR	8.4	NR	$30–1*10^3$	6	15 *s*	Aspartic acid, Glutamic acid	50	NR	[102]
5	Nylon membrane	Lysine oxidase	Ammonium electrode	Covalent binding	NR	NR	30–300	NR	NR	NR	NR	Pharmaceutical samples	[45]
6	Nylon membrane	Lysine oxidase	NH_4+ electrode	NR	NR	NR	NR	NR	NR	NR	NR	Feed samples	[46]
7	NR	Lysine oxidase	NH_4+ electrode	NR	NR	NR	0.1–5	$11*10^{-3}$	NR	NR	NR	Pharmaceutical preparations	[103]
8	Nylon membrane	Lysine oxidase	Ammonium electrode	NR	NR	NR	NR	NR	NR	NR	NR	NR	[47]
9	Ammonium selective poly(vinylichloride) membrane	Lysine oxidase	Ammonium electrode	NR	7.5	NR	10 to 10^5	NR	30–60 *s*	NR	45 days	Pharmaceutical tablets and capsules	[48]

lysine with lysine oxidase. Under the impact of high voltage, H_2O_2 (the product) is broken down into $2H^+$ ions, O_2 and electrons ($2e^-$). The electrons thus released are detected as current. The series of reactions of amperometric L-lysine biosensors is given as under:

$$L-\text{lysine}+O_2+\text{Lysine oxidase}\rightarrow\alpha-\text{keto}-\varepsilon-\text{aminocaproate}+H_2O_2+NH_3$$

$$H_2O_2\rightarrow 2H^++O_2+2e^-(\text{under high voltage})$$

These amperometric L-lysine biosensors can be classified into following sub-sub classes:

3.1.2.1. DO metric L-lysine biosensors. An amperometric flow through analyzer was developed to determine L-lysine based on the immobilization of lysine-2-monooxygenase onto the dissolved oxygen (DO) electrode. There was linearity in the concentration range 5.5-55 mM. The optimum pH was 8.2, the response time of 15.40 s and stability of 2 months. This was used to determine L-lysine in fodder and fermentation broth. The enzyme lysine-2-monooxygenase has the catalytic activity of decarboxylation and oxidation based on the following reaction:

$$L-\text{lysine}+O_2\rightarrow\delta-\text{valeramide}+H_2O+CO_2$$

By determining the concentration of oxygen, the L-lysine concentration was detected [49].

Vrbova and Marek, 1992 [50] revealed a L-lysine biosensor based on Clark type O_2-sensor. The sensor utilized cyclohexylisocyanide and glutaraldehyde for the co-immobilization of lysine oxidase and catalase using the ugi reaction onto the collagen membrane or nylon net. The optimum working conditions were pH 7.5, temperature 45 °C, response time 5 s. The sensor lost 50% of its activity on its continuous usage after 6 months. The linearity was observed in the working concentration range $6.7*10^{-6}$ to $6.7*10^{-4}$ M. It was used to determine L-lysine in the wheat extracts. In another study, lysine oxidase was immobilized amidst cellulose and polypropylene foil utilizing poly-urethane resin. This electrode worked on the basis of Clark electrode and was used for L-lysine determination in fermentation control [51].

A silica gel-based flow injection amperometric L-lysine biosensor was proposed. Lysine oxidase was covalently immobilized onto the silica gel and worked on the principle of O_2clark electrode. A stability of 150 days was observed with a linearity in the concentration range 0.2 to 5.5 mM, assay time ~ 2 min and response time 14 s [52].

Chen and coworkers (1996) [53] have used automated flow injection apparatus consisting of hydrogen peroxide electrode. There was linearity in the range of tens of micromolar to millimolar. The sensor worked well at an optimum temperature 25 °C and was stable for a time duration of 6 months. The sensor was used for the leaching of L-lysine from fish feed rich in L-lysine content.

Another amperometric L-lysine biosensor was based on lysine oxidase, immunodyne-ABC nylon membrane and oxygen electrode. It exhibited a linear range of 10–250 μM, response time 5 s and was highly sensitive, stable and specific. It showed low interference and better repeatability. It was applied to determine L-lysine in dry-ham and sausage [54].

3.1.2.2. Current based L-lysine biosensors

3.1.2.2.1. Platinum electrode-based L-lysine biosensors. The use of platinum as an electrode material has increased over years due to its inert behavior, stability, higher conductance, reactive nature, lower background noise, better catalytic behavior and easy fabrication [55]. Lysine dehydrogenase known to catalyze the conversion of L-lysine to L-α-aminoadipic-δ-semialdehyde in the presence of NAD^+, was immobilized on Pt electrode within gelatin support on a cellulose membrane by the entrapment method. The entrapment technique of enzyme immobilization used in the biosensors involves the trapping of enzyme molecules within the lattice of gelatin (Fig. 3), so that the products and the substrates of the reaction can easily be transported, preserving the enzyme activity without affecting the structure of enzyme. A potential of 0.4 V was applied at the electrode. The liberated NADH was sensed amperometrically by the biosensor. The sensing is accelerated by the presence of redox mediating ferricyanide ion. The main advantage of using dehydrogenase was its high specificity for L-lysine and lack of interference by other L-amino acids, alcohols or carbohydrates. This biosensor was used for L-lysine determination in fermentation broth, mammalian cell culture preparations and biological fluids [56].

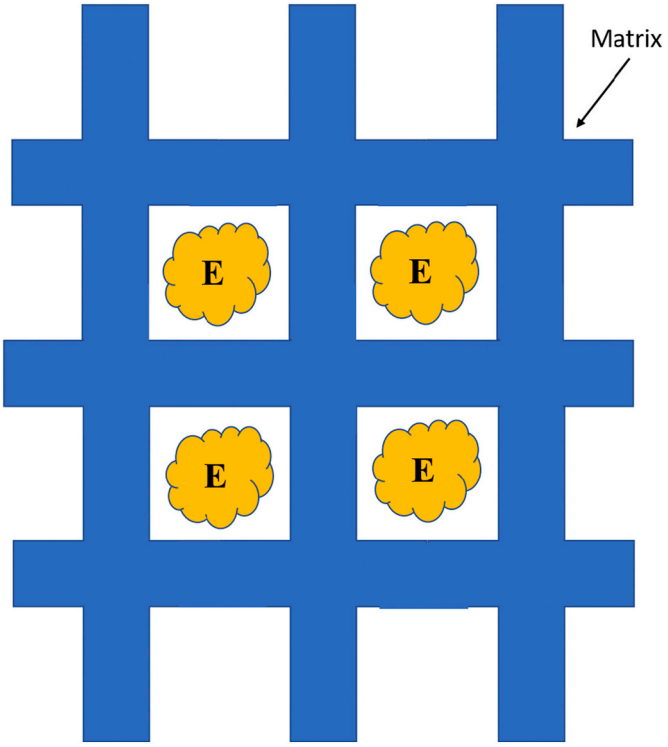

Fig. 3. Schematic representation showing the entrapment of enzyme particles within the matrix. (E: Enzyme particles).

Lavagnini et al., 1993 [57] used a preactivated polymeric support onto a Pt electrode followed by immobilization of lysine oxidase. This electrode was polarized at 0.6 V. The biosensor determined L-lysine in the range 10^{-6} to $2*10^{-3}$ M with response time 2 min, limit of detection 5×10^{-7} M and a stability greater than 3 months. Ornithine and arginine were the interfering molecules. The biosensor measured L-lysine in feed samples.

A L-lysine biosensor was constructed by immobilizing lysine oxidase on the hydrogen peroxide-based Pt electrode. The sensor worked at an optimum pH 7.0, optimum potential 0.6 V with linearity in the concentration range 0.01 to 0.5 mmol l^{-1} and interference was observed from arginine and phenylalanine. It was used to determine L-lysine in the food samples bovine serum albumin, farro sieved meal and farro whole meal [58].

A highly sensitive and stable amperometric L-lysine biosensor was fabricated by Guerrieri et al., in 2007 [59] by immobilizing lysine oxidase onto Pt electrode, polarized at optimum potential 0.7 V. The changes in pH and mass transport were done to study the hydrodynamic behavior of the electrode. The kinetic as well as analytical behavior of sensor were studied. It exhibited a response time <6 s, sensitivity 4.4 μA mM^{-1} cm^{-2}, 1 μM limit of detection with linearity in the concentration range 1 μM to 0.6 M. It was used to determine L-lysine in pharmaceutical samples.

