Unit 11

DIAGNOSTIC ENZYME

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11.1 INTRODUCTION

In the last unit you come across several techniques for the isolation and purification of enzymes. Purified enzymes have lot of applications in the industry and diagnostics is one of them. Enzyme diagnostics is one of the branches of enzymology. In this unit we will discuss about the different applications of enzymes as well as diagnostic enzymes and their related aspects. Enzymes are considered to be valuable markers in different stages of diseases and provide insights into the metabolic and pathological processes during the course of disease. As you know, these enzymes are vital indicators of cellular damage and have diverse applications in diagnostics, including immunoassays and biosensors. Their ability to detect specific conditions makes them valuable in diagnostic methodologies. In the clinical field, the enzymes perform a variety of roles, including treatment of enzyme deficiencies, diagnosis of diseases, managing wounds, and conducting analytical tests. They are also useful in detecting cellular disorders and tissue damage based on their levels or activity in biological fluids. Enzymes are used as markers for various diseases, like cancer, heart attacks, jaundice, pancreatitis, and neurodegenerative disorders. Furthermore, enzyme-based biosensors are employed in monitoring several diseases, drug discovery, and in detecting pollutants, disease-causing microorganisms, and disease indicators in fluids like blood, urine, saliva, and sweat. Enzymes are also among the most widely used biomolecules in research laboratories. Let us discuss the some of the applications of multifaceted enzymes.

Expected Learning Outcomes

After studying this unit, you should be able to:

- describe the application of enzymes in diagnostics;
- uses of enzymes as analytical agents;
- discuss the application of enzymes in medicine, agriculture and research;
- describe about enzyme therapy, and
- explain the role of enzymes as biosensors.

11.2 APPLICATIONS OF ENZYMES IN DIAGNOSTICS

Enzymes are utilized in diagnostic applications such as immunoassays and biosensors. These applications are categorized as enzymatic diagnostics, immunodiagnostics, and DNA diagnostics.

1. **Enzymatic diagnostics:** In your visit to the hospital, you may come across biochemical tests prescribed by the physician for the determination of disease or its progression. Many enzymes present in plasma or serum are routinely tested in clinical labs as these enzymes are of great diagnostic value. Each plasma enzyme has a normal

concentration range due to the balance that is set up between its rate of arrival and its rate of removal in the blood stream. These enzymes have a normal range of activity. If a particular tissue is affected by disease or gets damaged, some of enzymes may leak into the bloodstream at an increased rate and will be found in the plasma in elevated amounts. The detection and measurement of enzymes using biochemical methods helps in detection of disease via their correlation with the symptoms, progression of disease and even cure from the disease.

Diagnostic enzymes are crucial for disease diagnosis and monitoring using medical samples like urine, serum, and other body fluids (Table 11.1). The enzymatic reactions are measured either by the rate of substrate consumption or the rate of product formation. For instance, glutamate can be estimated using NADH produced in a glutamate dehydrogenase reaction (equation 1).

$$\text{2-Oxoglutarate} + NH_4^+ + \text{NAP(P)H} \xrightarrow[\text{Glutamate dehydrogenase}]{} \text{Glutamate} + \text{NAD(P)}^+ + H_2O \quad (1)$$

Glucose can also be measured by a coupled enzymatic reaction of hexokinase (equation 2) and glucose-6-phosphate dehydrogenase (equation 3).

$$\text{D-Glucose} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{Hexokinase}} \text{D- Glucose-6-phosphate} + \text{ADP} \quad (2)$$

$$\text{D- Glucose-6-phosphate} + \text{ADP} \xrightarrow[NAPD^+ / NAPDH]{\text{Glucose-6-phosphate dehydrogenase}} \text{6-Phosphogluconate} \quad (3)$$

Table 11.1: Diagnostic enzymes and their potential uses.

Enzyme	Abbreviations	Use
Serum glutamic oxaloacetic Transaminases/ Aspartate transaminase	SGOT/AST	Heart Attack, Liver dysfunction
Serum glutamate-pyruvate Transaminases/ Alanine transaminase	SGPT/ALT	Liver dysfunction
Creatine Kinase	CK	Brain and muscle disorders
Urease		Renal function test

Cholesterol Oxidase		Hypercholesterolemia, Heart attack
Lactate Dehydrogenase	LDH	Myocardial Infraction
Alkaline Phosphatase	ALP	Bile and Liver diseases
Acid Phosphatase	ACP	Prostate Cancer
Glucose Oxidase		Diabetes Mellitus, Hyperglycemia

2. **Immunodiagnostics**: Enzyme immunoassay depends on both immunological and enzymatic reactions, and is classified as heterogenous or homogenous assays.

   - *Heterogeneous assays* use solid-phase support for immobilizing antigens or antibodies, requiring a step to separate analyte and reaction products before analysis using enzymes like peroxidase, β-galactosidase and alkaline phosphatase.

   - *Homogeneous assays:* In these type of assays, enzyme label activity is inhibited either directly through site-specific attachment of a hapten-antibody complex or indirectly by substrates, inhibitors, or cofactors. The free analyte competes for the bound antibody, releasing the inhibited enzyme reaction and generating a signal proportional to the analyte's concentration in the sample. This method simplifies the assay process, generating detection signals directly without separation steps, and offers rapidity, automation, along with minimal separation and washing steps.

3. ***DNA-diagnostics*****:** This method involves the multiplication of target molecules and is currently used for detecting nucleic acids like polymerase chain reaction and nucleic acid sequence-base amplification (NASBA). DNA diagnostics are rapidly developing, particularly for identifying genetic, malignant, and infectious diseases, as well as individuals using DNA finger-printing.

