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A review on L-lysine metabolism with detailed description of biosensors and deseases associated with L-lysine

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ABSTRACT

L-lysine is a necessary amino acid that must be received through diet as the body is unable to synthesize it. As well as having uses in medicine and diagnostics, the Determination of L-Lysine is significant for the pharmaceutical and food sectors. In a healthy human serum sample,
150–250 µmol/l of L-lysine is considered normal. In certain illnesses, there is an imbalance in the amounts of L-lysine. It could therefore be a biomarker for diagnosis. There are several fundamental techniques for determining L-lysine, including chromatographic, radioisotope dilution, colorimetric, fluorometric, and voltammetric techniques. Some drawbacks of these procedures include the need for specialized staff, sample preparation, expense, and time. Because of their great sensitivity, stability, and specificity, biosensors help to overcome these limitations. The current review paper addresses the fundamentals, benefits, and drawbacks of several analytical techniques for determining L-lysine, with a focus on biosensors. pH levels between 5 and 10, potential ranges between 0.05 and 1.5 V, temperature ranges between 25 and 40 ◦C, linear ranges between 0.01 and 5500 µM, detection limit between
0.000004 and 650 µM, and response times of 2 are excellent for L-lysine biosensors.
The best conditions for L-lysine biosensors to function include pH 5 to 10, the potential range
~0.05 to 1.5 V, temperature 25 to 40 ◦C, linear range 0.01 to 5500 µM, detection limit
0.000004 to 650 µM, and response time 2.

Keywords: Pencil Graphite(PG) electrode, Lysine,Serum, L-Lysine oxidase nanoparticles(LoxNPs), Lysine biosensor.

INTRODUCTION

An α-amino acid that is utilized in the production of proteins is lysine. It is classified as a basic, charged, aliphatic amino acid because it has three different types of amino acids: αamino, α-carboxylic acid, and side chain lysyl.. The α-carbon is a chiral and lysine may refer to either enantiomer or a racemic mixture of both (Drechsel 1889). IUPAC name is (2S)-2,6Diaminohexanoic

acid (L-lysine) (2S)-2,6-Diaminohexanoic acid (D-lysine). Lysine is a type of essential amino acid that cannot be synthesized in human body. It is obtained from diet in form of meat, fish, diary products, parmesan, wheat germ, fruits etc (Dresden 1891). Human nutritional requirements varies from 64mg/kg/day in infancy to 30mg/kg/day in adults (Tome and Bos, 2007). It has been observed that the cancerous tissues like those of normal tissues require lysine for their growth. Lysine play several role in human like helping the body absorb calcium, iron, and zinc, help in promoting collagen growth, produce enzymes, antibodies, and hormones. Lack of lysine causes several diseases like fatigue, poor concentration, hair-loss, anorexia and problems with reproductive system (Dresden 2018). Overdose of lysine caused by ineffective catabolism, can cause severe neurological issues (Drechsel 1889). Increasing the dosage of lysine leads to nausea, stomach cramps, gallstones and higher cholesterol (Dresden 1891).

Lysine oxidase (LOx) is also called as protein-lysine 6-oxidase, is an enzyme in human that is encoded by LOx gene. Lysine oxidase (LOx), a copper amine oxidase catalyzes the oxidative deamination of lysine and hydroxylysine residues in collagens and elastin (Vallet et al., 2019). (Kagan et al., 1991). Lysine oxidase remains activated unceasingly as it plays role in interlocking of collagen and elastin via oxidizing lysine residues.

Lysine oxidase play role in alternating tumorsbehavior (Mishra et al., 2017).Many analytical techniques, such as liquid or gas chromatography combined with MS, UV or fluorescence detection, reverse phase chromatography, high performance liquid chromatography (Trapiella 1990), spectrophotometry, fluorimetry, and chemiluminiscence, have been proposed for the quantitative determination of lysine. (Peruschoff et al.,1994).

2. Point- of- care regarding L-Lysine detection, metabolism and its disorders

Peptidyl lysine is oxidized by lysine oxidase to alpha-aminoadipic-deltasemialdehyde, which is the starting point for covalent crosslinking, which stabilizes collagen and elastin fibers. This enzyme has an o-quinone-consistent carbonyl cofactor in addition to copper.

2.1 Metabolism of Lysine

Lysine, classified as an indispensable amino acid (IAA), plays a crucial role in our dietary requirements and is essential for animal growth (Rose et al., 2014). In 1955, William Rose and colleagues conducted seminal research demonstrating the necessity of lysine in human diets to achieve a positive nitrogen (N) balance. This approach to assessing amino acid requirements, known as the N balance method, builds upon the foundational principle established by Block and Mitchell (Mitchell & Block, 1946).

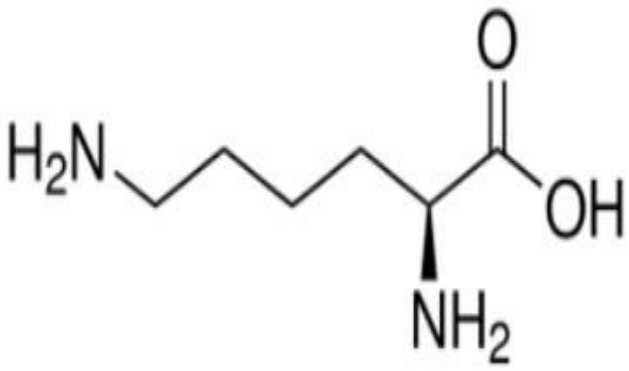


Figure 1: Structure of Lysine

The concept revolves around the composition of proteins in the body, which contain varying amounts of amino acids. Protein synthesis relies on the availability of all necessary amino acids. If any single amino acid is deficient in the diet, protein synthesis becomes limited, leading to catabolism and the formation of urea nitrogen (Rose et al., 2014).Restricting the intake of even one indispensable amino acid will hamper whole-body protein synthesis, resulting in the catabolization of excess dietary amino acids. This, in turn, leads to a negative nitrogen balance, highlighting the critical role of lysine and other indispensable amino acids in sustaining optimal protein synthesis and overall metabolic health.

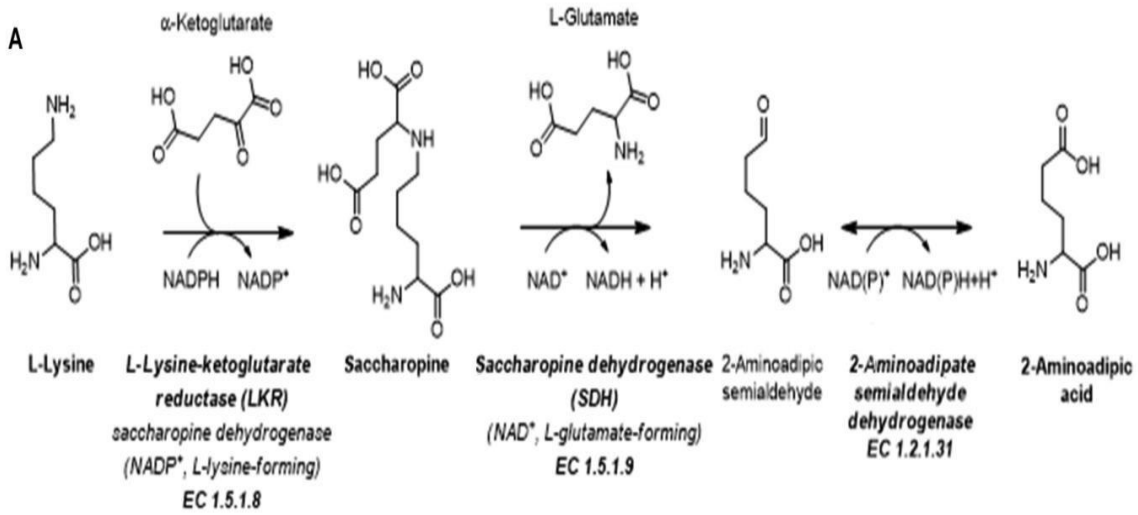
2.1.1 Key Features of Lysine Metabolism

- Lysine exhibits lower activity and does not initiate reactions like transamination or oxidative deamination compared to other amino acids. During oxidative demethylation of lysine, the resulting derivative undergoes irreversible reactions.
- The carbon skeleton of lysine can be assimilated into other amino acids such as glutamic acid, aspartic acid, and arginine through specific metabolic pathways.
- Two pathways facilitate this conversion: direct conversion to α-ketoglutarate and an alternative pathway involving acetate passing through acetyl-CoA.

2.1.2 Metabolic Fate of Lysine

Tracer techniques by Schoenheimer using N15 confirmed that dietary lysine has limited participation in transamination or protein synthesis. The α-keto derivative of lysine was also suggested to be inactive in nutritional factors. Despite irreversible lysine oxidative deamination, reducing lysine intake does not negatively impact athletic performance. Miller and Bale traced the decomposition pathway of lysine's carbon skeleton using carbon (Hommes FA, Kitchings L and Eller, 1983) labeling, revealing its incorporation into liver and plasma proteins.

The carbon-14 from lysine was found in substantial quantities in glutamic acid, aspartic acid, and arginine, implying a common progenitor for these amino acids.Additional studies show concentrated labeling on the alpha carbon of glutamic acid, opposite to the 6th carbon of lysine. Activity was also observed in the α-carboxyl carbon of aspartic acid. The carbon skeleton of lysine, particularly at the C2 and C3 positions, contributes to the carbon structure of aspartic and glutamic acids.