A comparative study of two amperometric L-lysine sensors was carried out by Sahin et al., 2013 [60]. They developed polyvinylferrocenium (PVF^+) and PVF^+/Pt electrodes, followed by further immobilization of lysine oxidase by ion exchange method. The limit of

detection for PVF^+ based electrode was 5×10^{-4} mM and that of PVF^+/Pt electrode was 6.5×10^{-4}mM with optimum pH 7.5 and optimum potential 0.6 V, linearity upto 1.3 mM (PVF^+) and 3 mM (PVF^+/Pt). The sensor showed a response time < 30 s with interference from arginine, ornithine and cysteine. It was used for determination of L-lysine in pharmaceutical samples.

Lysine oxidase was immobilized onto an overoxidized polypyrrole film deposited on a Pt electrode to construct L-lysine biosensor. The electrode was polarized at an optimum potential 0.7 V. The interference study for common interferents was done and the sensor showed interference from phenylalanine, tyrosine and histidine. It showed better sensitivity (41 nA mM^{-1} mm^{-2}), linearity (0.02–2 mM) and limit of detection (4 μM). It was used for L-lysine analysis in cheese and pharmaceutical samples. It showed a stability of 40 days [61]. Ciriello et al., 2015 [62] used the same biosensor to analyze L-lysine content in various cheese samples. Ciriello et al., 2018 [14] also measured L-lysine in untreated human urine using this biosensor. The sensor showed a detection limit of 2 μM with linearity upto 4 mM and sensitivity 0.11 μA mM^{-1} mm^{-2}. Interference was observed from ornithine and arginine. The working potential for platinum-based biosensors prepared for the determination of hydrogen peroxide is remarkable in terms of metabolite interference. It had no interference at 0.4 V. But when the working potentials is above 0.4 V, i.e. at 0.6 or 0.7 V, few amino acids like ornithine, arginine, cysteine, phenylalanine and tyrosine had interference. It indicates Pt electrodes at low potential had no interference by metabolite, while those working at high potential, exhibit interference by certain amino acids.

3.1.2.2.2. Gold electrode-based L-lysine biosensors. Gold electrode have larger cathodic potential range and are inert in nature. They have good conductivity, surface area, stability and are easy to modify. Gold electrodes have limited uses in the positive potential range due to the surface oxidation. A combination of flow injection analysis system and L-lysine biosensor developed by immobilizing lysine oxidase onto gold-poly(*m*-phenylenediamene) electrode embedded in a flow-through cell was reported. The flow through cell was joined to a peristaltic pump using a sample injector. This sensor provided linearity in the range 1×10^{-3}-5×10^{-5} M with better stability and reproducibility. It was used to estimate L-lysine in food samples like wheat flour, pasta etc. [63].

3.1.2.2.3. Carbon electrode-based L-lysine biosensors. Carbon electrodes have good anodic potential range. Glassy carbon (GC) is the most common carbon electrode form. GC have good mechanical properties and better chemical resistance. Carbon paste electrodes are another form of carbon electrode which are formed from fine granules of carbon mixed with oil. They are inexpensive, have wide potential range and lower background noise. Saurina et al., 1999 [45] investigated the use of two electrodes i.e., graphite methacrylate and graphite methacrylate modified with peroxidase enzyme. The former electrode was used to monitor the oxidative detection and the latter was used for reductive detection. The sensor exhibited sensitivity of 11,300 μA/M with linearity in the range 8.2×10^{-7} M to 1.6×10^{-4} M, limit of detection $8.2*10^{-7}$ M, 1.8% repeatability and response time 42 s. These conducting composite based biosensors were utilized to study L-lysine content in pharmaceutical tablets used as dietary supplements.

Another L-lysine biosensor was constructed based on H_2O_2 sensor fabricated by ruthenium and rhodium electrodeposition onto the carbon surface. The sensor thus developed was interference free from ornithine, arginine and phenylalanine with % interferences of 3.4, 1.1 and 0.7% respectively. It showed a lower limit of detection of 2 μM with linearity in the concentration range 2 to 125 μM. The sensor was applied to determine L-lysine in food samples i.e., pasta and milk samples [2].

The assembly of glassy carbon (GC) microspheres, mineral oil and Prussian blue (PB) formed Prussian blue modified glassy carbon paste (PB-GCP) to use it for construction of L-lysine biosensor. PB-GCP shows increased sensitivity, selectivity, stability, electrochemical activity and wide potential range. Ricci et al., 2003a [64] fabricated PB-GCP to detect concentration of glucose, lactate and L-lysine. The optimum working conditions for L-lysine based PB-GCP were 8 (optimum pH), 2.5 μM (detection limit), 2.5–50 μM (linear range), 30 s (response time), 68.9 μA mM^{-1} cm^2 (sensitivity) and 5% reproducibility.

Another L-lysine biosensor was developed by co-immobilization of lysine decarboxylase, diamine oxidase and horseradish peroxidase onto graphite electrode (GE) modified with Os polymer. The biosensor worked well at the optimum pH 7.0, when polarized at −50 mV potential vs Ag/AgCl. The limit of detection was 5 μM with a linear range 0.005 to 0.5 mM. The sensor was utilized to determine L-lysine in feed samples and pharmaceutical samples [65].

Polyvinylferrocene and graphene were deposited onto glassy carbon (GC) electrode and further modified by immobilization of lysine oxidase to construct a L-lysine biosensor. The sensor worked optimally at pH 10. It exhibited better sensitivity of 8.55 μA mM^{-1} cm^{-2} and good repeatability. The response time was short and limit of detection as, 2.3×10^{-7} M with linearity in concentration range 9.9×10^{-7} to 3.1×10^{-4} M. The sensor was applied for L-lysine analysis in cheese as well as pharmaceutical samples [66].

3.1.2.2.4. Polymer based L-lysine biosensors. The polymer-based electrodes have low redox potentials and high energy density which in turn provide a high rate of performance. They are inexpensive and not harmful to the environment. The non-conducting polymers like 1,2-diaminobenzene (DAB) act as better insulators, due to their uniform thickness. They provide resistance to interferents such as acetaminophen, uric acid and ascorbic acid. Curulli and coworkers, 1998 [67] designed a L-lysine biosensor by immobilization of lysine oxidase onto the DAB polymer film by passive adsorption. The sensor showed a response time of 12–16 s at an optimum pH 7.5 with 2×10^{-7} mol l^{-1} detection limit and linearity in the range 1×10^{-5}-1×10^{-3} mol l^{-1}.

3.1.2.2.5. Membrane based L-lysine biosensors. A polyazetidine prepolymer mediated lysine oxidase immobilization onto a nylon membrane was done to obtain partly disposable L-lysine biosensors. The free and bioavailable L-lysine concentration were determined in food material before and after thermal treatment. The optimum working conditions for the biosensor were 37 °C temperature, 5.0 pH (0.1 M citrate buffer), a response time of 1 min, linearity in the range 1 to 33 ppm with limit of detection 0.5 ppm and limit of quantitation 1 ppm with 1.8% repeatability [68].

An improved amperometric L-lysine biosensor for enantiometric analysis was fabricated by immobilizing lysine oxidase by physical adsorption onto the diamond paste. It showed a lower detection limit 4 pmol l^{-1} with linearity in the concentration range 1 to 100 nmol l^{-1}. It was used to determine L-lysine in pharmaceutical and serum samples and was highly selective and sensitive. The use of the diamond paste decreased the noise and thus increased signal to noise ratio. It is best utilized for in vivo studies to determine L-lysine [69].

A silicon-gold strip electrode was electropolymerized with *o*-phenylenediamine membrane and then immobilized with lysine oxidase. The biosensor exhibited a linear range of 0.1–10 μM. It showed less interference to common interferents. The fabrication process for this sensor was simple and showed a stability of 40 days [70].