11.2.1 Enzymes as Analytical Agents

Enzymes are essential analytical reagents due to their specific nature and efficiency, which enable quantitative determination of substrate concentration. Enzyme metabolism is crucial for all species' survival, and abnormalities can lead to metabolic disorders. Enzymes' biocatalytic properties make them useful in diagnosing various diseases, as they are associated with specific changes in enzyme activity. Elevated enzyme levels in the blood can indicate liver damage, heart attacks, or muscle disorders (Table 11.2). Enzymes are also preferred markers for detecting diseases like myocardial infarction, jaundice, pancreatitis, cancer, and neurodegenerative disorders, providing insight into the disease process and response therapy assessment. Enzymes

are also components of the analytical agents that are used in the diagnosis of certain conditions. Examples of such enzymes are given in the Table 11.2.

Table 11.2: Examples of various enzymes being used as analytical agents

Enzyme	Diagnosis
Acid phosphatase	Prostrate carcinoma
Arginase	L- arginine levels in plasma and urine
ALP	Brain neoplasms
Alcohol dehydrogenase	Brain neoplasms
ALT	Hepatitis
Amylase, Cholinesterase lipase (LPS)	Pancreatic disease
Cholesterol esterase/oxidase	Hyper/hypolipedemia
Creatine kinase	Cardiac and skeletal malfunction
Glucose oxidase	Hyper/ hypoglycaemia
Glycerol-3- phosphate dehydrogenase	Triglycerols
Hexokinase/ Glucose-6- phosphate dehydrogenase	Hyper/ hypoglycaemia
Lactate dehydrogenase	Heart attack or liver disease
Lipoprotein lipase	Hyperlipedemia
Urease	Renal function assessment
Glycerol kinase	Hyperlipedemia
Uricase	Gout

SAQ 1

Do as Directed

a) Which of the following enzymes is involved in the diagnosis of gout?

i) Amylase

ii) Uricase

iii) Alcohol dehydrogenase

iv) Urease

b) Enzymes used for diagnostic purpose involve

i) Enzyme diagnostics

ii) Immunodiagnostics

iii) DNA diagnostics

iv) All of the above

c) ……. enzyme is used for the diagnosis of heart attack or liver disease (Fill in the Blanks).

d) Identification of genetic diseases involve …….. (Fill in the Blanks).

11.3 APPLICATIONS OF ENZYMES IN MEDICINE

Enzymes play a crucial role in medicine, assisting in diagnosis and treatment. Estimation or determination of the enzyme activities in serum is essential for differential diagnosis, disease prognosis, and early disease detection. As already discussed, enzymes are also vital in extracellular body fluids like blood plasma, urine, digestive juices, amniotic fluid, and cerebrospinal fluid. Enzymes leak through membranes due to energy imbalances, raising fluid levels in the circulatory system. Evaluation and assessment of these enzymes can help to identify damaged cells as well as to indicate the level and extent of injury. Enzymes as therapeutics are better than non enzymatic drugs on account of their specificity towards targets as well as multiple substrate conversion. Unlike drugs enzymes bind and act on their targets with great affinity. Moreover they are specific which make them potent drugs that have multiple therapeutic uses. Table 11.3 enlists some of these enzymes available that can be used for several types of treatment of diseases:

Table 11.3: Examples of enzymes used in the treatment of dieases

Enzymes	**Treatment**
Algucerase	Gauche's disease Type I
Amylase	Chronic pancreatitis and gastro intestinal disorders
Asparaginase	Acute childhood leukaemia
Bacterial asparaginase	Leukaemia

Enzymes	Treatment
Collagenase	Clean up wounds by removing dead tissues of severe burns and dermal ulcers
Chymotrypsin	Opthamalogy by dissolving the ligaments of the lens at the time of extraction
DNase	Cystic fibrosis
Elastase	Chronic pancreatitis and gastro intestinal disorders
Glutaminase	Leukaemia
Hyaluronidase	Promoting subcutaneously injected drug absorption
α-Lactamase β-Lactamase	Penicillin allergy
Lipase	Chronic pancreatitis and gastro intestinal disorders
Lysozyme	Antibiotic
Penicillinase	Penicillin allergy
Pepsin	Chronic pancreatitis and gastro intestinal disorders
Prolactazyme	Lactose intolerance
Ribonuclease	Antiviral therapy
Streptokinase	Myocardial infarction which dissolves purulent material or the clot
Trypsin peptidase	Chronic pancreatitis and gastro intestinal disorders
Uricase	Gout
Urokinase	Myocardial infarction which dissolves the clot

Therapeutic enzymes have a wide variety of specific uses:

- Oncolytics: Pegasparaginase (Oncaspar) is a medication used to treat patients hypersensitive to L-asparaginase, a substance that affects

malignant cells that rely on asparagine for survival. It exploits a metabolic defect in the asparagine synthesis of some malignant cells.

- Diphtheria toxin, an oncolytic enzyme, aids in protein synthesis by transferring NAD's ADP-ribose moiety to elongation factor 2, making it 100–10,000 times more sensitive in tumor cells compared to normal cells. Neuroaminidase removes sialic acid residues from the surface of neoplastic cells, thereby altering their immunogenicity and rendering them sensitive to immune responses.
- Thrombolytics (thrombolytic therapy): The enzymes in this category are streptokinase, tissue plasminogen activator (rt-PA), urokinase, retavase, tenecteplase, and TNK-tPA (TNKaseTM).
- Debridging agents effectively clean open wounds by removing foreign matter and any surrounding dead tissue.
- Proteolytic enzymes also help to degrade host during invasion such as:

  Trypsin (from mammalian pancreas) hydrolyzes peptide bonds involving arginine and lysine;

  Papain (from the leaves and the unripe fruit of the papaya tree) hydrolyzes peptide bonds involving the basic amino acids - lysine, arginine, and histidine.