Aminoadipate-semialdehyde synthase (AASS)

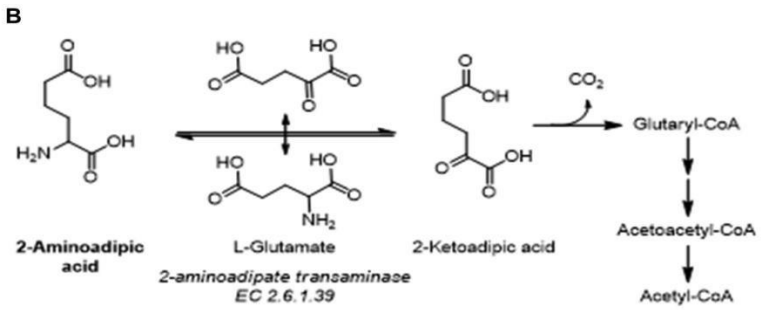


Figure 2: The metabolic pathway of lysine catabolism in mammals involves two main steps. In the first step, lysine is converted to saccharopine, which is then reduced to 2aminoadipic semialdehyde. This process releases a glutamate molecule containing the ϵN of lysine. Both conversion steps are catalyzed by enzymes known as saccharopine dehydrogenases. In the second step, 2-aminoadipic acid undergoes transamination with α-ketoglutarate to form glutamate and 2-ketoadipic acid. Subsequently, a dehydrogenase catalyzes the decarboxylation of 2-ketoadipic acid, producing glutarylCoA. Glutaryl-CoA is further metabolized to acetyl-CoA through a series of CoA esters.

In these experiments, the arginine was isolated and broken down to ornithine. A large portion of the radioactivity was found within this fragment. Nevertheless, the precise pathway of lysine's carbon chain conversion to arginine is unknown according to the data available. There is a hint that glutamic acid might be involved in this pathway, which is also supported by the fact that Womack and Rose had corrected a relative deficiency of arginine in rats' diets by supplementing with glutamic acid. It implies that there is a metabolic pathway in lysine metabolism where the carbon skeleton of lysine is used to form other amino acids with unbranched chains of five or six carbon atoms. Furthermore, glutaric acid is suggested to act as an intermediate in lysine transformation to ketoglutaretic acid and glutamic/aspartic acids, as well as an alternative pathway using acetate to methionine for amino acid incorporation.

Lysine shows activity in the isolated arginines of the liver and plasma proteins implying that it is involved in arginine synthesis, which may be through a pathway that involves glutamic acid. Consequently, lysine serves the dual purpose of being a building block for proteins and a

metabolic precursor for indispensable amino acids. The study of these pathways provides a better understanding of the nutritional and metabolic functions of lysine.

2.2 DISORDERS ASSOCIATED WITH ELEVATED LEVEL OF LYSINE CONCENTRATION IN BODY FLUIDS

Disorders associated with elevated levels of lysine concentration in body fluids are typically caused by metabolic abnormalities or genetic mutations affecting lysine metabolism pathways. These conditions can result in various symptoms ranging from developmental delays to intellectual disabilities.

2.2.1 Hyperlysinemia

A malfunction in the major catabolic route of the vital amino acid L-lysine causes hyperlysinemia, an autosomal recessive inborn error of metabolism. Acetyl-CoA is produced by this route, which mostly functions in the liver (Figure 1A). Firstly, lysine-ketoglutarate reductase (LKR, EC 1.5.1.8) transforms lysine and α-ketoglutarate into saccharopine.

Saccharopine dehydrogenase (SDH, EC 1.5.1.9) then oxidizes saccharopine to α-aminoadipic semialdehyde and glutamate. Interestingly, in mammals and other eukaryotes, a single bifunctional enzyme termed α-aminoadipic semialdehyde synthase—encoded by the AASS gene—catalyzes both enzyme functions. Hyperlysinemia has been connected to mutations in AASS.

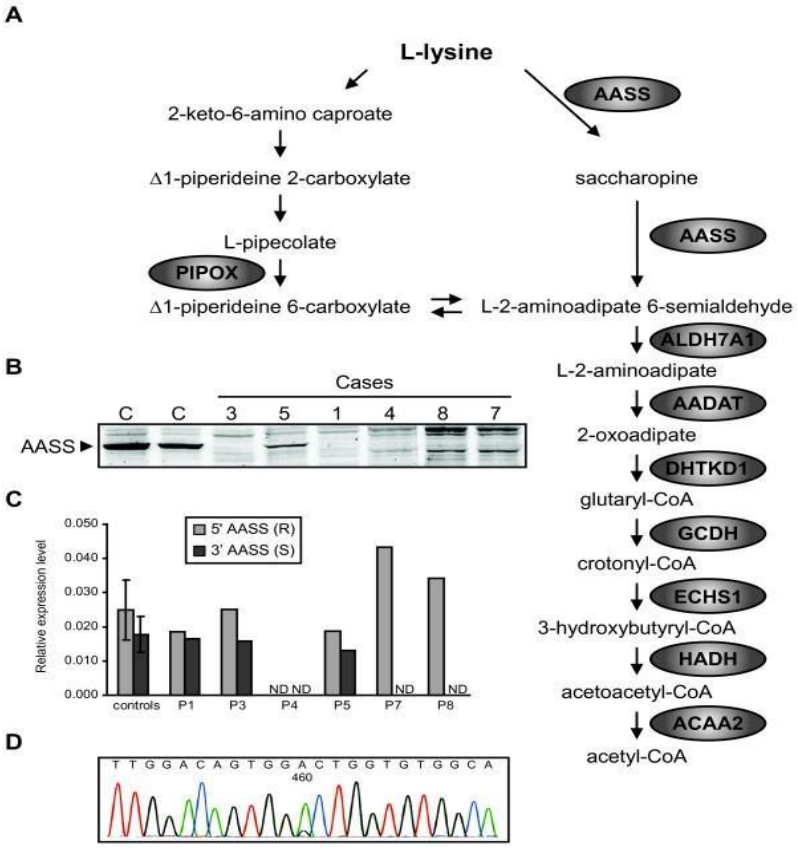


Figure 3: Molecular and biochemical studies in hyperlysinemia patients A) The figure displays the lysine degradation pathway and the two ways it could look with the enzymes involved. Some of the most critical enzymes are those that act as a catalyst in certain disorders. B) Western blot reaction to fibroblasts of the patient was either absent or with much less level of AASS enzyme compared to controls. C) qPCR revealed severely reduced or no detectable mRNA expression of patient samples compared to controls. D) Sequencing analysis uncovered one of the patient shotgun mutation of

having the exonicduplication of allele that led to premature termination of the protein and probably nonsense mediated decay.

Elevated plasma lysine levels above 600 µmol/L, with a maximum of 2000 µmol/L (adult reference range: 111–248 µmol/L), are the hallmark of hyperlysinemia. When α-ketoglutarate is less available, as in urea cycle diseases, pyruvate carboxylase insufficiency, methylmalonic aciduria, and propionic aciduria, plasma lysine levels are raised but still fall below 600 µmol/L. Lysine buildup triggers a number of different metabolic processes. For example, in the urea cycle, homoarginine can be produced when lysine takes the place of ornithine. Additionally, the use of alternate or detoxifying metabolic processes results in the accumulation of N-ϵ-acetyl-L-lysine, N-α-acetyl-L-lysine, and pipecolic acid. Saccharopinuria is another symptom that certain hyperlysinemic people may have when SDH is low but LKR activity is high. At first, hyperlysinemia was linked to mental retardation and neurological impairment, but later studies have called into doubt this connection. Moreover, there is still uncertainty regarding the effectiveness of dietary lysine restriction, and there have been reports of unaffected kids delivered to sick women. Based on these data, it is likely that hyperlysinemia is a benign metabolic variation, highlighting the potential for accidental correlations between biochemical abnormalities and nonspecific clinical indications.

The toxicity of lysine or lysine-derived metabolites remains unclear, although in vitro studies have shown adverse effects such as oxidative damage to proteins and lipids at high lysine concentrations.

2.2.2 Saccharopinuria

Saccharopinuria is a rare metabolic disorder that is characterized by elevation of lysine and saccharopine in urine which often cause spastic diplegia and intellectual disability. This disease, in which the bifunctional enzyme alpha-aminoadipate semialdehyde synthase (AASS) is deficient, is caused by an AASS gene mutation and is called saccharopine dehydrogenase deficiency or saccharopinemia. This enzyme is a key player in the lysine metabolism pathway, being the first two enzymes involved in the degradation of lysine. In the case of Saccharopinuria the lack of the lysine-ketoglutarate reductase (LKR) activity of AASS results in the impairment of lysine metabolism and accumulation of lysine and its metabolite, saccharopine.The presentation of Saccharopinuria is very variable, but commonly it presents with intellectual disability, spastic diplegia, histidinuria, hyperlysinuria, and EEG abnormalities. The intensity of symptoms can vary from mild to severe and there are some individuals who may face only mild intellectual impairment while others may have profound neurological deficits. Saccharopinuria diagnosis is carried out through a thorough evaluation which includes physical exam, history taking, lab tests and imaging studies. Lysine and saccharopine levels may be shown to be high in the urine during laboratory examinations, which is a diagnostic indicator of the disease.

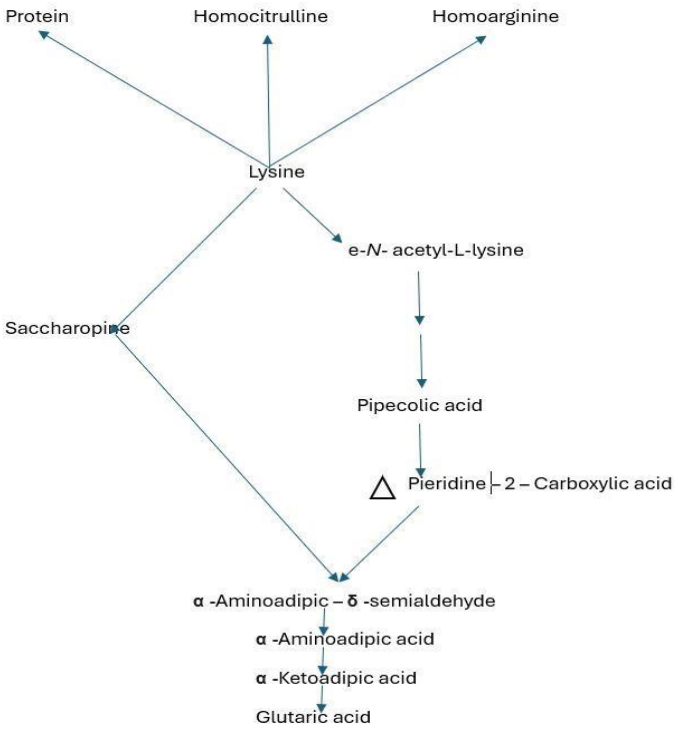


Figure 4: Schematic flowchart of known lysine pathways

The genetic AASS gene mutation, which is located on chromosome 7q31.3, underlies Saccharopinuria. These mutations interfere with the normal AASS enzymatic functioning, thus resulting in a partial deficiency of the lysine-ketoglutarate reductase activity and disturbed lysine metabolism. The gene responsible for saccharopinuria is located on autosomes and therefore is inherited in an autosomal recessive mode, which means that the individual must inherit two copies of the mutated gene (one from each parent) to develop the disorder. Carrier status, where a person carries one copy of the mutated gene, usually does not manifest symptoms, but can be diagnosed by genetic testing.