3.1.2.2.6. Chip L-lysine biosensor. Chip biosensors using thin film metal electrode (TFME) have been constructed to improve the handling. TFME's construction involves the deposition of metal films using vapour and electron beams onto the oxidized wafers of silicon utilizing the metal masks. This gives a low-cost biosensing device. A study of amperometric chip biosensors was reported by Hintsche et al., 1991 [71]. The surface of the electrodes was TFME consisting of nanometer to micrometer scale chips of Pt, Au, Ti, Ag, silicon/silica. The working range for the L-lysine biosensor was 0.005 to 0.040 mmol l^{-1} with a sensitivity of 24 mA/mmol and lifetime >3 days.

3.1.2.2.7. Screen printed L-lysine biosensors. Screen printed biosensors involve the layer-by-layer accumulation of the ink onto a solid support by using a screen/mesh that actually defines the structure of the biosensor. Silver and carbon ink pastes are generally used for the purpose of printing. The constituent of different inks utilized for the

printing of electrodes decides the sensitivity as well as selectivity of the sensor. The constituents of the carbon ink i.e., graphite particles, polymer binding agent and other additives are used as dispersive, printable and adhesive agents. Due to its cheaper, easily modifiable and chemically inert nature, carbon paste is the most preferred material in screen printed biosensors. Some other materials like Pt and Au were also used but lesser than the carbon due to their high cost.

Screen printed lysine oxidase-based biosensors were developed using a platinum working electrode, an Ag/AgCl pseudo reference and a carbon counter electrode. The sensitivity of the sensor was 1.72 nA/μM ± 0.27 nA/μM with a linear range from 10 μM to 250 μM L-lysine and correlation coefficient greater than 0.999. The stability of the sensors was at least for 6 months at 4 °C and for 2 months at room temperature without notable decrease of sensitivity. The sensors were used for determination of L-lysine in samples of fermentation broth. The sensor showed a stable response to L-lysine and observed to be free from interference from components like glucose, sucrose, maltose, trehalose, malic acid, lactic acid, glycerol, acetic acid, α-ketoglutaric acid and antifoam additive [72].

Another screen-printed L-lysine biosensor was constructed with combination of glucose biosensor based on hydrogen peroxide detection. Commercially available carbon ink was used as the crucial element of screen-printed electrode along with 10% PB altered GC particles. The enzymes i.e., glucose oxidase and lysine oxidase were immobilized onto the PB modified screen-printed electrode using glutaraldehyde, Nafion and bovine serum albumin. The L-lysine biosensor showed a detection limit of 5×10^{-3}mmol l^{-1} and achieved a linear range of 6×10^{-3} to 0.7 mmol l^{-1}. The biosensor exhibited a sensitivity of 52 mA (mol l^{-1} cm^2). There was improved stability due to the use of Prussian blue modified glassy carbon particles [73].

Advantages: These biosensors showed improved performance, lower cost, better reproducibility and provided miniaturized version for point-of-care detection.

Disadvantages: The organic solvents found in the buffers or mobile phase may lead to the dissolution of inks which decreases the electron transfer rate and thus the sensitivity and detection limit of the biosensor.

3.1.2.3. Nanomaterials based electrochemical biosensor. Nanoparticles have gained utmost significance due to their unique physical and chemical properties such as higher surface area, lower melting point, specific magnetic property, the mechanical strength and specific optical properties. Due to their size in nanometers i.e., diameter in the range of 1–100 nm, these have been proved to be a newer range of structures to develop the biosensors for disease diagnosis. Nanomaterials are utilized as support for the direct immobilization of the enzymes onto the electrode surface to enhance the electrochemical effect as well as for the signal amplification [74]. The unique properties of nanoparticles make them suitable candidates for the construction of biosensors with improved specificity and sensitivity. Such biosensors, called nanobiosensors have more pros than the conventional biosensors. Different kinds of nanomaterials have been used in the fabrication of nanobiosensors i.e., iron-oxide based nanoparticles, silver nanoparticles, gold nanoparticles, single and multi-walled carbon nanotubes, quantum dots, magnetic nanoparticles etc. A number of L-lysine biosensors have been fabricated using nanomaterials including gold nanoparticles, platinum nanoparticles, tin oxide nanoparticles and carbon nanotubes. There is a brief discussion about these nanomaterials-based biosensors in the below mentioned sections.

3.1.2.3.1. Gold nanoparticles (AuNPs) and carboxylated multiwalled carbon nanotubes (c-MWCNT) polymer-based L-lysine biosensor. Carbon nanotubes (CNTs) due to their increased surface area, good structural, electrical and mechanical characteristics, biocompatible nature, easy synthesis and ability to renew their surface have gained attention for the construction of biosensors. On the other hand, the AuNPs are used to enhance the electron transfer rate and increase the wiring of biomolecules onto the electrode surface. Polyaniline (PANI) has been was used as a conducting polymer because of its simplistic synthesis, higher conductance and stability in the environment. Addition of the CNTs to a conducting polymer like PANI reduces the mass transfer impedance and the charge transfer resistance. Chauhan et al., 2012 [1] used two conducting polymers along with other nanomaterials to construct L-lysine biosensors and compared their different parameters. Lysine oxidase was immobilized onto the AuNPs and c-MWCNT nanocomposite modified Au electrode with polymers, PANI and poly-1,2-diaminobenzene (DAB). The optimum current response for the PANI based modified Au electrode was noticed within 2 s under optimum pH 7.0, temperature 25 °C, while the DAB based electrodes gave optimum response within 4 s under optimum of pH 7.0, and incubation temperature of 30 °C. It showed a lower detection limit of 5 μM for PANI-based electrode with linearity in the concentration range 5–600 μM and detection limit of 20 μM for DAB based electrode with a linearity 20–600 μM. Overall, the PANI based electrode showed better results than the DAB based Au electrode. So, it was used for detection of L-lysine in serum samples, milk and pharmaceutical tablets. It also showed a better half-life (i.e., 120 days) than the DAB based electrode (i.e., 90 days), while being stored at 4 °C (Fig. 4).

3.1.2.3.2. Gold NPs (AuNPs) and platinum NPs (PtNPs) based L-lysine biosensor. In addition to higher conductivity, AuNPs also provides a higher surface area and have a biocompatible exterior to immobilize the biological molecules. PtNPs have extraordinary electrochemical and catalytic properties and enhance the ability for redox reactions. In a study by Chauhan et al., 2013 [75], a combination of AuNPs and PtNPs was electrodeposited on the surface of an Au electrode utilizing the cross-linking chemistry of glutaraldehyde and 3-aminopropyltriethoxysilane (3-APTES). This modified enzymatic biosensor detected L-lysine levels as low as 1 μM within 4 s at optimum pH 7.5, temperature 30 °C, when polarized at 0.2 V vs Ag/AgCl in the sodium phosphate buffer. The working linear range for this biosensor was 1–600 μM and was used for detection of L-lysine levels in various samples. This biosensor lost 50% of its original activity over a time duration of 120 days, while being stored dry at 4 °C (Fig. 5).

3.1.2.3.3. TiO_2NPs and MWCNT based non enzymatic L-lysine biosensor. Gholivand et al., 2014 [76] fabricated a non-enzymatic sensor for L-lysine determination by immobilizing MWCNT and TiO_2NPs onto GC electrode. The sensor was used for detection of L-lysine in commercial syrup and urine samples. The method was simple not including any enzyme special electron special mediator or any particular reagent. The biosensor showed a sensitivity of 0.1795 μAμM^{-1} and detection limit 390 nM. A linear electrocatalytic response was observed in the concentration range 500–5500 nM. This sensor showed better catalytic activity and antifouling ability to L-lysine and its product.

In another study, TiO_2NPs and CNTs nanocomposites were developed by hydrothermal method and modified with Yttrium acetate. These Yttria modified TiO_2NPs/CNTs composites were immobilized onto GC electrode. The sensor exhibited good sensitivity, recovery and stability for the detection of L-lysine within the concentrations 5–25 mM [77].

3.1.2.3.4. Tin oxide NPs (SnO_2NPs) and cMWCNT based L-lysine biosensor. Recently, metal oxide nanoparticles (MONPs) have gained attention due to their excellent surface area to volume ratio, better electroconductivity, mechanical activity, biocompatibility and stability. Out of the various MONPs, SnO_2NPs are different due to their unique photoelectronic characteristics, gaseous sensitivity as well as better conductivity than the other MONPs [78]. Various biosensors have been constructed recently using SnO_2NPs [79,80].