  Colllagenase (from culture extracts of various animal cells or from various Clostridium species (pathogenic) breakdown collagen at its peptide bond regions under physiological pH and temperature condition.
- Antiinflammatory agents: Chymotrypsin (a zymogen produced in the pancreas) is converted to active form in the small intestine and Bromelians, which are plant proteases from pineapple stem or fruit, have anti-inflammatory properties due to their ability to break down protein-based inflammatory mediators.

Enzyme replacement therapy provides relief from the disorders by supplying missing or deficient enzymes in the body especially in persons with an inherited enzyme deficiency syndrome.

- Metabolic storage disorders due to insufficient activity of housekeeping enzymes.
- Gaucher's disease due to glucoce rebrosidase absence (isolated from human placenta) leads to glycolipid accumulation in cells, especially in macrophages.
- Fabry's disease in which kidney, heart, gastrointestinal tract and peripheral nerves are damaged. Fabrazyme restores levels of alpha-galactosidase A to provide relief from the disease.
- Patients suffering from pompe's disease in which the heart, skeletal muscles and brain are involved can seek relief by regular, lifelong infusions of alglucosidase alfa in the form of Lumizyme drug.

- Hurler's disease and Maroteaux- Lamy syndrome in which the eyes, liver, joints and skeleton are usually affected can be treated by Laronidase (Aldurazyme).

    - Digestive aids: Most digestive aid preparations are based on depolymerises responsible from the breakdown of polysaccharides, proteins and lipids. Such preparations may include a single enzyme or multiple enzymes. Some of the examples are given below:

- Alpha amylase hydrolyzes α-1,4-glycosidic bonds, and its oral administration is used to aid digestion.
- Lactase hydrolyzes lactose;
- Various proteolytic enzymes e.g., papain, pepsin;
- Pancreatin (extracted from the pancreas), are used in deficiencies related to the secretion of pancreatic enzymes (e.g., chronic pancreatitis, pancreatic carcinoma, cystic fibrosis).

SAQ 2

Do as Directed

a) Trypsin and papain fall in which of the following categories of enzymes?

i) Anti inflammatory agents

ii) Proteolytic enzymes

iii) Oncolytic enzymes

b) Enzyme replacement therapy is available for which of the following disorders?

i) Gaucher's disease

ii) Niemann Pick disease

iii) Mucolipidosis

iv) Metachromic leukodystrophy

c) …… enzyme is used in antiviral therapy (Fill in the Blanks).

d) …… is an example of anti inflammatory agent (Fill in the Blanks).

11.4 APPLICATIONS OF ENZYMES IN AGRICULTURE

Enzymes are crucial for growth and development in living organisms, including plants, and are increasingly important in agriculture. They play a role in organic matter transformation, nutrient cycle, and nitrogen uptake, increasing crop production, soil fertility, and food protection. Agricultural enzymes are

bioactive proteins that maintain soil health by balancing biological, chemical, and physical components. They break down plant residues and organic matter, providing nutrients and aiding in seed development. The vital role of enzymes in soil can help to downplay ecosystem disturbances thereby aiding sustainable soil management. Soil enzymes are used as indicators of soil health, fertility, and productivity, and can improve crop resistance to water stress and nutrient assimilation. They also act as natural herbicides, pesticides, and fertilizers to enhance crop growth and yield. Enzymes are also sprayed on animal farms to reduce infection and keep insects away.

The most important enzymes used in plant growth and soil fertility includes

Phosphatases, whichare crucial in the phosphorus cycle, aiding in plant growth by hydrolyzing phosphates and making phosphorus available in the soil.

Dehydrogenases are responsible for the biological oxidation of soil by hydrolyzing organic material and transferring hydrogen to inorganic acceptors.

Urease breaks down urea-based fertilizers in the soil, providing nitrogen components for plants and absorbing them for soil mineralization. It also decomposes ammonia, nitrite, and nitrate excreted by aquaculture animals, preventing toxic nitrogen waste accumulation in the pond water.

Amylase is a vital enzyme that breaks down starch-based organic substances from food waste, animal waste, and livestock products, improving the environment and habitat of domestic animals, and aid in their nutritional metabolism, digestion and growth.

Cellulase breaks down cellulose in plant cell walls into monosaccharides or polysaccharides. Most animals have limited fibre digestion, except herbivores and a few species like termites. Cellulase helps to hydrolyse fibre and carbohydrates in feed ingredients for aquatic animals, promoting nutrient absorption, health, and faster growth. It also treats plant-based organic substances, such as dead algae and wastewater from livestock and aquaculture activities. Only a few species produce cellulase to consume fibre.

Researchers have identified enzymes involved in producing auxin, a plant growth hormone that impacts cell division and flowering.

In agronomy, soil microbes and their enzymes are crucial for assessing soil health, fertility, and crop yield. They play a role in nitrogen, sulphur, and phosphorus cycling, as well as organic waste degradation. Enzyme-microbe complexes are sustainable ways to increase crop production and soil fertility, leading to improvement in the crop quality.

SAQ 3

Do as Directed

a) Which of the following enzymes is involved in providing nitrogen requirement to plants?

   i) Amylase

ii) Urease

iii) Cellulase

iv) Phosphatase

b) Cellulase breaks down cellulose in plant cell wall to

i) Monosaccharides

ii) Disaccharides

iii) Oligosaccharides

c) Phosphatase help in hydrolysing……..in the soil (Fill in the Blanks).

d) Microbes are preferred sources of enzymes than plants and animals (True/False).