The treatment of Saccharopinuria is mainly directed at symptom control and palliative care since there is no cure for this condition. A low-lysine diet could be suggested to lower lysine levels in the body, and consequently to mitigate the symptoms. Nevertheless, the effectiveness of dieting may be variable among individuals, and other treatments should be considered for the treatment of specific symptoms or complications. Continuous monitoring and follow-up care are important to access the progression of the disorder and to adjust the therapy approaches, if needed.Prognosis of patients with Saccharopinuria is dependent on the degree of manifestation of symptoms and the presence of any complications. Mild cases may have a comparatively better prognosis with the people having a relatively normal life with appropriate management and support.

2.2.3 Pipecolic Acidemia

Pipecolic Acidemia is a rare metabolic disorder characterized by elevated levels of pipecolic acid, a metabolite of lysine, in the blood and urine. This condition arises from a deficiency of the enzyme alpha-aminoadipic semialdehyde dehydrogenase. Pipecolic acid plays a role in the degradation of lysine, and its accumulation disrupts normal metabolic processes. The symptoms of pipecolic acidemia can vary but may include developmental delays, seizures, intellectual disability, and liver

dysfunction. The mechanism involves the impaired breakdown of lysine, leading to the accumulation of pipecolic acid and other metabolites, which can have toxic effects on various tissues and organs.

2.2.4 Lysinuric Protein Intolerance (LPI)

Lysinuric Protein Intolerance (LPI) is a genetic disorder characterized by impaired transport of lysine, arginine, and ornithine across the cell membrane. This defect results in elevated levels of lysine in the blood and urine. Lysine is an essential amino acid involved in protein synthesis, and its abnormal accumulation disrupts normal cellular functions. Symptoms of LPI may include growth failure, muscle weakness, hepatosplenomegaly (enlargement of the liver and spleen), and osteoporosis (reduced bone density). The mechanism of LPI involves dysfunction in the transport proteins responsible for importing lysine, arginine, and ornithine into cells, leading to their buildup in the bloodstream and urine, and subsequent metabolic disturbances.

2.2.5 Pyrroline-5-carboxylate synthase deficiency

Pyrroline-5-carboxylate synthase deficiency is a rare metabolic disorder characterized by an accumulation of proline and hydroxyproline in the urine. This condition results from a deficiency in the enzyme pyrroline-5-carboxylate synthase, which is involved in the synthesis of proline from glutamate. Proline is an amino acid essential for protein synthesis and various cellular functions. The accumulation of proline and hydroxyproline disrupts normal metabolic processes and can lead to intellectual disability and seizures. The mechanism of this deficiency involves impaired conversion of glutamate to proline, leading to the buildup of proline and its metabolites in the body.

2.3 LYSINE OXIDASE ENZYME STRUCTURE AND FUNCTION (EC 1.4.3.14)

Lysine oxidase (EC 1.4.3.14) is an oxidoreductase enzyme which is known to catalyze the conversion of L-lysine, molecular oxygen (O2) and water (H2O) into 6-amino-2oxohexanoate, ammonia (NH3) and hydrogen peroxide (H2O This enzyme is key to lysine degradation and is involved in many physiological processes. It is also known as L-lysine αoxidase and L-lysyl-α-oxidase.

Lysine oxidase exists in two forms, one from Trichoderma viride Y 244-2 and another from Trichoderma harzianum Rifai. The enzyme's molecular weight lies between 100 and 120 kDa and its isoelectric point is between 4.25 and 5.6. Every subunit of the enzyme contains flavin adenine dinucleotide (FAD) as a coenzyme. Lysine oxidase has typical optical absorption spectra with peaks at 360 nm, 460 nm and 280 nm. The enzyme is composed of carbohydrate residues and is thermally stable, especially when held in a diluted solution.The two forms of lysine oxidase, which are derived from the different sources, show different structural features. These proteins have different percentages of α-helix and β-sheet secondary structures. Moreover, the amino acid composition is not the same for both forms, with glycine, tryptophan, and arginine residues varying.

Thermal stability of lysine oxidase differs by its source. The enzyme from Trichoderma harzianum Rifai has lesser thermal stability than that from Trichoderma viride Y 244-2. Nevertheless, thermal inactivation can be overcome by storing the enzyme as a diluted solution, but stability will be maintained for long-term storage under proper conditionsLysine oxidase has a special role in the degradation of L-lysine and a low Michaelis constant (Km). The enzyme catalyzes the oxidative deamination of lysine with the use of molecular oxygen to produce ammonia, hydrogen peroxide,

and α-keto-ϵ-aminocaproic acid. Lysine oxidase's physiological effects are based on the reduction of lysine and the production of hydrogen peroxide.

2.3.1 Physiological Effects

In vitro studies have shown various physiological effects of lysine oxidase, such as impairment of DNA, RNA and protein synthesis in leukemic and cancer cells. The enzyme shows anti-invasive, antibacterial, and antiviral properties, preventing the virus from replicating like Herpes simplex. In vivo animal experiments demonstrated that lysine oxidase exhibits immunomodulating, antitumor, and antimetastatic effects, thus making it a potential therapeutic agent in cancer treatment.

Lysine oxidase is used in the industry for the selective determination of lysine content in dietary supplements and end products. Lysine oxidase immobilized, together with horseradish peroxidase, is used for lysine determination on porous support membranes. This enzymatic assay provides a sensitivity from 20 to 120 μM and a reaction time of 2-20 minutes, thus, giving a convenient and reliable method for lysine quantification.

2.4 VARIOUS METHODS OF LYSINE DETECTION

Lysine, a major and indispensable amino acid that is essential for the growth of mammals, is also involved in protein synthesis and metabolism. It cannot be produced in the body but must be obtained through diet, thus, its measurement and quantification are crucial for nutritional assessment and research. Different analytical methods are used for lysine determination, such as ion-exchange chromatography coupled with post-column ninhydrin derivatization which has been a traditional and reliable choice. Nevertheless, the procedure is laborious and necessitates specific tools that may not be available everywhere. Liquid chromatography (LC) instruments provide an alternative but some challenges such as sensitivity and interference from other amino acids like proline have been experienced. Nevertheless, the exact determination of lysine concentrations is necessary for the comprehension of its role in physiological processes as well as for assessment of nutritional needs.

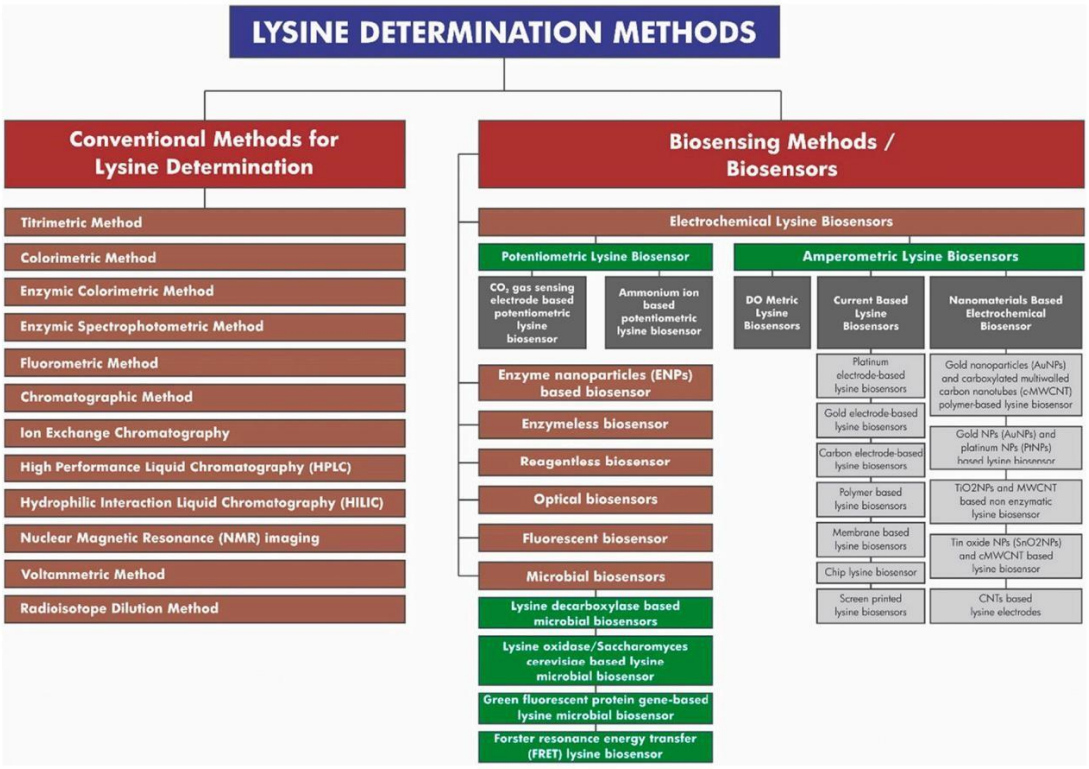


Figure 5: various methods for detection of Lysine

A comparison of various traditional methods for the determination of L-lysine is presented in Table 1. These methods include titrimetric analysis, colorimetric assays utilizing gold nanorods, enzymatic colorimetric detection of hydrogen peroxide, enzymatic spectrophotometric assays, fluorometric techniques, chromatographic approaches using reverse-phase C18 columns, and voltammetricdetection methods based on derivatized products formed with acetaldehyde. Each method offers distinct advantages and limitations in terms of sensitivity, working range, and sample types.