Kacar et al., 2017b [81] reported the use of SnO_2NPs, cMWCNTs, graphene and sol to form a nanocomposite for the construction of L-lysine biosensor. The nanocomposite was immobilized onto the GC electrode followed by the covalent immobilization of lysine oxidase. This modified GC electrode exhibited a linearity in the concentration range 9.9×10^{-7} M to 1.6×10^{-4} M with a lower limit of detection 1.5×10^{-7} M. The biosensor showed higher sensitivity i.e., 55.2 μA mM^{-1}

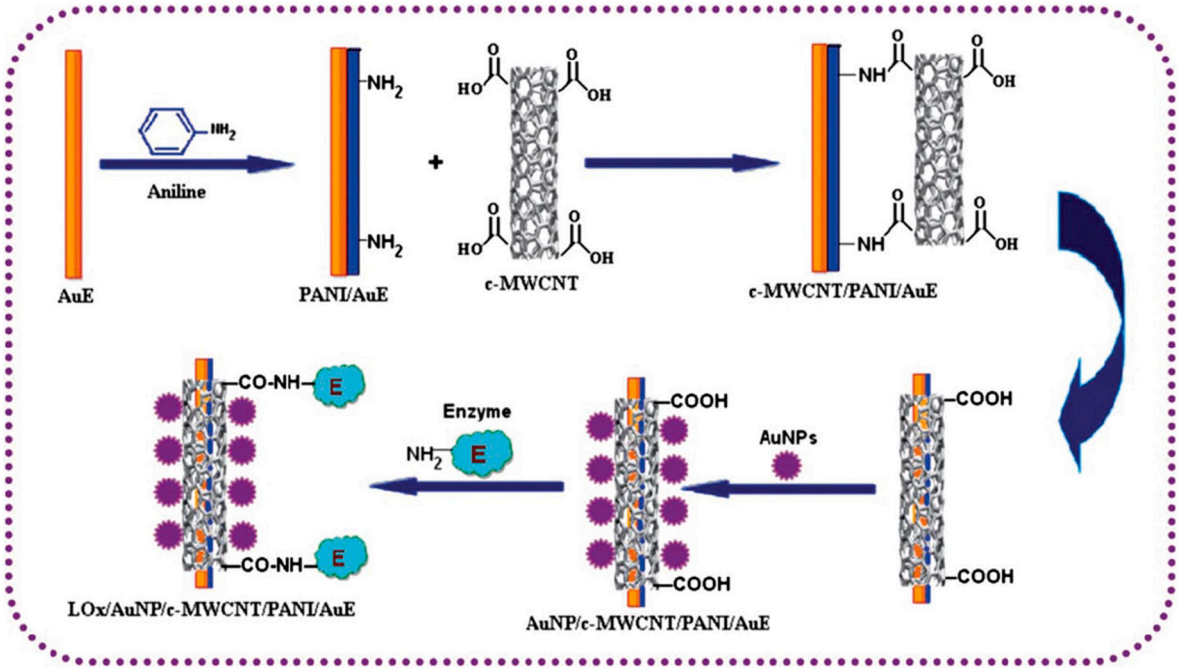

Fig. 4. Schematic representation of the chemical reactions involved in fabrication of LOx/AuNPs/c-MWCNT/PANI/Au electrode. (LOx: Lysine oxidase, AuNPs: Gold nanoparticles, c-MWCNT: Carboxylated multiwalled carbon nanotubes, PANI: Polyaniline, Au: Gold) (Source: N. Chauhan, A. Singh, J. Narang, S. Dahiya, C.S. Pundir, Analyst. 137 (2012) 5113–5122) [1].

cm^{-2} within <25 s when polarized at 0.7 V vs Ag/AgCl electrode. This biosensor was utilized for detection of L-lysine levels in 2 dietary supplements.

3.1.2.3.5. CNTs based L-lysine electrodes. Graphene, CNTs and conducting polymers like polyvinylferrocene (PVF) have excellent properties to enhance sensitivity, conductivity and stability of biosensors when they are used alone. But, the combination of these composites provides better performance of the biosensors.

Kacar et al., 2017c [82] investigated two different electrodes for determination of L-lysine. They constructed 2 enzyme electrodes based on PVF, MWCNTs and gelatin and the other one based on PVF, MWCNTs, gelatin and graphene as the composites immobilized onto the GC electrodes utilizing the glutaraldehyde, bovine serum albumin crosslinking. The electrode based on gelatin alone showed a detection limit 1.8×10^{-7} M with the linear range 9.9×10^{-7} to 7×10^{-4} M and a good sensitivity of 13.51 μA mM^{-1} cm^{-2}. While, the electrode using both the gelatin and graphene had a much lower detection limit of 9.2×10^{-8} M with linearity in the same concentration range as that of gelatine alone and a better sensitivity 17.8 μA mM^{-1} cm^{-2}. They observed that the gelatin graphene composite showed 1.3 times more sensitivity than the MWCNTs part. These electrodes were used for determination of L-lysine in pharmaceutical samples and cheese samples.

Advantages of amperometric biosensors: Amperometric biosensors have better sensitivity, operational stability, short response time and longer storage time. Ammonia interference is not an issue in case of amperometric biosensors.

Disadvantages of amperometric biosensors: Less coupling of biological recognition materials with the transducer alters the stability, selectivity, dynamic range and sensitivity of biosensors.

A comparison of analytical parameters of amperometric L-lysine biosensors is summarized in Table 3.

3.2. Enzyme nanoparticles (ENPs) based biosensor

In L-lysine biosensors, the enzyme immobilization was performed on to distinct matrices, for the determination of L-lysine in various samples. However, direct enzyme immobilization of enzyme on different matrices can lead to their degradation, in turn causing loss of enzymic strength and activity. The above concern was overcome by using enzyme nanoparticles (ENPs) rather than indigenous enzyme molecules. ENPs are synthesized as a result of agglomeration of the enzyme molecules in the nanometer range exhibiting remarkable physio-chemical attributes. Due to their exceptional mechanical, catalytic, optical, thermal, electronic and electrical properties along with increased surface area, the ENPs have displayed remarkable characteristics which in turn increases the electrodes performance. So, due to the benefits of ENPs, they have been used in place of native enzymes for the fabrication of amperometric biosensors. This upgraded the analytic properties of biosensors and also provided a simple process for the fabrication of enzyme electrodes [74]. An improved version of amperometric L-lysine biosensor was developed in our laboratory by immobilizing covalently ENPs of lysine oxidase onto gold electrode (AuE). The ENPs modified AuE displayed good conductivity and low background current. The working electrode showed optimum current at 0.8 V, within 3.5 s, pH 6.5 and temperature 35 °C. The ENPs/Au electrode showed linearity between the substrate concentration and current in the range 10–800 μM and LOD as 10 μM. The analytical recovery of added L-lysine in serum at 10 and 20 μM were 98.39% and 98.23% respectively. Within and between batch coefficients of variations were 0.0751% and 0.0637% respectively. This biosensor was used to measure L-lysine levels in milk samples, pharmaceutical tablets and serum samples of healthy people and cancer patients. A good correlation was found between the L-lysine levels in sera and milk samples (R^2 = 0.999 and R^2 = 0.98 respectively) with the standard spectrophotometric method. The ENPs based working electrodes on the continuous use for a period of 200 days, stored at 4 °C lost only 14% of the initial activity [83] (Fig. 6). Another L-lysine-based biosensor was developed in our laboratory by immobilizing the lysine oxidase nanoparticles (LOxNPs) onto the graphene oxide nanoparticles (GrONPs) embodied onto the pencil graphite (PG) electrode. The altered PG electrode showed better conductivity, enhanced electrochemical characteristics, lower fabrication cost and an easily available disposable electrode. This electrode displayed optimum current response at 0.7 V within 3.95 s at an optimum pH 6.5, temperature 35 °C. The modified PG electrode exhibited lower detection limit i.e., 0.01 μM. Compared to