11.5 APPLICATIONS OF ENZYMES IN RESEARCH

11.5.1 Immobilized Enzymes

Nowadays immobilized enzymes are being used increasingly in the industry and research due to their ability to be used continuously and reused without a difficult recovery process. You will read more in detail about the different processes of enzyme immobilization in the last unit of this course**.** Some of the examples of well-known immobilized enzymes and their applications are given in the Table 11.4

Table 11.4: Immobilized enzymes and their applications

Immobilized Enzyme	**Application**
Amino acid acylase	Resolution of racemic mixture
Aspartase L-Arginine deaminase Histidine ammonia lyase	Amino acid interconversion
α-Amylase	Production of corn syrup
β-Lactase	Production of lactose- free milk
Exo-1,4-α-D-glucosidase Xylose isomerase	Production of High -fructose corn syrup from starch

Immobilized Enzyme	Application
Flavoprotein oxidase	Nitrogen oxidation of drugs containing hydrazine groups
Flavoprotein oxidase	Production of prednisolone
Glucose coamylase	Production of glucose from corn syrup
Glucose oxidase	Fabrication of Biosensors e.g., Glucose biosensor
Invertase	Production of invert sugar
Invertase Inulinase	Conversion of sucrose into glucose and fructose
L-amino acid oxidase	D-amino acid production
Lipase	Production of cocoa butter substitute
Nitrile hydratase	Production of Acrylamide
Pectinate	Treatment of fruit juice
Penicillin amidase	Production of precursors of semi-synthetic penicillin
Protease	Hydrolysis of whey proteins, cheese ripening
Ribonuclease	Nucleotide production from RNA
Tannase	Hydrolysis of tannins
Thermolysin	Production of Artificial Sweetening Agent
Urease	Estimation of urea

11.5.2 Biocatalytic Applications

Immobilized enzymes from microorganisms have high catalytic efficiencies with a wide range of extensive applications (Table 11.5).

Table 11.5: Immobilized enzymes as biocatalytic agents

Immobilized Enzyme	Applications
Alcohol dehydrogenase	Biotransformation
Acetylation	Biotransformation in the production of antibiotics
Cytochrome P450 (Drug Metabolising Enzymes)	Biotransformation of drugs in the body
Hydroxylation	Biotransformation of steroids

11.6 DIAGNOSTIC ENZYMES

Diagnostic enzymes are enzymes used for disease detection, diagnosis, or prognosis. They are used as reagents to determine normal and pathological components in substances like serum, urine, and gastric juice. These enzymes are often used in assays and tests to diagnose diseases, monitor health conditions, or evaluate the effectiveness of treatments. The enzymes are divided into three groups:

-*Cellular enzymes:* These enzymes enter blood from various organs, and their activity in serum depends on their content, molecular weight, intracellular localization, and rate of elimination.

-*Secretory enzymes*: These enzymes are synthesized by cells and then enter the blood stream, and fulfill their specific functions in the circulatory system. These are the enzymes of the coagulation system and fibrinolysis, choline esterase, etc.

-*Excretory enzymes*: These enzymes are synthesized by the glands of the GIT and enter the blood (e.g., amylase, lipase).

Enzymes, located in various cellular compartments, can indicate the severity of cellular damage. Diseased states often cause moderate or extensive tissue damage. The bloodstream releases enzymes specific to diseased organs, exhibiting enhanced activity. Measurement of enzyme activities in blood, plasma, or other body fluids is used for diagnosis, as they exhibit better sensitivity and accuracy, and changes in enzyme activity can be detected earlier than clinical signs or other diagnostic indicators.

Commonly used enzymes in clinical practice aid in diagnosing, prognosing, and monitoring pathologies. Their determination in blood serum has high clinical significance. Some of the well-known examples of diagnostic enzymes are:

11.6.1 Amino Transferases (Transaminases)

These are a group of enzymes that carry out the interconversion of amino acids and oxyacids by transferring amino groups. Two types of amino transferases are used in diagnostic enzymology. These are alanine amino transferase (ALT), formally called glutamate pyruvate amino transferase (GPT), and aspartate amino transferase (AST), formerly called glutamate oxaloacetate amino transferase (GOT).

Alanine amino transferase (ALT): ALT speeds up reversible transamination of L-amino acid and 2-oxoglutamate into pyruvate and glutamate inside the cell's cytoplasm. ALT is a key indicator of liver, heart, and muscle damage. It's a key marker in diagnosing liver disorders like hepatitis, hepatocirrhosis, viral hepatitis, and toxic liver necrosis. Elevated ALT levels are linked to an increased risk of hepatocellular carcinoma. In healthy adults, ALT concentrations range from 5–35 U/L, while higher levels indicate diseased organs.

Aspartate amino transferase (AST): AST is an enzyme that converts L-aspartate and 2-oxoglutarate into oxaloacetate and glutamate. It is found in various tissues and can be used to monitor heart and muscle diseases. The AST-to-platelet ratio index can also be used to screen for liver fibrosis in chronic hepatitis B. Elevated AST levels are more specific than ALT in evaluating hepatic disorders in large animals, but ALT plasma levels may exceed those of AST in hepatitis.

11.6.2 Alkaline Phosphatase (ALP)

ALP is an enzyme that breaks down phosphate ester bonds from nucleotides and proteins under alkaline conditions, leading to its increased serum levels. It is primarily produced in the liver, bones, intestine, and kidney. The placenta in pregnant women produces ALP. Serum ALP levels are higher in children and correlate with bone growth rates. Men have slightly higher ALP values than women, but after age 60, they are equal to or higher than women. ALP concentrations increase during puberty, pregnancy, and after menopause. Serum ALP activity is increased in bone and liver diseases, including osteomalacia, rickets, Paget's disease, carcinoma, cholestasis, lesions, tumours, hyperthyroidism, and hyperparathyroidism. Higher levels of ALP are also linked to hepatic fibrosis in patients with NASH and type 2 diabetes. Low ALP activity is less common and more likely to be related to genetic conditions or nutritional deficiency.