S. No.	Methods	Principle/based on	Limit of detection (mM)	Working range (mM)	Samples
1	Titrimetric	Detection of L-lysine based on titration of samples against	NR	NR	Soyabean meal
		the indicators brilliant green and solid red			and milk
					samples
2	Colorimetric	Detection of color change of gold nanorods from red to blue	1.610^−3	510^−3 – 1	Food samples
		due to their reaction with Llysine, 11-mercaptoundecanoic			(cookies, milk
		acid and Eu3+			and bread)
3	Enzymic colorimetric	Detection of hydrogen peroxide produced due to the reaction	NR	0-0.02	Human plasma
		of L-lysine with lysine-ϵ-oxidase			
4	Enzymic	Detection of NADH or formazan	NR	510^−4 -	Rabbit

		produced due to the reaction		10^−4	serum
	spectrophotometric	of L-lysine with lysine-edehydrogenase			
5	Fluorometric	Reaction between L-lysine and ortho-phthaldialdehyde	0.00004	NR	Milk samples
6	Chromatographic	Detection of L-lysine using reverse phase C18 column and diode array detector	NR	0.03-0.325	Rice samples
7	Voltammetric	Detection of L-lysine based on derivatized product formed	410^−4 samples 110^−2	6 *10^−4* - with acetaldehyde	Nutrition

2.4.1 Chemical Method

2.4.1.1 Ninhydrin-Ferric Method

The determination of lysine using the ninhydrin-ferric reagent method involves a specific chemical reaction that is highly sensitive to lysine and can be performed without the need for sample dilution. The method relies on the reaction of ninhydrin with lysine in the presence of ferric ion at a pH of 1.0. Ferric ion serves to inhibit the reaction of ninhydrin with other amino acids such as proline, ornithine, glycine, arginine, and histidine, ensuring the specificity of the assay for lysine.The determination of lysine using the ninhydrin-ferric reagent method involves several steps and reactions:Lysine samples are prepared by dissolving in hydrochloric acid (HCl) solution.The concentration of lysine in the sample should be within the linear response range of the method (0.0625 to 0.5 mg lysine.HCl).The prepared lysine samples are mixed with ninhydrin-ferric reagent at a pH of 1.0.The ninhydrin-ferric reagent specifically reacts with lysine, forming a coloredcomplex.Thecolored complex formed by the reaction of lysine with ninhydrin-ferric reagent is measured spectrophotometrically at a specific wavelength. The intensity of the color is directly proportional to the concentration of lysine in the sample. The lysine concentration in the sample is determined using a calibration curve constructed with known concentrations of lysine standards. The absorbance values obtained from the spectrophotometric measurement are compared to the calibration curve to determine the lysine concentration in the sample.This method offers high specificity for lysine and can be used to determine lysine samples of high concentration without the need for dilution. The inhibition of the reaction with proline, ornithine, glycine, arginine, and histidine by ferric ion enhances the specificity of the method for lysine detection.

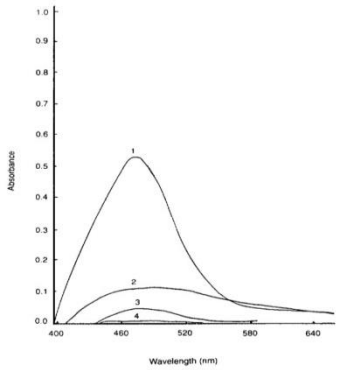
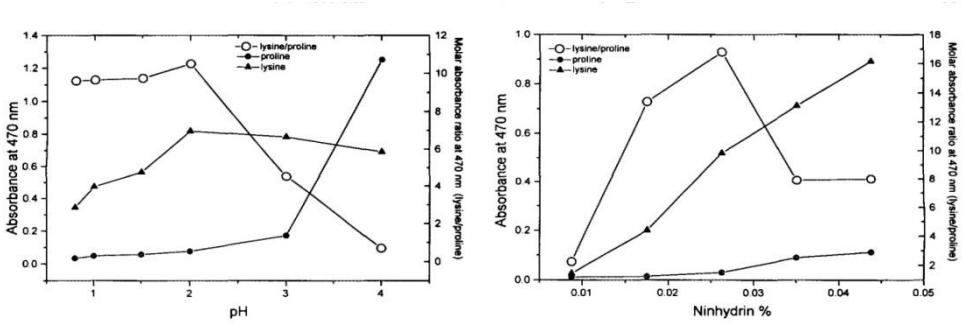


Figure 6: The absorption spectra of products of a ninhydrin-ferric reagent reacting with lysine (1), proline (2), histidine (3).

Figure 7(a) illustrates the influence of pH on the specificity of the colorimetric reaction with lysine. The pH range tested extends from 0.8 to 4.0, using a sample containing 200 mM of lysine. It is observed that at higher pH levels within this range, the specificity of the reagent to lysine significantly diminishes. This decrease in specificity suggests that pH plays a crucial role in determining the effectiveness of the colorimetric reaction with lysine.



(a)(b)

Figure 7: (a) Effect of pH on the colorimetric reaction of ninhydrin ferric reagnt to lysine and proline; (b) The effect of ninhydrin concentration.

As the concentration of ferric ion increases beyond 0.068%, there is a noticeable decrease in the color yield of the ninhydrin reagent to lysine.The intensity of the color developed is directly proportional to the concentration of lysine in the sample. Therefore, the absorbance of the colored complex is measured spectrophotometrically at a specific wavelength to quantify the amount of lysine present. The method exhibits a linear response over a range of lysine concentrations, typically from 0.0625 to 0.5 mg lysine.

2.4.2 Colorimetric Method Using Novel Meldrum's Acid-Furfural Conjugate

The colorimetric method for rapid detection of lysine and arginine utilizes a novel conjugate of Meldrum's acid and furfural (MAFC) as a chemosensor. This approach offers a swift and selective means of identifying trace amounts of lysine, an essential amino acid crucial for various biochemical processes.MAFC undergoes a photoswitching reaction, forming intensely colored donor-acceptor Stenhouse adducts (DASA) upon interaction with nucleophilic secondary amines. This reaction leads to a spontaneous color change, from colorless to magenta, in the presence of lysine and its structural analogue arginine, making them ideal targets for detection.

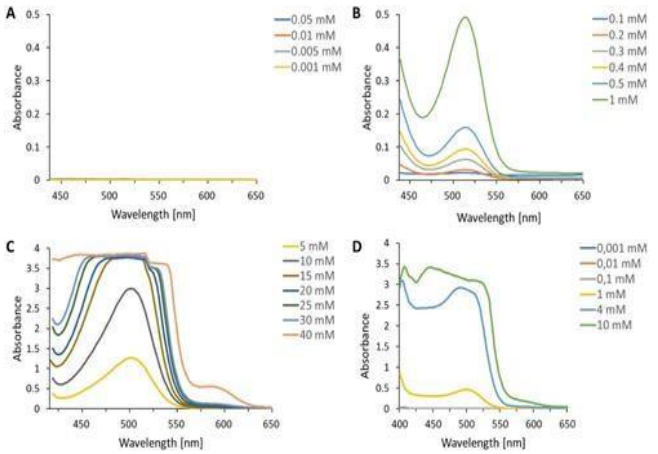


Figure 8: UV/Vis absorption spectra of A)-C) MAFC-lysine solutions with concentrations of 0.001-40 mM at pH 7 and D) MAFC-Arginine solutions with concentrations of 0.001-10 mM. [Zeubel Et al., 2021]

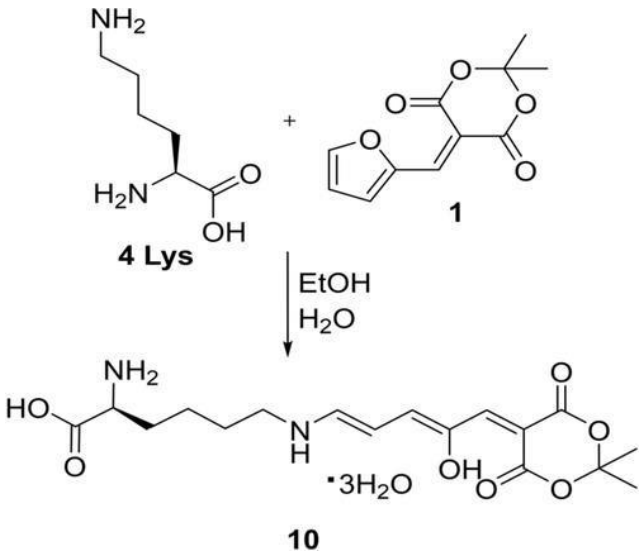


Figure 9: Proposed structure for the colored product formation by the reaction between lysine and MAFC at neutral pH.

Through UV-Vis spectroscopy, the sensitivity of the sensor was evaluated across a range of lysine concentrations (0.001–40 mm) at neutral pH, revealing a detection minimum at a concentration of 100 µm.

This method offers several advantages. The first and foremost advantage of this technique is its simplicity. Unlike many other technologies, it does not require expensive or specialized equipment to work, which makes it available for several different uses.

2.4.3 Spectrophotometric Enzymatic Method

The spectrophotometric enzymatic method for lysine detection is a valuable analytical technique utilized in various fields, including biochemistry, clinical diagnostics, and food quality control. This method relies on the enzymatic activity of lysine oxidase (EC 1.4.3.14), which catalyzes the oxidation of lysine in the presence of oxygen and water. Through this enzymatic reaction, lysine is converted into 6-amino-2oxohexanoate, along with the production of ammonia and hydrogen peroxide. The detection of lysine is indirectly achieved by quantifying the production of hydrogen peroxide, a byproduct of the enzymatic reaction, using a spectrophotometer set to a specific wavelength.

Principle: Lysine naturally yields α-keto-ϵ-aminocaproate (KAC) through enzyme lysine oxidase. The reaction of KAC with a chromogenic substrate e.g. oaminobenzaldehyde is then employed to produce a colored complex that is spectrophotometrically measured.