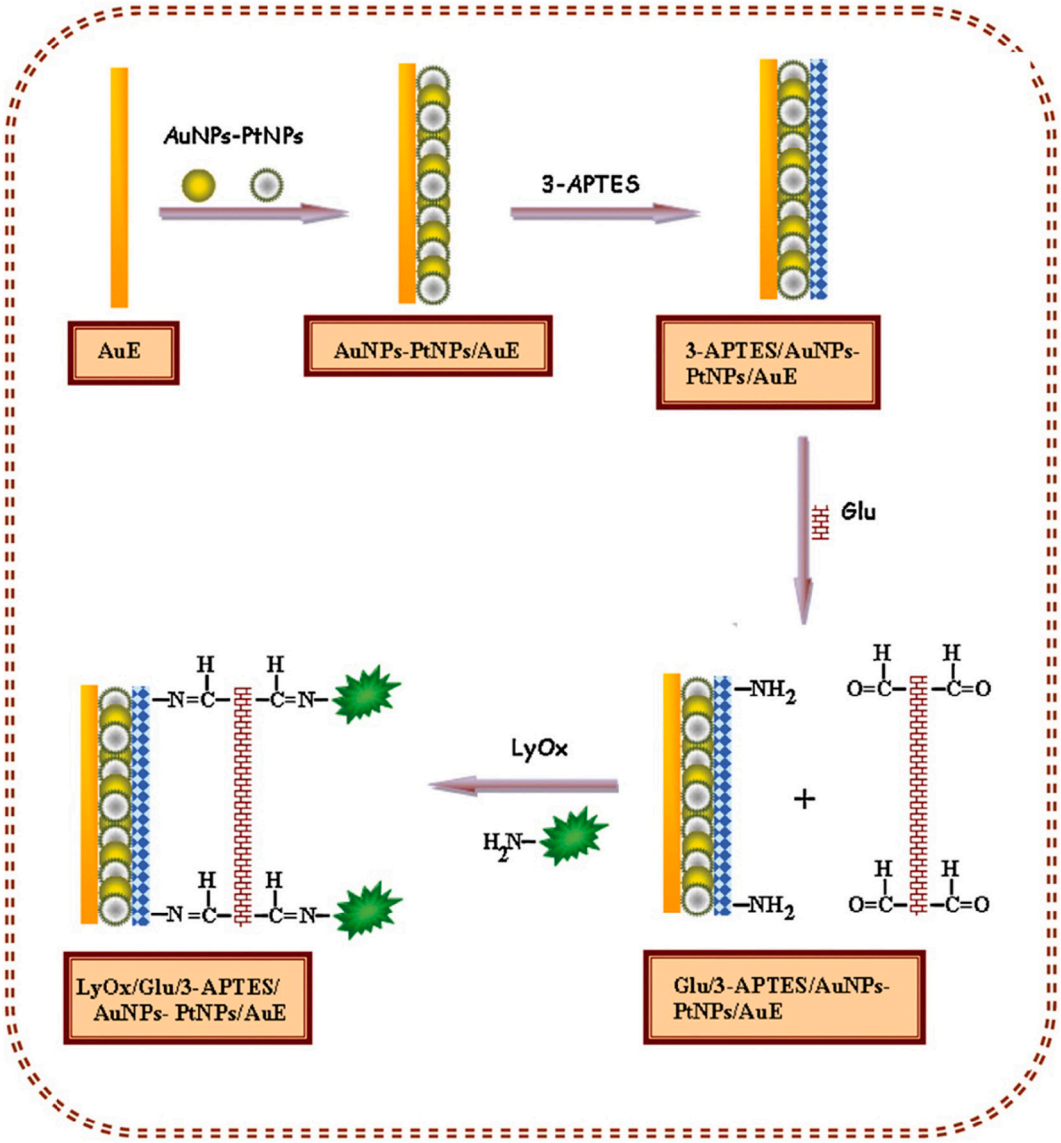

Fig. 5. Schematic representation of steps involved in construction of LOx/AuNPs/PtNPs/AuE. (LOx: Lysine oxidase, AuNPs: Gold nanoparticles, PtNPs: Platinum nanoparticles, AuE: Gold electrode) (Source: N. Chauhan, J. Narang, Sunny, C.S. Pundir, Enzyme and Microbial Technology. 52 (2013) 265–271) [75].

the earlier ENPs based L-lysine sensor, this sensor had a wider linear range i.e., 0.01–1000 μM and an analytical recovery od added L-lysine in the serum samples was 97%. The sensor had a good correlation with standard spectrophotometric method with correlation coefficient of R^2 = 0.989. Within and between coefficients of variation were 0.068 and 0.074%. This improved amperometric biosensor was used for the determination of L-lysine concentrations in the serum samples of healthy persons and HIV infected patients. It lost 35% of its original performance on its continuous usage for a period of 180 days, when stored dry at 4 °C [84] (Fig. 7).

3.3. Enzymeless biosensor

In a study by Bobreshova et al., 2009 [85], a potentiometric L-lysine sensor was developed to improve the sensitivity. They developed MF-4SK altered perfluorinated sulfonic cation-exchange membranes by treating the MF-4SK membranes with ethylene glycol for increasing the biosensor sensitivity. The principle behind the use of these membranes to determine L-lysine is the protolytic reaction. Due to this reaction, the L-lysine ions with single charge from the aqueous solution were shifted to the double-charged ions in the membrane and the protons were almost entirely moved from the membrane. The biosensor showed a response time of about 10–15 min. This sensor was used to determine L-lysine monohydrochloride in mixture of amino acids.

Bobreshova et al., 2010 [86] presented another method to determine L-lysine in the aqueous suspensions consisting of potassium chloride and sodium chloride. They developed a multi-sensor array which consists of a cross-sensitive sensor whose response was based on Donnan potential. This potential was measured at ion-selective electrodes, Ag/AgCl electrodes and ion-exchange polymer interface. A label-free L-lysine chemosensor was developed based on dye (murexide)/metal ion complex. It is a simple, rapid colorimetric assay-based method with better selectivity and sensitivity. The indicator is displaced from the dye/metal ion complex by histidine and the complex formation of L-lysine with coordination sites of the zinc atom of dye-metal ion complex leads to the changes in absorbance. It exhibited a lower limit of detection i.e., 9.4 nmol l^{-1}. This chemo-sensor was used to determine L-lysine and histidine in urine samples [87].

Advantages: Suitable for L-lysine determination in mixed aqueous suspension of neutral amino acids and sodium and potassium chlorides.

Disadvantages: Complex method and have less recognition positions.

Table 3
A comparison of analytical properties of amperometric L-lysine biosensors.