11.6.3 Acid Phosphatase (ACP)

Acid phosphatase, a protein found in the body, is primarily found in the prostate gland, where it is 100 times more abundant than in other tissues. It is used to monitor prostatic cancer progress. There are five types of ACP, including prostatic, erythrocytic, macrophagic, lysosomal, and osteoclastic. ACP is more concentrated in semen, making it a common test for rape victims. Increased ACP activity is also observed in patients with Gaucher's disease.

11.6.4 Lactate Dehydrogenase (LDH)

This enzyme is present in all body cells but is most abundant in the liver, heart, pancreas, and kidney, catalyzing the reversible interconversion of pyruvate and lactate. There are five well defined LDH isoenzymes according to the type of tissues in which they reside:

LDH1 in the heart and red blood cells;

LDH2 in white blood cells;

LDH3 in the lungs;

LDH4 in the kidney; and

LDH5 in the liver and skeletal muscles.

Serum electrophoresis is the primary method for identifying isoenzymes, which are tetramers of H and M, which can form different isoenzymes. Elevations of LDH1 and LDH2 are common after myocardial infarction, acute leukaemia, and liver or skeletal muscle damage.

LDH gene expression is unregulated in various human malignant tumours, including colorectal, lung, breast, oral, prostate, germ cell, and pancreatic cancers. It serves as a prognostic marker for acute leukaemia and sickle cell disease, and is crucial for testicular germ cell malignancy monitoring.

11.6.5 Creatine Kinase (CK)

Creatine kinase (CK) is an intracellular enzyme that converts creatine phosphate to ADP to generate ATP after muscle cell depletion. It consists of three isoenzymes: CK-MM, CK-MB, and CK-BB. CK-MM is found in skeletal muscles and increases in cases of heart, brain, and skeletal damage. CK-MB is present in the heart and increases after heart attacks, inflammation, and muscle dystrophy. CK-BB is mostly found in the brain and increases in cases of brain injury, meningitis, abnormal cell growth, stroke, and hyperthermia. CK levels can increase to up to 2.0 U/ml during myocardial infarction, muscular dystrophy, and inflammatory reactions.

11.6.6 Amylase

Salivary alpha (α) amylase is a digestive enzyme found in high concentrations in pancreatic juice and saliva. It cleaves starch and glycogen into maltose, making it a sensitive marker in acute pancreatitis. Additionally, it serves as a biomarker for heart failure, chronic stress, and kidney function monitoring. Some important enzymes of clinical significance are listed in the Table 11.6:

Table 11.6: Distribution and application of clinically important enzymes

Enzymes	Tissues	Clinical applications
5‘- nucleotidase	Liver	Pancreatic diseases
Alanine amino transferase	Liver	Hepatoparenchymal diseases

Enzymes	Tissues	Clinical applications
Alkaline phosphatase	Liver, bone, intestinal mucosa, placenta	Liver and bone diseases
Amylase	Salivary glands, pancreas	Pancreatic diseases
Aspartate amino transferase	Liver, skeletal muscle, heart, erythrocytes	Hepatic parenchymal disease, muscle disease
Cholinesterase	Liver	Organophosphorus insecticide poisoning
Creatine kinase	Skeletal muscle, heart	Muscle disease
Gamma glutamyl transferase	Liver	Hepatobillary disease, Marker alcohol abuse
Lipase	Pancreas	Pancreatic diseases
LDH	Heart, liver, skeletal muscle, erythrocytes, lymph nodes, platelets	Hepatoparenchymal diseases, muscle diseases, hemolysis, tumour markers

SAQ 1

Do as Directed

a) Which of the following enzyme is not used to diagnose hepatobilliary disease?

   i) Alkaline phosphatase

   ii) 5'-nucleotidase

   iii) Alanine aminotransferase

   iv) α-glutamyl transferase

b) The group of enzymes that catalyze the interconversion of amino acids to keto acids by transfer of amino groups is referred to as

   i) Alkaline phosphatase

   ii) 5'-nucleotidase

   iii) Transaminase

   iv) α-glutamyl transferase

c) …… hydrolyses α-1,4-linkages in starch and glycogen to yield maltose (Fill in the Blanks).

d) An increase in serum level of LDH1 relative to LDH2 is an indication of …….. (Fill in the Blanks).

11.7 ENZYME IMMUNOASSAY

How diagnostic enzymes are used in clinical laboratories? What do you think? The answer is that several labs run ELISA assays using diagnostic enzymes to determine or to monitor the presence of specific antigens or antibodies in a given sample. Enzyme immunoassay methods are sensitive and versatile, serving as an alternative to radioisotopes in immunoassays. They can help to determine protein and hormone levels, and are not hazardous to health. Enzymes can be detected using more accessible techniques. Two common examples of enzyme immunoassay (EIA) procedures are:

-Enzyme- linked immunosorbent assay (ELISA)

- Enzyme multiplied immunoassay test (EMIT)

11.7.1 ELISA

ELISA is a solid-phase enzyme immunoassay used to detect the presence of a specific protein in blood samples. It uses enzymes like ACP, Horse Radish Peroxidase (HRP), lactoperoxidase, and α-galactosidase to detect the binding of an antigen or antibody.

- *Antigen-antibody reactions,* in which the presence of antigen or antibody is detected in a sample;

  Enzymatic chemical reactions determine antigen or antibody quantity by determining the antigen-antibody complex formation rate catalyzing a colorless substrate to produce a colored product.

- *Signal detection and quantification*: the intensity of the colored product generated by the enzyme and substrate is detected and measured.

ELISA serves as a diagnostic tool for diagnosing both non-infectious diseases such as hormones, drugs, and autoimmune ones, and infectious diseases such as HIV, Lyme disease, tuberculosis, Rocky Mountain spotted fever, rotavirus, squamous cell carcinoma, syphilis, and toxoplasmosis.