Procedure: Prepare a reaction mixture containing:Prepare a reaction mixture containing: Lysine oxidase enzyme, O-aminobenzaldehyde, Buffer solution, standard solutions or samples are serving.The incubation time of the reaction mixture should be set at 37°C, which is the sufficient temperature for the enzyme reaction to take place.Registrate the absorbance of 440 nm with help of a spectrophotometer. The number of yellow complexes has the same proportion with the lysine concentration. Please prepare a standard curve by taking absorbance reading for standard lysine

solutions with concentration known in advance. Follow the same procedure as it was followed in the test samples to identify the concentration of lysine.

This technique also enables precise, sensitive, and specific measurement of lysine concentration in samples as the enzyme is accompanied with the colorimetric detection system.The spectrophotometric enzymatic method offers several advantages for lysine detection. Firstly, it demonstrates high specificity for lysine, as the enzymatic reaction is specifically catalyzed by lysine oxidase. This specificity ensures minimal interference from other compounds present in the sample matrix, leading to accurate and reliable results. Additionally, the method exhibits sensitivity to low concentrations of lysine, allowing for the detection of lysine even at trace levels. This sensitivity is particularly beneficial in applications where precise quantification of lysine is required.

2.4.4 Flow Injection System

The enzymatic determination of lysine offers enhanced selectivity compared to other methods. While L-amino acid oxidase from snake venom is unspecific, lysine decarboxylase, lysine dehydrogenase, saccharopine dehydrogenase, L-lysine αketoglutarate e-aminotransferase, and L-lysine α-oxidase have been identified as potential enzymes for lysine determination. Among these, lysine decarboxylase exhibits the highest selectivity, catalyzing the reaction lysine + cadaverine + CO2. However, challenges arise from high and variable CO2 levels in real samples, making assays unreliable for practical applications. Therefore, L-lysine α-oxidase (LysOD) was chosen for further investigation due to its reliability and selectivity.

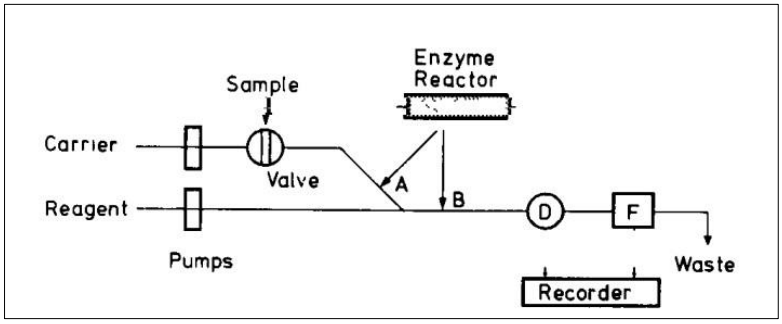


Figure 10: Schematic diagram of experimental setup of flow injection.

LysOD is a flavin adenine dinucleotide (FAD) – dependent enzyme and catalyses the following reaction:

L-lysine + O_2 + H_2O →∝-keto-∈ - aminocaproate + NH_3 + H_2O_2

To facilitate rapid and automated detection, flow-injection techniques were employed for enzymatic lysine determination. These techniques offer the advantage of permanent control of detector drift and are increasingly being utilized in process monitoring and control. Experimental procedures involved the manual assay of Llysine using a coupled test with LysOD and horseradish peroxidase (POD) in a potassium phosphate buffer. The assay measured the formation of a red quinoneimine compound at 500 nm using a spectrophotometer.The enzymatic reaction catalyzed by LysOD involves the conversion of L-lysine to α-keto-ϵ-aminocaproate, which cyclizes spontaneously into 1-piperidine-2-carboxylate. The detection of hydrogen peroxide (H2O2) resulting from this reaction is commonly achieved through its oxidation of chromogenic substances catalyzed by POD. This yields a red quinoneimine product, which is measured photometrically at 500 nm.

2.4.5 Chromatographic techniques

2.4.5.1 High performance Liquid Chromatography

L-lysine is the essential amino acid which is the structural component of protein hence it is involved in the repair and growth of tissues in the body. Lysine is critical amino acid that can only be supplied from animal sources such as fish, beans, poultry, and eggs. L-lysine can be used as dietary supplement to enhance effectiveness of anxiety, hypertension, and viral infections treatment. This approach employs HPLC with a UV detector for the determination of the content of L-lysine in food, plants and animal samples.

In high-performance liquid chromatography (HPLC), the separation and detection of L-lysine from food samples involve several key steps. Initially, the amino acids present in the sample undergo hydrolysis to break down complex proteins into their individual components. Following hydrolysis, derivatization is carried out using ophthaldialdehyde and 9-fluorenylmethyl chloroformate. These derivatization agents react specifically with the amino groups of amino acids, including L-lysine, forming stable and detectable derivatives. Once derivatized, the amino acids are separated on a chromatographic column, such as the Zorbax Eclipse-AAA column, which is specifically designed for the analysis of amino acids. This column provides efficient separation based on the unique properties of each amino acid, including size, polarity, and interaction with the stationary phase. As the derivatized amino acids pass through the column, they interact differently with the stationary phase, leading to their separation. Finally, the separated amino acids, including L-lysine, are detected using a diode detector. This detector monitors the eluent exiting the column and detects the presence of the derivatized amino acids based on their characteristic absorbance or fluorescence properties. By measuring the signals produced by the derivatives, the concentration of L-lysine in the sample can be quantified accurately.

2.4.5.2 Gas Chromatography

Gas chromatography (GC) offers a powerful method for the precise detection and quantification of lysine, an essential amino acid crucial for various biological processes. Through derivatisation and chromatographic separation, lysine can be effectively analyzed in complex sample matrices. Here, we present a focused investigation on the detection of lysine using gas chromatography, highlighting its reliability and sensitivity in accurately determining lysine content.

Reagents used areLysine, trifluoroacetic anhydride and orthomethyl-isourea hydrogen sulphate, n-butanol-stearate in n-butanol. For the detection of lysine, a lysine standard solution of known concentration was prepared. Guanidation of lysine was accomplished by using the procedure published by Mauron&Bujard (1963). Following the guanidation, the protein was precipitated, hydrolysed with 6N HCl, and then derivatised as explained by Kaiser et al. (1974). Derivatisation process was used for the transformation of lysine to its derivative which is suitable for analysis by gas chromatography.The separation of lysine derivatives was achieved under chromatographic conditions used. Gas chromatograph was used to separate the lysine derivatives from the other compounds present in the sample matrix. The peak relating to the lysine derivative was clear and repeatable, which allowed for an accurate measurement of the lysine content in the sample.

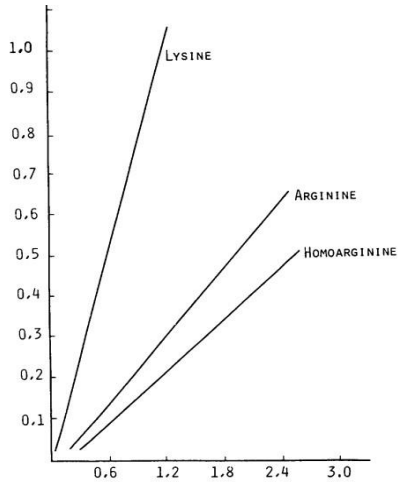


Figure 11: calibration curve for lysine and other compounds (arginine and homoarginine)

The figure 11 shows a response linearity graph for lysine. Different dilutions of a known lysine solution were prepared, derivatised, and analysed by gas chromatography. The peak area corresponding to lysine derivative was a linear function of the lysine amount injected, which means the method is linear during the tested range.

2.4.5.3 Ion exchange chromatography

Ion exchange chromatography is one of the best methods of detection and separation of lysine, an amino acid which is essential for different biological processes. In this technique, the adsorption of charged molecules in a mobile phase onto a stationary phase with opposite charge characteristics is a reversible process. The choice of ionic strength and pH values of the mobile phase governs the adsorption of molecules onto the stationary phase, which gives selective desorption based on the ionic strength.

In this procedure, the initial conditions are set so that lysine molecules, which are positively charged, get attached to the column that is packed with a resin containing negatively charged groups. The lysine molecules bind to stationary phase and the rest of the solution passes through the column. Succeeding alterations in the ionic strength or pH of the mobile phase neutralize the charge on the lysine molecules, which are released from the column. The resin has a selective mechanism of releasing molecules depending on their binding strengths with the stronger binding molecules desorbing last.The quantity of the resin, which is determined by the number of charged groups per liter of resin, decides the capability of the resin to bind lysine under experimental conditions. Variables like temperature, buffer strength, pH, and flow rate influence the resin's available capacity. Moreover, the characteristics of the substance to be separated, for instance, molecular weight and charge, also affect the resin capacity.The charges of lysine, which can be characterized by its pKa values, are the ones that determine its behavior in the ion exchange chromatography. At pH levels below its pKa, lysine carries a positive charge, which enables it to bind to the negatively charged resin. As the pH increases, lysine's charge gets more and more negative. The chromatography column effectively separates lysine from the other compounds in the mixture, as lysine has a pI value that is higher than the rest of the components and requires a much higher pH buffer for elution.

To measure lysine, the saccharopine dehydrogenase reaction can be utilized, which is the depletion of NADH at a wavelength of 340 nanometers. This reaction provides the basis for lysine measurement, which is based on the decrease in NADH during the reaction.The experimental procedure involves Lysine separation by anion exchange chromatography, where the choice of

resin is based on its efficiency and compatibility with the experimental setup. Chromatography parameters, such as flow rates and volumes of the solution, are optimized to obtain efficient separation and detection of lysine.

2.5BIOSENSORS FOR LYSINE DETECTION

Biosensors have emerged as advanced analytical tools, combining biological sensing elements, transducers, and electronic systems to convert the response of substances being measured into measurable signals. According to the recent definition by the International Union of Pure and Applied Chemistry (IUPAC), a biosensor is a self-contained integrated device capable of providing specific quantitative and semi-quantitative analytical information using a biological recognition element in direct spatial contact with a transducer element.These versatile devices find widespread applications in various fields including diagnostics, medical research, quality control, agriculture, veterinary sciences, and more.

However, the utilization of biosensors typically requires expensive equipment and trained personnel.

In recent years, considerable attention has been devoted to the development of biosensors for the determination of L-lysine in different biological samples. These L-lysine biosensors encompass a variety of classes, including electrochemical biosensors, optical biosensors, microbial biosensors, screen-printed sensors, enzymeless sensors, reagentless sensors, and nanomaterials-based electrochemical biosensors.