S. no.	Support for immobilization	Enzyme	Working electrode	Method of immobilization	Working potential (V)	Optimum pH	Optimum temperature (°C)	Linear Range (μM)	Detection limit (μM)	Response time (sec)	Sensitivity	Interfering compound	Storage stability (days)	Samples used	Reference
1	Bovine serum albumin matrix/gas permeable membrane/Calcium alginate gel	Lysine decarboxylase	Oxygen electrode	NR	NR	6.0	33 °C	25–400	NR	60–180 s	NR	NR	NR	NR	[89]
2	Silica gel	L-lysine-2-monooxygenase	Oxygen electrode	NR	NR	8.2	25 °C	0.55–55*10^6	NR	15–40 s	NR	Ornithine, phenylalanine and arginine.	90	Fermentation broth and fodder.	[49]
3	Nylon net/collagen membrane	Lysine oxidase and catalase	Clark type oxygen electrode	Crosslinking	NR	7.5	45 °C	6.7–670	NR	5 s	NR	Ornithine, arginine and cysteine.	180	Wheat extracts.	[50]
4	Gelatin/cellulose membrane	Lysine dehydrogenase	Platinum electrode	Entrapment	0.4 V	9.7	NR	7*10^{-2} - 700	7*10^{-2}	30 s		Cysteine	25	NR	[56]
5	Cellulose acetate/ polycarbonate membrane	Lysine oxidase	Platinum electrode	NR	NR	7.0	25 °C	0.5–500	0.5	120 s	NR	Ornithine, arginine	90	Feed samples	[57]
6	Cellulose/polypropylene/ polyurethane	Lysine oxidase	Clark oxygen electrode	NR	NR	7.0	NR	NR	NR	NR	0.5 mol m^{-3}	Aspargine, methionine and ornithine.	NR	NR	[51]
7	Silica gel	L-lysine-α-oxidase	NR	Covalent linking	NR	8.0	25 °C	200–5500	200	14 s	NR	Ornithine, arginine and phenylalanine	150	NR	[52]
8	Immobilon-AV membrane/cellulose membrane/polycarbonate membrane	L-Lysine-α-oxidase	H_2O_2 platinum electrode	NR	NR	7.0	NR	10–500	NR	NR	NR	Arginine and phenylalanine.	NR	Faro meals, BSA.	[58]
9	1,2-diaminobenzene	L-Lysine-α-oxidase	Platinum electrode	Passive adsorption	NR	7.5	NR	10–1000	0.2	12–16 s	NR	Ascorbic acid, acetaminophen and cysteine.	NR	NR	[67]
10	Nylon membrane	Lysine oxidase	(i) Graphite-methacrylate composite electrode	NR	1 V	7.5	40 °C	2.2–120	2.2	38 s	9850 mA/M	Tryptophan, tyrosine and cysteine.	18	Pharmaceutical samples.	[45]
			(ii) Peroxidase-modified graphite-methacrylate biocomposite electrode		−0.15 V	7.5	40 °C	1.7–40	1.7	30 s	440 mA/M				
			(iii) Hydroquinone-mediated peroxidase-graphite-methacrylate biocomposite electrode		−0.1 V	7.5	30 °C	0.82–160	0.82	42 s	11,300 μA/ M		25		
11	Ruthenium/rhodium/1,2-diaminobenzene polymer	L-Lysine-α-oxidase	Glassy carbon electrode	NR	0.1 V	7.0	30 °C	2–125	2	NR	NR	Ornithine, arginine and phenylalanine.	NR	Milk and pasta samples.	[2]
12	Poly(*o*-phenylenediamine) membrane	Lysine oxidase	Silicon-Gold strip electrode	NR	NR	9.0	37 °C	0.1–10	NR	NR	NR	Tyrosine and cysteine.	40	NR	[70]
13	Prussian blue	Lysine oxidase	Glassy carbon paste electrode	NR	NR	8.0	NR	2.5–50	2.5	30 s	68.9 μA mM^{-1} cm^2	NR	NR	NR	[64]
14	Poly(*m*-phenylenediamine)	Lysine oxidase	Gold electrode	Crosslinking	0.65 V	8.0	NR	5–1000	NR	NR	NR		31	Food samples.	[63]

(*continued on next page*)

Table 3 (*continued*)

S. no.	Support for immobilization	Enzyme	Working electrode	Method of immobilization	Working potential (V)	Optimum pH	Optimum temperature (°C)	Linear Range (μM)	Detection limit (μM)	Response time (sec)	Sensitivity	Interfering compound	Storage stability (days)	Samples used	Reference
												Ornithine, arginine and cysteine.			
15	Glutaraldehyde/bovine serum albumin	Lysine oxidase	Platinum electrode	Crosslinking	NR	7.5	NR	1 to $6*10^5$	1	<6 s	4.4 μA mM^{-1}	NR	40	Pharmaceutical samples.	[59]
16	Teflon membrane	Lysine oxidase	Oxygen electrode	Crosslinking	NR	7.5	30 °C	1–10	NR	60 s	NR	NR	14	NR	[10]
17	Nylon and cellulose acetate membrane	L-lysine-α-oxidase	(Platinum) H_2O_2 sensitive electrode	NR	NR	5.0	37 °C	$0.03*10^{-3}$-$1*10^{-3}$		60 s	NR	NR	120	Foodstuff	[68]
18	Diamond paste	L-lysine oxidase	(Silver wire) H_2O_2 sensitive	NR	NR	8.0	NR	$1*10^{-3}$-$1*10^{-1}$	$4*10^{-6}$	NR	NR	NR	NR	Serum samples and pharmaceutical compounds	[69]
19	(i) Polyaniline	Lysine oxidase	Gold electrode	Covalent linking	NR	7.0	25 °C	5.0–600	5.0	2 s	NR	NR	120	Milk, pharmaceutical tablets and sera.	[1]
	(ii) Poly 1,2-diaminobenzene					7.0	30 °C	20–600	20	4 s			90		
20	3-APTES/AuNPs-PtNPs	Lysine oxidase	Gold electrode	Crosslinking	0.2 V	7.5	30 °C	1.0–600	1.0	4 s	0.2 μA/μM	None	120	Sera, milk and amino acid tablet.	[75]
21	(i) Polyvinylferrocenium (PVF^+)	Lysine oxidase	NR	0.6 V	NR	7.4	40 °C	0.5–1300	0.5	<30 s	NR	Arginine, ornithine and cysteine.	22	Pharmaceutical samples	[60]
	(ii) Platinum deposited PVF^+					7.4	50 °C	650–3000	650			Cysteine	30		
22	Overoxidized polypyrrole film	L-Lysine-α-oxidase	Platinum electrode	Crosslinking	NR	5.0	NR	20–2000	4	NR	41 nA/ ($mM*mm^2$)	Phenylalanine, tyrosine and histidine	40	Pharmaceutical and cheese sample	[61]
23	TiO_2 NPs/MWCNT	NR	Glassy carbon electrode	NR	NR	7.0	NR	5–110	0.39	NR	0.1795 $\mu A\mu M^{-1}$	Proline and tryptophan.	NR	NR	[76]
24	Overoxidized polypyrrole film	L-Lysine-α-oxidase	Platinum electrode	Crosslinking	NR	5.0	NR	20–2000	4	NR	NR	NR	NR	Dairy products	[62]
25	Os polymer	Lysine decarboxylase, diamine oxidase and horseradish peroxidase	Graphite electrode	NR	−0.5 V	7.0	NR	5–500	5	NR	NR	Cadaverine and putrescine	NR	Pharmaceutical products and feed samples.	[66]
26	Immunodyne ABC nylon membrane	Lysine oxidase	Oxygen electrode	Crosslinking	NR	7.6	37 °C	10–250	10	5 s	NR	NR	NR	Cured meat samples.	[54]
27	Graphene and redox polymer poly (vinylferrocene)	L-Lysine-α-oxidase	Glassy carbon electrode	Crosslinking	0.7 V	10.0	RT	0.99–310	0.23	<5 s	8.55 μA mM^{-1} cm^{-2}	NR	30	Pharmaceutical and cheese sample	[66]
28	cMWCNT/graphene/ chitosan composite	Lysine oxidase	Glassy carbon electrode	Covalent linking	0.7 V	10	RT	0.99–160	0.15	<25 s	55.20 μA mM^{-1} cm^{-2}	Ornithine, arginine, phenylalanine and proline	30	Dietary supplements 1	[81]
29	(i) PVF/MWCNTs-GEL	Lysine oxidase	Glassy carbon electrode	Crosslinking	0.7 V	10	RT	0.99–700	0.18	<5 s	13.51 μA mM^{-1} cm^{-2}	Ornithine, arginine, phenylalanine	30	Cheese and pharmaceutical samples.	[82]
	(ii) PVF/MWCNTs-GEL/ GR							0.99–700	0.092		17.8 μA mM^{-1} cm^{-2}				
30	Overoxidized polypyrrole film	L-Lysine-α-oxidase	Platinum electrode	Crosslinking	NR	5.0	NR	2–4000	2	<300 s	0.11 μA/ mM mm^2	Ornithine and arginine	40	Human serum	[14]
31	–	Lysine oxidase nanoparticles	Gold electrode	Crosslinking	0.8 V	6.5	35 °C	10–800	10	3.5 s	NR	NR	200	Milk, pharmaceutical	[83]

(*continued on next page*)

Table 3 (*continued*)

S. no.	Support for immobilization	Enzyme	Working electrode	Method of immobilization	Working potential (V)	Optimum pH	Optimum temperature (°C)	Linear Range (μM)	Detection limit (μM)	Response time (sec)	Sensitivity	Interfering compound	Storage stability (days)	Samples used	Reference
32	Graphene oxide nanoparticles	Lysine oxidase nanoparticles	Pencil graphite electrode	Crosslinking	0.7 V	6.5	35 °C	0.01–1000 μM	0.01	3.95 s	NR	NR	180	and serum samples. Serum samples	[84]

Abbreviations: NR: Not reported, TiO2NPs: Titanium oxide nanoparticles, MWCNT: Multiwalled carbon nanotubes, cMWCNT: Carboxylated multi walled carbon nanotubes, PVF: Polyvinylferrocene, GEL: Gelatine, GR: Graphene.