11.7.2 EMIT

The enzyme multiplied immunoassay technique (EMIT) is a method used for determining therapeutic and recreational drugs and proteins in serum and urine. It involves a drug competing with an enzyme to bind to an antibody, with an increased drug concentration causing a color change.

Immunoassays are used in life science research to track proteins, hormones, and antibodies, and in industry to detect contaminants in food and water. They differ from enzyme-linked immunosorbent assays (ELISA) in two ways:

enzymes play a key role throughout the assay process and require little enzyme technology knowledge. The EMIT therapeutic drug monitoring tests provide accurate information on drug concentrations, such as immunosuppressant drugs and antibiotics.

SAQ 5

Do as Directed

a) Which of the following techniques are used in enzyme immunoassay?

   i) Immunofluorescence

   ii) Radio immunoassay

   iii) ELISA

b) In an enzyme immunoassay, the enzyme

   i) is bound by the antibody's antigen-binding site

   ii) is attached to the wall of micro titre plate

   iii) is conjugated to the suspect antigen

   iv) is bound to the constant region of the secondary antibody

c) ELISA allows for rapid screening and quantification of the presence of…… in the sample (Fill in the Blanks).

d) ELISA stands for …….(Fill in the Blanks).

11.8 ENZYME THERAPY

Enzyme therapy is the use of enzymes to treat medical conditions such as enzyme deficiencies and other health issues. In the enzyme therapy, enzymes are added to change the existing abnormal or diseased state owing to the lack or short supply of same enzyme in the body. Enzymes are given most likely in an immobilized manner but biodegradable form. Enzymes aid in digestion, detoxification, strengthens immune system, muscle contraction, and reduces stress on vital organs like the pancreas. They have numerous applications, including treatment of pancreatic insufficiency, cystic fibrosis, metabolic disorders, lactose intolerance, and cancers. Enzyme therapy has been involved in the degradation of necrotic tissue by usage of proteolytic enzymes such as trypsin or chymotrypsin. Enzymes can be administered orally, topically, or intravenously and can be non-systematic or systematic.

Enzymes have also been used as drugs in the treatment of specific medical problems.

- **Streptokinase,** an enzyme from Streptococcus, is crucial for clearing blood clots in the lower extremities and treating heart attacks by activating the fibrinolytic proenzyme plasminogen, which attacks fibrin and breaks it down into soluble components.

- **Asparaginase therapy** is used for certain adult leukaemia types, as tumour cells require asparagine, which is depressed by administering asparaginase, thereby affecting their viability.
- **Micro-plasminogen activator**, formerly urokinase, is an enzyme extracted from human urine that can be infused into the bloodstream of individuals at risk for pulmonary embolism. It stimulates a cascade system for active plasmin, a proteolytic enzyme that digests fibrin.
- **Immobilized enzymes:** Artificial kidney machines use immobilized enzymes to remove waste products from the body, such as urea, due to kidney disease. Urea enters the machine through harm-dialysis, converted to CO_2 and NH_4^+ by immobilized urease, and toxic NH_4^+ is trapped on resins or incorporated into glutamate for coenzyme recycling before returning to the bloodstream.
- Enzyme therapy has been used in the diagnosis of necrotic tissue using proteolytic enzymes such as **trypsin** or **chymotrypsin**.
- **Adenosine deaminase (ADA)** is utilized in treating inherited immunological disorders by breaking down excess adenosine in patients' blood, thereby reducing the damage to the immune system.
- **α-glucocerebrosidase**, an enzyme involved in glycolipid metabolism, causes Gaucher's disease, a lysosomal storage disorder characterized by glucocerebroside accumulation due to gene mutations.
- **Sacrosidase** treats congenital sucrose-isomaltase deficiency (CSID), enabling patients to digest sucrose, a disaccharide, and consume sucrose-containing foods, thereby improving their digestive abilities.
- Enzymes are used as **digestive aids,** i.e., to cure the digestive problems induced by some sugars.

- The α-galactosidase enzyme is used as a digestive aid for individuals experiencing bloating, gas, and diarrhea after consuming foods like beans and Brassica vegetables. It breaks down the terminal α-galactosidic residue of sugar substrates, potentially causing discomfort due to bacterial fermentation.

- Lactose supplements can treat lactose intolerance, a condition where insufficient lactase enzyme breaks down milk sugar lactose into glucose and galactose.

- PKU is an inheritable disorder caused by the absence of the phenyl alanine hydroxylase enzyme, which converts phenyl alanine to tyrosine. The recombinant yeast phenyl alanine ammonia lyase (PAL) is an oral treatment for this disorder.
- Lysozyme, a naturally produced bactericidal enzyme, has been found to selectively degrade viral RNA, making it a promising candidate for HIV treatment.

Limitations

The bulky structure and large molecular weight of enzymes exclude them from intracellular domains, and their high antigenicity causes rapid clearance from blood plasma. Parenteral enzymes require extensive purification which leads tohigher costs.

Enzymes and Inborn errors of Metabolism: Inborn errors of metabolism occur due to genetic mutations and are characterized by the loss of enzyme activity. More than 400 errors are known but they occur rarely, for example phenylketonuria, 1 in 12000 births; cystinuria, cystinuria, 1 in 14,000; galactosemia, 1 in 57,000. These errors are identified by enzyme assays or the presence or absence of particular metabolite in blood, tissue, urine or other biological samples. The advent of recombinant technology has led to the development of highly accurate and sensitive diagnostic procedure for determining enzyme activities. It is feasible to obtain a pre-natal diagnosis by carrying out enzyme assays in the amniotic fluid. Some examples of inborn errors of metabolism due to enzyme deficiencies are given in the Table 11.7.