2.6 WORKING PRINCIPLE OF LYSINE BIOSENSORS

2.6.1 Electrochemical Lysine Biosensors

Electrochemical biosensors have been considered as the most sensitive devices for detecting L-lysine in biological specimens. Several techniques are developed to boost electronic signals to detect an analyte in a trace amount. Predominantly, electrochemical sensors are of three types: potentiometric, amperometric, and conductometric. The working principles of different types of electrochemical biosensors are shown in Fig. 12. In the following section, the L-lysine biosensors based on the enzymes are discussed. The enzymes utilized in L-lysine biosensors include lysine decarboxylase, lysine dehydrogenase, and lysine oxidase. The enzyme lysine decarboxylase decarboxylates L-lysine with the production of carbon dioxide which is further detected by carbon dioxide-specific electrode. The enzyme Lysine dehydrogenase requires NAD+ as a cofactor together with the redox mediators. In this reaction, NAD+ is reduced into NADH+, which is an amperometric signal. The commonly used enzyme for l-lysine analysis is lysine oxidase. The method used in Lysine oxidase biosensors is the determination of oxygen using clark type oxygen electrode or hydrogen peroxide formation.

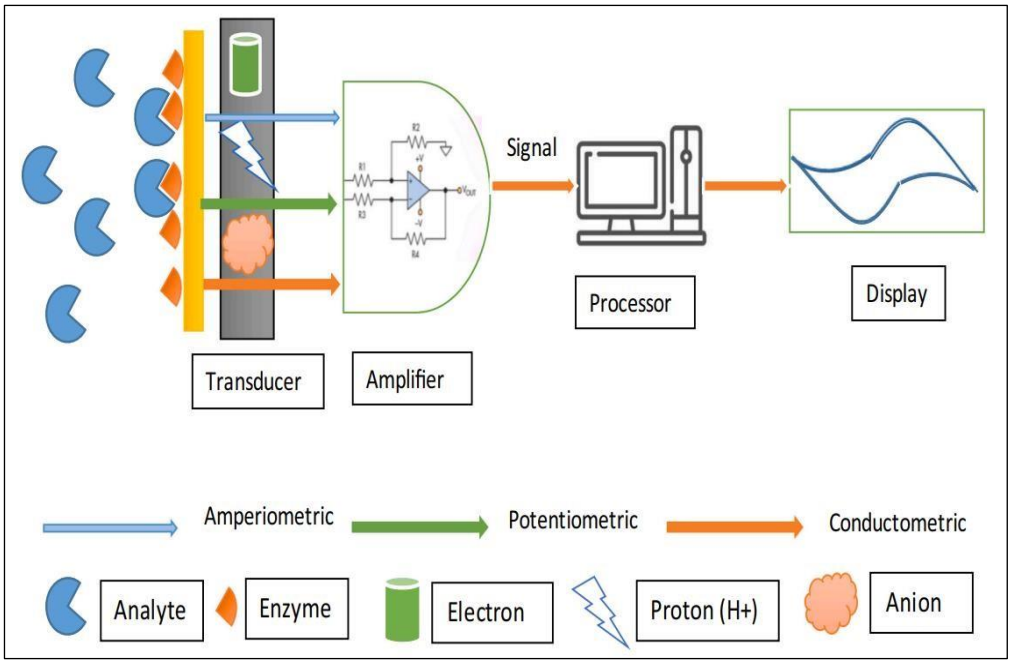


Figure 12: Schematic representation of principle of an electrochemical biosensor

2.6.1.1 Potentiometric L-lysine biosensor

The potentiometric biosensors measure the potential difference between the reference and working electrodes in the electrochemical cell when a current of zero or no flow occurs in the circuit. These are based on ion selective electrodes and ion sensitive field effect transistors. The output signal is because of the collection of ions at the chitosan-ion selective interface.

2.6.1.1.1 CO2 gas sensing potentiometric L-lysine biosensor based on electrode

The first potentiometric L-lysine biosensor was reported by White and Guilbault, 1978 [43] and was based on the carbon dioxide sensing electrode that was equipped with a Teflon membrane.

L-lysine → Cadaverine + CO2

The CO2 thus set free could be detected by the electrode. L-lysine decarboxylase was obtained from Escherichia coli B. It had a linear response 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 cornmeal samples. It did not yield a response to other amino acids.Another one of the potentiometric lysine decarboxylase-based L-lysine biosensors (enzyme electrode) was made with CO2 electrode as a model. The enzymic solution was placed between a dialysis and a gas-based membrane with plastic properties. To increase the measuring speed, two different types of measurement methods, namely quasi-steady-state and kinetic measurement were implemented. The measuring cycle has now been reduced by half, thanks to these principles. The linearity was discovered in the range 5 × 10^−4 to

10^−2 M with a detection time 3 to 5 min and stability for 3 to 6 weeks. This electrode was employed for process control of L-lysine fermentations.

Advantages: The electrode provided a fast and easy operation. In the process of sample analysis removing the insoluble material was not necessary. The electrode has a L-lysine specificity and the price is lower than earlier electrodes.

Disadvantages: Slow response time was a problem with the electrode. The CO2 in the test sample may obscure the L-lysine detection.

2.6.1.1.2 Ammonium ion-based potentiometric L-lysine biosensor

To detect ammonium ions, Saurina and the group created an ammonium electrode. The electrode was made by immobilizing lysine oxidase on the ammonium electrode using a chemical process. undefined

L-lysine + O2 → 2-ketoglutarate + H2O2 + NH3

The chemometric analysis of the data was done and the calibrated data was subjected to mathematical treatment to overcome the poor selectivity issue. The developed biosensor was then applied for the determination of L-lysine in pharmaceutical samples.In a further study, a potentiometric L-lysine biosensor array was constructed using 7 different ion-selective electrodes for the ions of K+, Mg2+, NH4+, H+, Na+, Li+, and Ca2+. The low specificity of the lysine oxidase to the other electrodes were the reason why the lysine oxidase membrane was immobilized onto the NH4+ electrode. Partial least square regression was one of the strategies applied for multivariate calibration to figure out the level of L-lysine in the feed samples (Garcia-Villar et al., 2001).Garcia et al., 2003 designed a lysozyme biosensor with the help of the flow injection technique and potentiometry. The mentioned biosensor was also based on the chemical immobilization of lysine oxidase on the nylon membrane attached to NH4+ electrode (Garcia et al., 2003). However, the newer biosensor was a more evolved device due to the employment of other techniques such as kinetic study, multivariate calibration, and differential potentiometry using twoelectrodes. The employment of these two methods in a combination resulted in the increased selectivity, sensitivity, accuracy, and durability of the biosensor for the determination of L-lysine in food samples.

Yarar and Karakus (2014) constructed a potentiometric L-lysine biosensor that consisted of ammonium ion electrode and lysine oxidase. The polyvinyl chloride, palmitic acid, nonactin, and bis(2-ethylhexyl) sebacate in tetrahydrofuran-based ammonium ion-selective electrode was prepared. Under the optimum conditions of pH 7.5 (10 mM Tris buffer), temperature of 25 ◦C, and response time of 0.5 to 1 min., the sensor was stable for up to 45 days with a good reproducibility and linearity in the concentration range 1 × 10^−5 to 1 × 10^−1 M. The

The fact that a mathematical approach was used meant that the endogenous ammonium was not removed because of the samples which served one of the major advantages to this technique.

Table 2 compares the analytical parameters of a potentiometric L-lysine biosensor.

Support for Immobilization	Enzyme	Workig Electrode	Method of Immobilization	Optimum pH	Response Time (sec)	Storage Stability (days)	Samples Used
Teflon membrane	L-Lysine decarboxylase	CO2 electrode	Crosslinking	~6.0	300-600	NR	Grains and foodstuffs
Gelatin and polypropyle	L-Lysineα-ne oxidase membrane	O2 electrode	NR	9.0	NR	180	Fermentation broth
Dialysis membrane and gas selective plastic membrane (Teflon and	L-Lysine decarboxylase	CO2 electrode	NR	NR	300 180–	NR	≥21

silicon)

Nylon membrane	Lysine oxidase	Ammonium electrode	NR	8.415	Aspartic acid, Glutamic acid	50
Nylon membrane	Lysine oxidase	Ammonium electrode	Covalent binding	NR NR	Pharmaceutical samples	NR
Nylon membrane	Lysine oxidase	NH4+ electrode	NR	NR NR	Feed samples	NR
NR	Lysine oxidase	NH4+ electrode	NR	NR NR	Pharmaceutical preparations	NR
Nylon membrane	Lysine oxidase	Ammonium electrode	NR	NR NR	NR	NR
Ammonium selective poly(vinyl chloride) membrane	Lysine oxidase	Ammonium electrode	NR	7.530–60	NR	45 days

2.6.1.2 Amperometric Biosensor for lysine detection

In a nutshell the mechanism of an amperometric L-lysine biosensor is based on the reaction between L-lysine and lysine oxidase that produces hydrogen peroxide. The outcome of the high-voltage effect is that H2O2 (the product) is broken down into 2H+ ions, O2 and electrons (2e−). Electrons as the result of the collisions are registered as a current. The series of reactions of amperometric L-lysine biosensors is given as under:

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

H_2O_2 -> 2H+ + O_2 + 2e− (under high voltage)

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

To detect the L-lysine, an amperometric flow was designed to pass through the analyzer. The immobilization of Lysine 2-monooxygenase on the DO electrode was carried out. There was a linearity in the concentration range 5.555 mM. The optimum pH was at 8.2, the response time of 15.40 s and the stability for 2 months was observed. This was applied to evaluate the concentration of 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:The enzyme lysine-2monooxygenase has the catalytic activity of decarboxylation and oxidation based on the following reaction:

L − lysine + O2→δ − valeramide + H2O + CO2

The oxygen concentration was first calculated, and then the L-lysine concentration was measured.