3.4. Reagentless biosensor

A reagentless L-lysine biosensor was constructed by layer-by-layer alternate adsorption of ferrocene labeled high molecular weight coenzyme derivative and L-lysine-6-dehydrogenase on gold electrode interface. L-lysine-6-dehydrogenase was obtained from thermophilic organism *Geobacillus stearothermophilus*. In this biosensor, all the components i.e., enzyme, coenzyme and electron transfer mediator were restricted in the layer-by-layer film. The sensor exhibited sensitivity of 0.02 μA cm^{-2} mM^{-1} and linear current response proportional to L-lysine concentration in the range 1–120 mM. After one-month storage, the sensor response decreased to 30% from the original activity. This method is based on alternating adsorption of positive and negative polymers into the solutions. Electrostatic interaction between positive and negative charges helps in the immobilization of biological materials and all the steps of adsorption are performed in gentle conditions in the aqueous suspension without the requirement of complex instruments [88].

Advantages: The use of the labeled coenzyme derivative averts the need of costly native coenzyme NAD^+ during each analysis.

Disadvantages: The biosensor response decreased on one-month storage.

3.5. Microbial biosensors

Microbial biosensors involve the use of microorganisms onto membranes or electrodes. Some of the microbial biosensors have been developed based on the use of bioluminescent materials utilizing the genetically modified microorganisms. Microorganisms used in these biosensors have the ability to metabolize various compounds. They have the ability to adapt to unfavorable conditions and are responsible to genetic alterations like mutations or recombinant DNA technology. Some of the L-lysine microbial sensors are discussed in the following subsections:

3.5.1. Lysine decarboxylase based microbial biosensors

A lysine decarboxylase based amperometric biosensor was fabricated based on CO_2 sensors. The decarboxylation of L-lysine produces CO_2, which diffuses through the membrane and approaches autotrophic bacteria. There is increase in the rate of respiration and more consumption of oxygen. This in turn reduces the O_2 concentration in the O_2 electrode. This decrease in O_2 is detected and L-lysine concentration is quantified using the calibration graph.

Suzuki et al., 1990 [89] demonstrated the construction of L-lysine biosensors based on the above principle. The sensor worked in optimum pH 6, temperature 33 °C and showed linearity in the concentration range 25–400 μM with a response time of 1–3 min. In another study, Suzuki et al., 1994 [90] reported integrated amino acid biosensors using the same concept of lysine decarboxylase and autotrophic bacteria. The response time for L-lysine was 3–10 min, optimum pH 6, temperature 33 °C and a linear range 0.2 to 0.8 mM.

3.5.2. Lysine oxidase/Saccharomyces cerevisiae based L-lysine microbial biosensor

Saccharomyces cerevisiae due to its lysine oxidase activity, substrate specificity, easy manipulation and ability to grow fast in the culture medium is used for the biosensor fabrication. A microbial L-lysine biosensor was developed based on yeast *Saccharomyces cerevisiae* cells procured from the exponential phase of the growth curve and immobilized onto gelatin using glutaraldehyde as cross-linking agent. The activated yeast cells were immobilized onto O_2-based Teflon membrane. The optimum working conditions for the biosensor are pH 7.5, temperature 30 °C, microbial quantities $0.97*10^5$ CFU cm^{-2} and linearity in the concentration range 1–10 μM with a response time of 1 min [10].

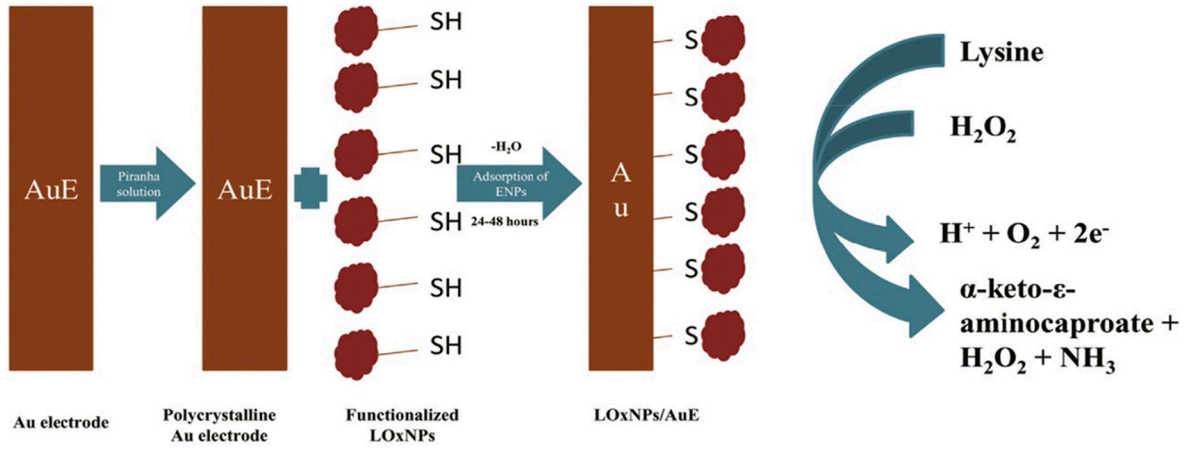

Fig. 6. Schematic representation of LOxNPs/AuE. (LOxNPs: Lysine oxidase nanoparticles, AuE: Gold electrode) [83].

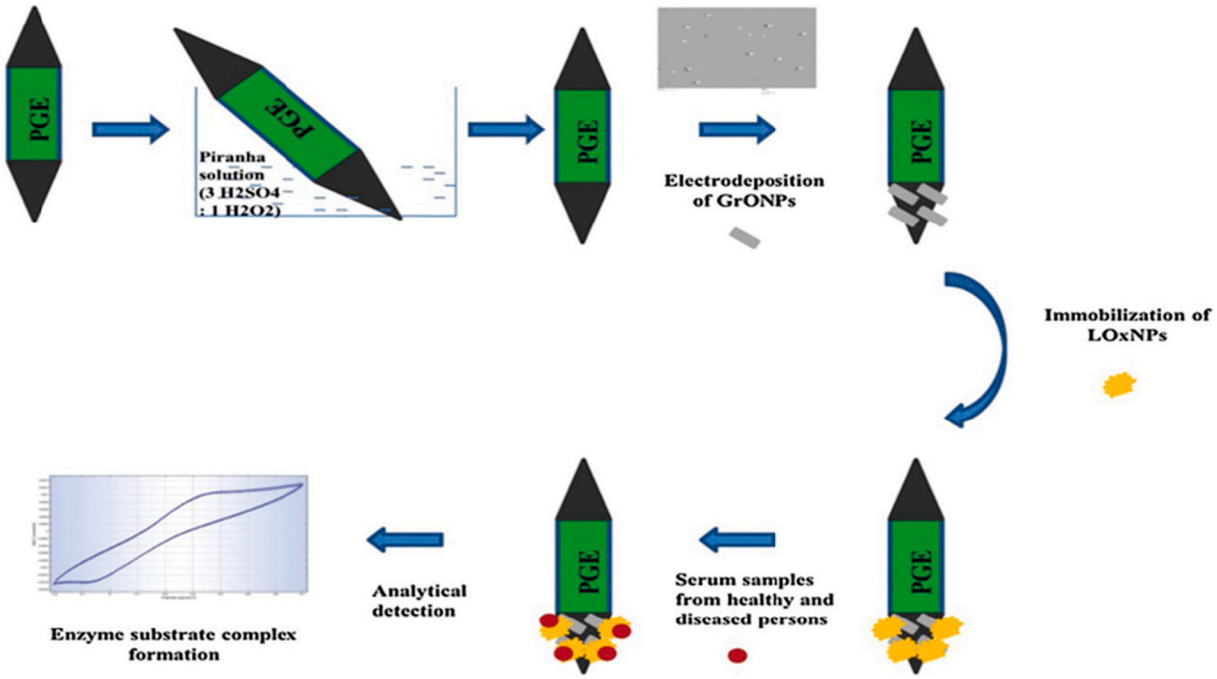

Fig. 7. Schematic representation of construction of LOxNPs/GrONPs/PGE. (LOxNPs: Lysine oxidase nanoparticles, GrONPs: Graphene oxide nanoparticles, PGE: Pencil graphite electrode) [84].