Table 11.7: Enzyme deficiency and inborn errors of metabolism

Inborn Error	Enzyme Deficiency
Alkaptonuria	Homogentisate1,2 –dioxygenase
Phenylketonuria	Phenylalanine 4 monooxygenase
Galactosemia	Galactose 1-phosphate uridyltransferase
Glycogen storage disease type V	Glycogen phosphorylase
Tay-Sachs	β-N-Acetyl-D-hexosaminidase

Treatment of inborn errors of metabolism usually involves reduction in the intake of substances that cannot be metabolized normally or supplementation of special diets to the patients. However, this kind of treatment is ineffective because of endogenous synthesis of metabolites in question or due to blockage of particular metabolic pathway vital for the body function. A successful treatment is possible only when normal enzyme could be administered at the appropriate site for example urokinase plasminogen activator (uPA) extracted from human urine can be infused in the patients suffering from pulmonary embolism. L-Asparaginase is also used in the treatment of leukemia.

SAQ 6

Do as Directed

a) Which of the following enzymes are not used in the treatment of gastrointestinal tract disorder and chronic pancreatitis?

   i) Pepsin

   ii) Cellulase

   iii) Amylase

   iv) Trypsin

b) Bacterial asparaginase is used in the treatment of

   i) Cleaning of wounds

   ii) Venus thrombosis

   iii) Leukemia

   iv) Traumatic or post operative edema

c) To dissolve blood clots during myocardial infarction, ……. enzyme is used (Fill in the Blanks).

11.9 Taq POLYMERASE

Taq polymerase itself isn't generally used directly as a diagnostic enzyme in the similar manner as enzymes like lactate dehydrogenase or creatine kinase, it plays a vital role in several diagnostic applications through its usage in PCR-based methods.

11.9.1 Characterization of Taq DNA Polymerase

Taq DNA polymerase is a heat-stable enzyme from *Thermus aquaticus*, with the highest activity at a pH of 9 and 75ºC. It incorporates nucleotides at 2-4 kilobases per minute and was discovered in Yellowstone National Park in 1969. Despite lacking 5' to 3' and 3' to 5' exonuclease activities, it is ideal for manual and automated DNA sequencing due to its fast, progressive nature, and broad temperature range.

- *Size, activity, and fidelity*: Taq, a DNA polymerase, has a C-terminus activity and 5' to 3' exonuclease activity, with an approximate size of 94 kDa.
- *Temperature dependency*: Taq's optimal temperature range is 75-80ºC, where its catalytic activity can reach 150 nucleosides per second due to its temperature-dependence.
- *Thermostability*: Taq, a heat-stable PCR tool, can be used in hot-start protocols, but its half-life decreases rapidly at high temperatures, causing

PCR protocols with denaturing steps at 95ºC to denature a portion of the Taq.

- *Monovalent and divalent cation dependencies*: Monovalent and divalent ions, like K^+ and Mg^{2+}, neutralise DNA's negative charges, reducing repulsive interactions. They are added in salt forms like KCl and $MgCl_2$, respectively. Divalent cations, like Mg^{2+} or Mn^{2+}, act as cofactors during polymerization, with $MgCl_2$ concentrations around 2 mM.
- *pHdependency*: The enzyme's optimal pH range is 8-9.4 units, varying with the buffer system. Common commercially available Taq buffers typically contain 10-50mM Tris-HCl for pH stability.

11.9.2 Applications

Taq polymerase is generally not considered a diagnostic enzyme per se but being integrated to PCR based diagnostic methods, it becomes essential for several range of molecular diagnostic applications. Some of these molecular applications are:

1. **PCR Amplification:**
    - **Disease Detection:**In clinical laboratories, during PCR, Taq polymerase is used to amplify DNA sequences from several pathogens (bacteria, viruses, etc.) in samples. For example, PCR can identify the presence of viral DNA in infections such as HIV, hepatitis, or SARS-CoV-2 (the virus causing COVID-19).
    - **Genetic Testing:** PCR can amplify specific genes or genetic mutations. It will be extremely helpful in diagnosing genetic disorders, determining hereditary conditions, thereby guiding personalized medicine.
2. **Quantitative PCR (qPCR):**
    - **Quantification of Nucleic Acids:** qPCR as the name suggests permits quantitative measurement of DNA or RNA in a sample. It uses Taq polymerase to determine and assess the viral load in infections, measurement of gene expression levels, or determination of the concentration of a specific genetic marker.
3. **Molecular Diagnostics:**
    - **Pathogen Identification:** PCR assays using Taq polymerase can identify definite pathogens by amplifying their exclusive genetic material. This application helps diagnose infectious diseases quickly and accurately.
    - **Biomarker Detection:** PCR-based techniques can detect biomarkers related to diseases, such as cancer-related gene mutations or specific bacterial or viral DNA.

11.9.3 Limitations

- Taq's performance is limited in challenging applications like high-fidelity synthesis of long amplicons and amplification of GC-rich sequences.
- Taq DNA polymerase's low disincorporation rate, estimated between one and 10,000 bases, is due to its lack of 3'-5' proof-reading activity.

SAQ 7

Do as Directed

a) Taq polymerase is a ——- polymerase

i) Heat stable

ii) Buffering

iii) Denaturant

iv) Large

b) …….. is used to extract the polymerase for PCR (Fill in the Blanks).

c) qPCR as the name suggests permits quantitative measurement of …….. in a sample (Fill in the Blanks).