Vrbova and Marek, (1992) proposed a L-lysine biosensor which is like Clark type O2 sensor. The sensor consists of cyclohexylisocyanide and glutaraldehyde for the co-immobilization of lysine oxidase and catalase which are obtained by the ugi reaction onto the collagen membrane or nylon net. The sensor was best at working under the conditions of pH 7.5, at 45 ◦C, with 5 s response

time. The sensor lost half of its activity after 6 months of continuous use. The linearity was observed in the work concentration range from 6.710−6 to 6.710−4 M. L-lysine was determined in the wheat extracts by this linearity. In a different case, lysine oxidase was immobilized in cellulose and polypropylene foil using poly-urethane resin. This electrode is a Clark-type electrode and is used for L-lysine monitoring in the fermentation control.A flow injection amperometric L-lysine biosensor based on silica gel was suggested. Lysine oxidase was physically bonded to the silica gel, and the principle of operation was like O_2clark electrode. A stability of 150 days was achieved with a linear concentration range 0.2-5.5 mM, assay time of ~2 min and response time 14 s.

The other amperometric L-lysine biosensor was designed based on immunodyne-ABC nylon membrane and oxygen electrode. It showed a linear range of 10-250 μM with the response time of 5 s; it was highly sensitive, stable and specific. It demonstrated the effect of low interference and repeatability. It was used to analyze L-lysine in dry-ham and sausage products.

2.6.1.2.1 Graphite electrode (L-lysine biosensors based on carbon electrodes)

Carbon electrodes have a wide range of anodic potential. GC is the most common carbon electrode type. GC exhibit high mechanical propertiesand better chemical resistance. Carbon paste electrodes is also an example of carbon electrode which are in the form of fine particles of carbon mixed with oil. They are cheap, can cover vast distances and have large carrying capacitylower background noise. Saurina et al., 1999 studied the effect oftwo electrodes such as graphite methacrylate and graphite methacrylatemodified with peroxidase enzyme. The ex-electrode was applied tooxidative detection is monitored and the latter is employed for reductive purposes. The sensor was found to have a sensitivity of 11,300 μA/M within the range of 8.2 × 10-7 M to 1.6 × 10-4 M, and limit of detection8.2 × 10−7 M, 1.8% repeatability and response time 42 s. These conducting composite based biosensors were employed to investigate L-lysinecontent of the pharmaceutical tablets that are used for dietary supplements.The construction of the other L-lysine biosensor was also based on H2O2 sensormade of ruthenium and rhodium electrolysis in the carbonsurface. The sensor therefore built was not affected by interference from ornithine, arginine and phenylalanine have % interferences which are

3.4, 1.1 and 0.7% respectively.It has a lower limit of detection of 2 μM and linearity.in the range of 2 to 125 μM. The construction of glassy carbon (GC) microspheres, mineral oil andPB - a constituent of Prussian blue modified glassy carbon paste (PB-GCP).The other L-lysine biosensor was created by co-immobilization of the enzymes lysine decarboxylase, diamine oxidase, and horseradish peroxidase attached to Os polymer modified graphite electrode (GE). The biosensor showed the best results at the optimum pH of 7.0 when polarized at −50 mV potential vs Ag/AgCl. The limit of detection was 5 μM, and the linear range was 5 to 500 μM, 0.005 to 0.5 mM. The sensor was employed to identify L-lysine in feed samples and drugs.

These materials were deposited onto glassy carbon by using polyvinylferrocene and graphene (GC) electrode, and then the enzyme lysine oxidase is immobilized on the electrode to build an L-lysine biosensor. The sensor had the best performance at pH 10. It showed better sensitivity of 8.55 μA mM−1 cm−2 and good repeatability. The response time was quick, and the limit of detection was 2.3 × 10^−7. The calibration curve for the method was obtained within the concentration range of 9.9 × 10^−7 to 3.1 × 10^−4 M with linearity. The sensor was also used for L-lysine monitoring in cheese and pharmaceutical samples.

2.6.1.2.2 Polymer L-lysine biosensors

The polymer-based electrodes have low redox potentials and high energy density that facilitates the discharge, which enables them to attain high-speed operation. They are cheap and they do not harm the environment. Nonconducting polymers such as 1,2-diaminobenzene (DAB) are the best insulators because of their homogeneous thickness. They serve as a hindrance to interferents such as acetaminophen, uric acid, and ascorbic acid. Curulli et al. and others, 1998created a biosensor for L-lysine by means of lysine oxidase immobilization onto the DAB polymer film. The adsorption is passive. The sensor showed a response time of 12–16 seconds at the optimal pH 7.5 with 2 × 10^−7 mol l^−1 limit of detection and linearity in the range of 1 × 10^−5 – 1 × 10^−3 mol l^−1.

2.6.1.2.3Screen printed carbon

Screen printing biosensors are based on the multilayerdeposition of ink onto a solid support using a screen or mesh as a mask that controls the biosensor formation. Usually, the silver and carbon ink pastes are used for the preparation of the sensor. The composition of these inks affects the sensitivity and selectivity of the sensor. Carbon paste made from graphite particles, polymer binding agents, and other additives, is preferred for its costeffectiveness, ease of modification, and chemical inertness. Although platinum and gold are also used in addition, carbon is the most preferred because of its economic price.

Lysine oxidase-based biosensors have been developed through screen printing technique involving platinum as a working electrode, Ag/AgCl as the pseudo reference and carbon as the counter electrode. The sensors exhibit a sensitivity of 1.72 nA/μM ± 0.27 nA/μM, which is a linear range from 10 μM to 250 μM L-lysine with a correlation coefficient exceeding 0.999. They ensure stability for at least 6 months at 4°C and for 2 months at room temperature without a significant decrease in sensitivity. These biosensors have been utilized in the determination of L-lysine in the fermentation broth, showing a stable response and a negligible response to the various components.The other screen-printed biosensor consists of a glucose biosensor based on hydrogen peroxide detection coupled with an L-lysine biosensor. Through this design, we use commercially available carbon ink and modified glassy carbon particles. Glucose oxidase and lysine oxidase are immobilized on the screen-printed electrode for analysis which has a detection limit of 5 × 10−3 mmol L−1 and a linear range of 6 × 10−3 to 0.7 mmol L−1. The biosensor shows up with a

sensitivity of 52 mA (mol L-1 cm2) and its stability was enhanced due to the utilization of Prussian, blue-modified particles.

These biosensors are the choice of most people owing to the reasons of better performance, lower cost, better reproducibility, and a miniaturized version for point-of-care detection. The issue, however, is that the organic solvents in buffers or mobile phases could dissolve the inks, which cause the electron transfer rate, sensitivity, and detection limit of the biosensor to decrease.

2.6.1.2.4Platinum electrode-based L-lysine biosensors

The use of platinum as an electrode material has made big progress over the years because of its inert behavior, stability, high conductance, reactive nature, lower background noise suppression, better catalytic activity, and simple fabrication. Lysine dehydrogenase, which is known to convert L-lysine to Lαaminoadipic-δ-semialdehyde in the presence of NAD+, was immobilized on Pt electrode within the gelatin support on a cellulose membrane. The confinement technique of enzyme immobilization utilized in biosensors involves the confinement of enzyme molecules inside the network of gelatin, so that the products and the enzyme molecules are separated from the surrounding medium. The reactants of the reaction can be easily transported and as such, the

process is conserved. Regulate enzyme activity while maintaining the structure of the enzyme. A potential of 0.4 V was applied on the electrode. The sensed NADH now became free amperometrically by the biosensor.

The process of sensing is also facilitated by the existence of oxidizing agent ferricyanide ion. The main advantage of the enzyme used was dehydrogenase as it had high specificity for L-lysine and did not have any side reactions interference from other L-amino acids, alcohols, or carbohydrates. This biosensor was used for the determination of L-lysine in fermentation broth, mammalian cell culture preparations, and biological fluids. The work of Lavagnini et al., 1993 was based on a preactivated polymeric support through the immobilization of lysine oxidase onto a Pt electrode. The working electrode was polarized at 0.6 V, and the biosensor measured L-lysine concentration in the range 10−6 to 210−3 M with the response time 2 min, the limit of detection is 5 × 10−7 M and a shelf life of more than 3 months.

Ornithine and arginine were the interfering molecules. The biosensor was for L-lysine in feed samples. The L-lysine biosensor was assembled by immobilizing lysine oxidase on the platinum (Pt) electrode that is based on hydrogen peroxide. The sensor started working at optimum pH 7.0, optimum potential 0.6 V, and linearity in the concentration range 0.01 to 0.5 mmol−1 and interference was experienced from arginine and phenylalanine. It was utilized to measure L-lysine in thefood samples bovine serum albumin, farro sieved meal and farro wholemeal.

A highly sensitive and stable amperometric L-lysine biosensor was developed, where lysine oxidase immobilized on the Pt electrode was polarized at an optimum potential of 0.7 V. pH and mass transport were manipulated to observe the hydrodynamics and behavior of the electrode, studying both kinetic and analytical aspects of the sensor. It displayed a reaction time of less than 6 seconds, sensitivity of 4.4 μA·mM^-1·cm^-2, and a detection limit of 1 μM with linearity in the concentration range from 1 μM to 0.6 M. This method was utilized for the determination of L-lysine in pharmaceutical samples.

Lysine oxidase bound to an overoxidized polypyrrole thin film on a Pt electrode was used to fabricate the L-lysine biosensor, polarized at the optimum potential of 0.7 V. The study of common interferants revealed interference from phenylalanine, tyrosine, and histidine. This sensor exhibited better sensitivity (41 nA·mM^-1·mm^-2), linearity (0.02–2 mM), and detection limit (4 μM), and was applied to L-lysine determination in cheese and pharmaceutical samples with a stability of 40 days.

Ciriello et al. (2015) applied the same biosensor to detect L-lysine in different types of food and cheese samples. In 2018, they also detected L-lysine in untreated human urine with this biosensor, indicating a detection limit of 2 μM, linearity up to 4 mM, and sensitivity of 0.11 μA·mM^-1·mm^-2, with interference occurring from ornithine and arginine.

The potential of platinum-based biosensors in the biomedical field is significant, given the unique interference of hydrogen peroxide in the determination of metabolites, which is not observed at 0.4 V. However, the current increases when the voltage reaches 0.7 V. This implies that only certain amino acids are active at high potential, while ornithine, arginine, cysteine, phenylalanine, and tyrosine are interfering compounds. Hence, there is no interference at low potential by the Pt electrodes, but interference is observed with metabolites when working at high potential, particularly influenced by specific amino acids.