3.5.3. *Green fluorescent protein gene-based L-lysine microbial biosensor*

A study by Zabala-Diaz et al., 2007 [91] demonstrated a green fluorescent protein (gfp) gene-based *E. coli* biosensor to quantify bioavailable L-lysine. The gene construct was prepared by inserting L-lysine inducible promoter in a promoterless mutagenesis system followed by insertion into *E. coli* genome using transposon mutagenesis. This whole cell L-lysine biosensor was based on the fluorescent response obtained due to the coupling of gfp with L-lysine inducible promoter. This fluorescent response was directly proportional to the amount of L-lysine. The developed fluorescent microbial biosensor was used to determine L-lysine levels in four feed samples.

3.5.4. *Forster resonance energy transfer (FRET) L-lysine biosensor*

FRET based biosensors measure the change in conformation of the central protein due to ligand binding and changes the FRET signal of the fluorescent proteins flanking the binding protein. Steffan et al., 2016 [92] reported the construction of a sensor toolbox to determine ratiometric L-lysine. The sensor was fabricated by utilizing binding protein L-lysine-/arginine-/ornithine- (LAO) obtained from *E. coli*. Enhanced cyan fluorescent protein (ECFP) and citrine were the two proteins flanking the binding protein sequence. These sensors were used for monitoring the extracellular L-lysine concentration of *Corneybacterium glutamicum*, a L-lysine producing bacteria in a cultivation device and to determine L-lysine in fermentation liquid media.

Advantages: Microbial cells can be genetically engineered for biocatalytic attributes avoiding the need of pure enzymes. Microbial biosensor's receptors have the ability of regeneration.

Disadvantages: The signal generation rate and selectivity are low in case of microbial biosensors.

3.6. *Optical biosensors*

Optical biosensors are the analytical devices constituting biological recognition sensing element along with optical transducers. The optical transducers are small size, highly specific, sensitive and cost effective. A study to develop optical L-lysine biosensor was discussed by Hong et al.,

1992 [93] to fabricate a biosensor based on the enzymatic reaction catalyzed by lysine decarboxylase. The biosensor contained a poly vinyl chloride (PVC) membrane consisting of lipophilic tartaric acid acting as amine carrier for the cadaverine sensor. The cadaverine cation is transported to the membrane leading to proton transport and thus a change in the spectrum of indicator dye which is proportional to the cadaverine concentration. The linear range for the sensor is 0.1–100 mM. This sensor was better than previous sensors based on lysine decarboxylase as it measured the amount of organic amine (cadaverine) instead of CO_2 produced, the advantage being the low background of amines in real samples [93]. Another study used flow injection system to determine L-lysine based on the enzyme lysine oxidase. They used optical fibre as the hydrogen peroxide (H_2O_2) detector. H_2O_2 was detected on the basis of peroxidase-luminol reaction. This sensing assay exhibited a linear range 10–1000 μM with a detection limit of 5 μM at an optimum pH 8.5. The average measuring time for a sample was 30–50 s. the rate of sampling was 90 h^{-1} [94]. To compare the fibre optic sensor, another optical L-lysine biosensor was constructed by Preuschoff et al., 1994 [95] using the photodiode chemiluminescent based sensing apparatus. This was a bienzymatic sensor involving the catalytic activity of microbial peroxidase and lysine oxidase. The coupled action of these two enzymes enhanced the sensitivity and selectivity of the sensor. The sensor thus developed was faster and exhibited quick response time < 10 s, a detection limit of 10^{-6} M, linear range of 10^{-3} to 10^{-5} M with 2.5% precision at an optimum pH 8.5. An electrochemiluminescence based biosensors array was constructed for the associated detection of choline, glucose, lactate, L-lysine and urate. The fabrication of the sensor was done using the immobilization of diethylaminoethyl (DEAE) on the glassy carbon foil in the PVA-SbQ photopolymer. The detection limit for the L-lysine was 1 μM and linearity in the range 1 μM to 0.5 mM. These multifunctional biosensors were used to determine the respective concentrations in human serum matrix and showed good correlation amidst the observed and expected values [96]. Endo et al., 2008 [97] reported an enzymatic optical biosensor by using the enzyme membrane of L-lysine oxidase isolated from rockfish (*Sebastes schlegeli*) and oxygen probe. The sensor worked at an optimum pH 7.5, temperature 35 °C, flow rate 0.3 ml min^{-1}, linear range of 0.1–3 mmol L^{-1}. The stability of the sensor was at least 35 days, while working at optimum conditions.

Advantages: Optical biosensors are of small size, flexible, cost-effective and fast. They provide higher sensitivity and specificity.

Disadvantages: It requires strenuous labelling process which may interfere with the normal functioning of biomolecule. Quantitative analysis is difficult as the number of fluorescent molecules binding on each biomolecule cannot be controlled precisely.

3.7. Fluorescent biosensor

A pentafluorobenzoyl derivative of (*R*)-1,1′-b-2-naphthylamine (BINAM) was used in a fluorescent biosensor as a probe to determine L-lysine. A para-position substituted reaction product is formed due to the nucleophilic substitution of the derived BINAM. There was enhancement in the fluorescence signal due to inhibition in PET process during the substitution of fluorine with the nitrogen atom. The fluorescent signal increased by adding increased L-lysine concentration. The sensor showed detection limit $5.1*10^{-5}$ M [98]. Sharma N and Yun K, 2020 [99] devised a method to detect tetracycline and L-lysine. The synthesized carbon dots from the seeds of *Nigella sativa* using the hydrothermal method. The presence of L-lysine in the samples led to the enhancement of fluorescent signal, while the addition of tetracycline led to less fluorescence. The mechanism of the increase of signal due to L-lysine is the enhancement of photoluminescent intensity, which is increased due to hydrogen bonds between carbon dots and L-lysine. The synthesized carbon dots were used as sensing element to detect L-lysine in sera samples. A linearity was observed in the concentration range 0–500 μM with detection limit of 453 nM.

Advantages: The specificity of these biosensors is high because of the unique optical properties of molecules. They can quantify analyte concentrations as fluorescence intensity.

Disadvantages: Some of the disadvantages are photostability and loss of recognition capability. They are also prone to autofluorescence.

4. Conclusion and future perspective

In the end, it can be concluded that biosensing methods as compared to traditional methods like spectrophotometric, enzymic spectrophotometric, colorimetric, enzymic colorimetric, fluorometric, chromatographic, voltammetric are better due to their simple procedure, better sensitivity, selectivity, reproducibility and stability. The L-lysine biosensors reported so far operate ideally in pH range 5 to 10, temperature 25 to 40 °C, response time 2 to 300 s, linear concentration range 0.01 to 5500 μM, limit of detection between 0.000004 and 650 μM and the potential range −0.05 to 1.5 V. The biosensors are suitable for long term monitoring and miniaturization, requiring no reagents and complicated instruments. Future research on the L-lysine biosensors could be focussed on the use of electrochemical paper analytical devices, lab on a chip (electronic chips) and other nanomaterials. There is no requirement of complicated three electrode system in case of lab on a chip device. Hence, these could be a better candidate for L-lysine determination. The use of paper-based devices is beneficial due to their simplicity, minimum analyte amount, lower detection limits and the ability to be fabricated by different methods such as wire application, pen drawing, evaporation deposition, screen printing or nanoparticles deposition. Microtechnology and silicon technology have emerged as new platforms for biosensor fabrication. The use of microfluidic systems reduces the reagent consumption. The future holds the use of biosensors which are economic, wearable, unobtrusive, sleek and versatile. Doubtlessly, the use of enzyme-based biosensors is very promising in the near future for point-of-care diagnosis. The captivating features of biosensors make them better analytical tools for metabolite determination in various biological fluids.

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