2.6.1.2.5 Gold electrode-based L-lysine biosensors

Gold electrodes have a wider cathodic potential range and are chemically stable, which is the reason for their use in many electrochemical processes. They have good conductivity, surface area,

durability, and are simple to adjust. However, gold electrodes have very limited functionality in the positive current region simply because they cannot handle high currents due to surface oxidation. An assembled approach using the flow injection analysis system and a Lysine biosensor, which was fabricated by the immobilization of lysine oxidase onto a gold-poly(m-phenylenediamine) electrode in a flow-through cell, was reported. A peristaltic pump was connected to a cell flow line using a sample injector. This sensor offers linearity in the range of 1 × 10^-3 to 5 × 10^-5 M due to better stability and reproducibility. It was used to predict L-lysine in foodstuffs such as wheat flour, pasta, etc.

2.7 NANOMATERIALS AND NANOCOMPOSITE USED IN LYSINE BIOSENSORS

Nanomaterials based electrochemical biosensor. Nanoparticles have become the most significant in the light of their specific physical and chemical properties like high surface area, low melting point, specific magnetic properties, mechanical strength and specific optical properties. The size of these nanomaterials, which is around 1-100 nm in diameter, has been proved to be the newest range of structures for the development of the biosensors in disease diagnosis. Nanomaterials are employed as supports for the direct immobilization of the enzymes on the electrode surface as well as for the signal enhancement. The distinctive features of nanomaterials ensure their use as the main materials for the fabrication of biosensors with enhanced selectivity and sensitivity. These nanosensors, termed as nanobiosensors, have more advantages than the conventional biosensors. Many kinds of various nanomaterials have been used in the production of nanobiosensors, for instance, ironoxide based nanoparticles, silver nanoparticles, gold nanoparticles, single and multi-walled carbon nanotubes, quantum dots, magnetic nanoparticles etc. Several L-lysine biosensors have been fabricated using nanomaterials including gold. In the following sections, a brief discussion about these nanomaterials-based biosensors is provided.

2.7.1 Gold nanoparticles (AuNPs) and carboxylated multiwalled carbon nanotubes (c-MWCNT) polymer-based L-lysine biosensor

CNTs have received a lot of interest for the creation of biosensors because of their larger surface area, excellent structural, electrical, and mechanical properties, biocompatibility, simplicity of production, and capacity to regenerate their surface. On the other hand, the wiring of biomolecules onto the electrode surface and the electron transfer rate are enhanced by AuNPs. The conducting polymer polyaniline (PANI) has been investigated because of its environmental stability, greater conductance, and ease of manufacturing. A conducting polymer such as PANI will become less resistant to charge and mass transfer when CNTs are added. Using two conducting polymers and other nanomaterials, Chauhan et al. (2012) created L-lysine biosensors and compared their different properties. Polymers were used to immobilize lysine oxidase onto the AuNPs-c-MWCNT nanocomposite modified Au electrode.

2.7.2 Gold NPs (AuNPs) and platinum NPs (PtNPs) based L-lysine biosensor

In addition, AuNPs have a biocompatible surface and a larger surface area to trap the target molecules. PtNPs support redox processes and have excellent electrochemical and catalytic qualities. Chauhan et al. (2013) report that the cross-linking chemistry of glutaraldehyde and 3-aminopropyltriethoxysilane (3-APTES) was used to electrodeposit AuNPs and PtNPs on the Au surface. When polarized at 0.2 V versus Ag/AgCl in the sodium phosphate buffer, this designed enzymatic biosensor could detect L-lysine concentrations as low as 1 μM in 4 s at optimal pH 7.5 and temperature 30 ◦C. The detection of L-lysine levels in various samples was done using a working linear range of 1-600 μM. After being stored for 120 days at 4 °C in dry circumstances.

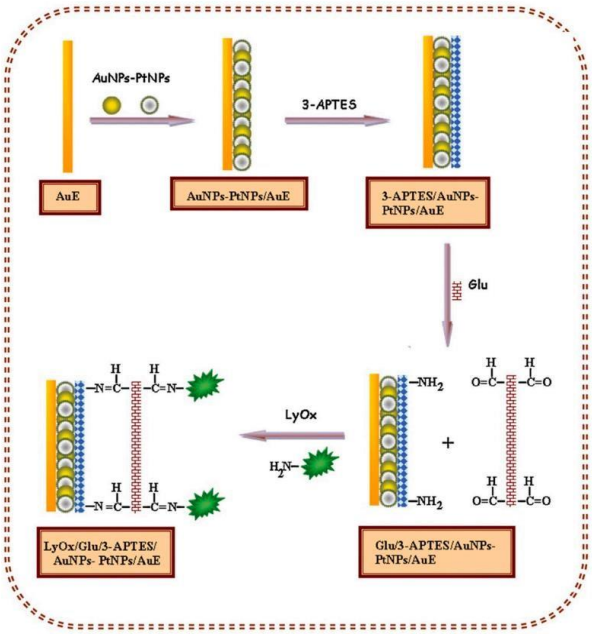


Figure 13: Schematic representation of steps involved in construction of L)x/auNPs/PtNPs/AuE. [N. Chauhan et al., 2013]

2.7.3 TiO2NPs and MWCNT based non enzymatic L-lysine biosensor.

In order to create a non-enzymatic sensor for measuring L-lysine, Gholivand et al. (2014) immobilized MWCNT and TiO2NPs on a GC electrode. L-lysine was found using the sensor in samples of urine and industrial syrup. The process was straightforward and didn't call for any unique reagents, enzymes, or electron-specific mediators. The biosensor demonstrated a 390 nM detection limit and a sensitivity of 0.1795 μAμM−1. In the 500–5500 nM concentration range, a linear electrochemical response was seen. In comparison to L-lysine and its product, the sensor was shown to have greater catalytic activity and antifouling capabilities.

2.7.4Tin oxide NPs (SnO2NPs) and carboxymethylated multi-walled carbon nanotubes (cMWCNT) based L-lysine biosensor

It has recently been discovered that metal oxide nanoparticles, or MONPs, are very desirable due to their excellent surface to volume ratio, superior electroconductivity, mechanical activity, biocompatibility, and stability. SnO2NPs: Among the several MONPs, they vary from the others due to their superior conductivity, unique photoelectronic properties, and gas sensitivity. Recently, SnO2NPs have been used to create a wide variety of biosensors. The use of SnO2NPs, cMWCNTs, graphene, and sol to create a nanocomposite for the construction of an L-lysine biosensor was described by Kacar et al., 2017b. After immobilizing the nanocomposite onto the GC electrode, lysine oxidase was further immobilized via covalent bonding. With a lower detection limit of 1.5, the biosensor displayed a linear range of 9.9 × 10− 7 M to 1.6 × 10− 4 M.

2.8 NANOCOMPOSITE BASED LYSINE BIOSENSORS

2.8.1 CNTs based L-lysine electrodes

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. WhereasLess coupling of biological recognition materials with the transducer alters the stability,

selectivity, dynamic range and sensitivity of biosensors which serves as one of the major disadvantages.

2.8.2 Enzyme Nanoaggregates based Biosensors

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 When utilized alone, graphene, carbon nanotubes (CNTs), and conducting polymers like polyvinylferrocene (PVF) offer great qualities to increase biosensors' sensitivity, conductivity, and durability. However, the biosensors' performance is improved by the mixture of these composites. L-lysine was determined using two distinct electrodes by Kacar et al., 2017c. Using glutaraldehyde and bovine serum albumin crosslinking, they created two enzyme electrodes: one based on PVF, MWCNTs, and gelatin, and the other based on PVF, MWCNTs, gelatin, and graphene as the composites adsorbed onto the GC electrodes. With a linear range of $9.9 \times 10-7$ to $7 \times 10-4$ M and a good sensitivity of 13.51 μA $mM-1$ $cm-2$, the electrode based solely on gelatin demonstrated a detection limit of $1.8 \times 10-7$ M. Using the gelatin and the electrode simultaneously exhibiting remarkable physio-chemical attributes. The amazing qualities of the ENPs, along with their enlarged surface area and excellent mechanical, catalytic, optical, thermal, electronic, and electrical capabilities, have resulted in an improvement in electrode performance. As a result, ENPs have been utilized in place of native enzymes in the creation of amperometric biosensors because of their advantages. This improved the biosensors' analytical capabilities and offered a straightforward method for creating enzyme electrodes [74].

In our lab, we created an enhanced amperometric L-lysine biosensor by covalently immobilizing lysine oxidase ENPs onto a gold electrode (AuE). The modified AuE using ENPs showed reduced background current and excellent conductivity. The working electrode displayed the ideal current at 0.8 V, pH 6.5, and 35 ◦C in 3.5 seconds. With a LOD of 10 μM, the ENPs/Au electrode demonstrated linearity between substrate concentration and current in the range of 10-800 μM. At 10 and 20 μM, the analytical recovery of added L-lysine in serum was 98.39% and 98.23%, respectively. The coefficients of variation within and between batches were 0.0751% and 0.0637%, respectively. The L-lysine content of milk samples, medication tablets, and blood samples from cancer patients and healthy individuals was measured using this biosensor.

By immobilizing the lysine oxidase nanoparticles (LOxNPs) onto the graphene oxide nanoparticles (GrONPs) embedded into the pencil graphite (PG) electrode, another L-lysinebased biosensor was produced in our lab. Better conductivity, improved electrochemical properties, reduced manufacturing costs, and an easily accessible disposable electrode were all displayed by the modified PG electrode. This electrode showed the best current response at 0.7 V in 3.95 seconds at the ideal pH of 6.5 and temperature of 35 ◦C. The adjusted PG electrode demonstrated a reduced detection limit of 0.01 μM. This sensor has a larger linear range, 0.01-1000 μM, and a 97% analytical recovery rate for added L-lysine in blood samples when compared to the previous ENPs-based L-lysine sensor. With a correlation coefficient of $R2 = 0.989$, the sensor exhibited good agreement with the conventional spectrophotometric approach.

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