11.10 ENZYME ELECTRODES AND ENZYME AS BIOSENSORS

Biosensors, or enzyme electrodes, are electronic devices that sense and analyse biological information using enzyme specificity and immobilization techniques. Biosensors have found an extensive usage in the analytical chemistry. They are used by auto analysers to estimate biochemical and pathological quantities in biological samples like plasma, serum, urine, and cerebrospinal fluid by converting the biological response to an electronic signal which is amplified, processed and displayed. Biosensor technology uses enzyme specificity and reactivity to transform metabolites into products, determining substrate usage or product composition. Biosensors offer reliability, sensitivity, accuracy, ease of handling, and low cost compared to conventional detection methods, making them ideal for biomedical applications like medical diagnostics, pathogen detection, food safety control, and environmental monitoring.

11.10.1 Components of a Biosensor

A biosensor is an analytical device that uses a biological or biologically derived sensing material to detect an analyte. It consists of a *biorecognition* device and a *biotransducer*, which converts biochemical activity into electrical energy (Fig.11.1). The analyte diffuses into the enzyme layer, where catalytic reactions occur. The signal is processed by a *processor* and displayed on a

screen, allowing for a better understanding of the analyte's presence and concentration.

The immobilization method, including adsorption, cross-linking, and self-assembly, significantly impacts enzyme activity and biosensor sensitivity, with enzymes incorporated into carbon-paste, polymers, and gels.

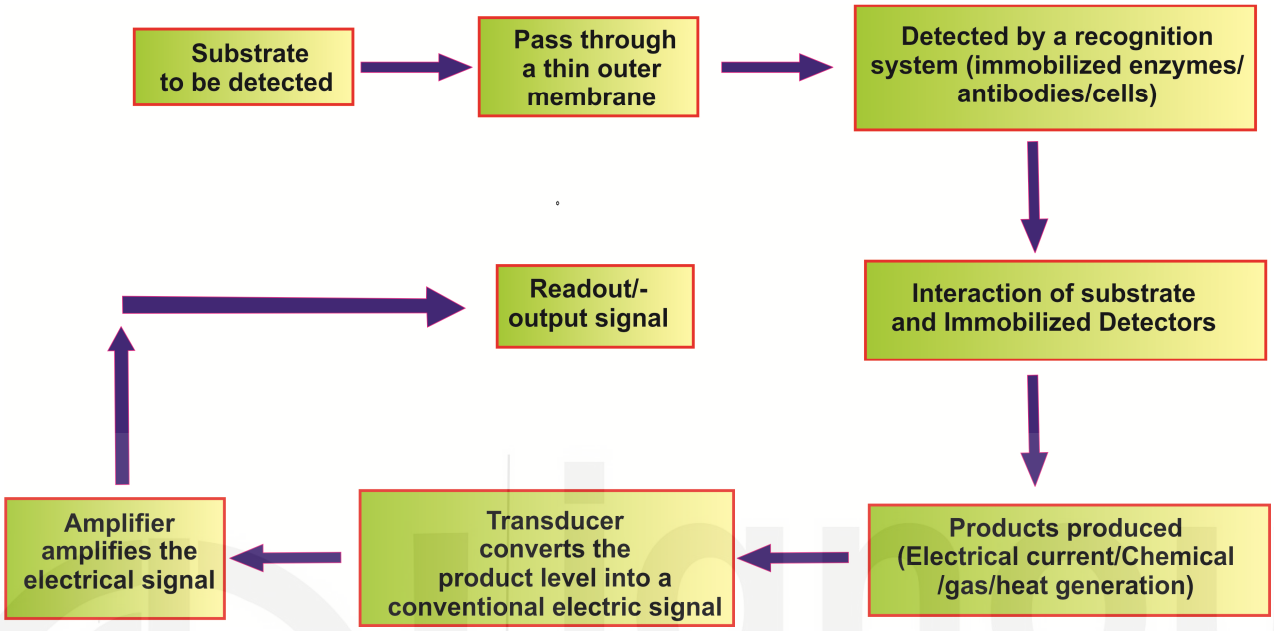

Fig.11.1: Components of Biosensor.

Enzyme-based biosensors have been developed over time, using various transducer systems and various shapes and sizes, as shown in Table 11.8.

Table 11.8: Types of Biosensors

Transducer	Example
Calometric	Thermistor, Thermopile
Electrochemical	Clark electrode, Mediated electrode, Ion selective electrode
Magnetic	Bead-based devices
Optical	Photodiode, Waveguide system, Integrated optical devices
Piezoelectric	Quartz crystal microbalance (OCM), Surface acoustic wave (SAW) devices

11.10.2 Types of Biosensors

There are several types of biosensors having different applications:

- *Calorimetric biosensor*: Calorimetric biosensors measure temperature changes in turbid and strongly colored solutions but maintain a constant sample temperature, making them widely applicable.

- *Potentiometric biosensor*: The altered electron distribution at the transducer surface produces an electrical potential, converting the biological reaction into an electric signal.
- *Amperometric biosensors*: Amperometric biosensors detect electron movement through redox reactions, producing current proportional to substrate concentration. They reduce reagent costs and enhance measuring devices' amplification.
- *Optical biosensor*: Optical biosensors measure light produced or absorbed during a reaction by analysing the change in fluorescence or absorbance caused by catalytic reactions.
- *Acoustic biosensors*: Piezoelectric devices, also known as transducers, detect changes in the mass of biological components due to reactions. Since they are covered in antibodies, they bind to complementary antigens in sample solutions. This lowers the vibrational frequency and helps to determine the amount of antigen present.

11.10.3 Examples of Biosensors

Some of the widely used biosensors are discussed below:

Biosensors for Blood Glucose:

The biosensor is a valuable tool for diabetics, measuring blood glucose concentration. They are of different types:

- Glucose dehydrogenase-based glucose biosensors,
- Cellulose dehydrogenase-based glucose biosensors,
- Glucose-oxidase-based glucose biosensors.

Diabetic patients must monitor their blood glucose levels to detect potential hyperglycemia and hypoglycemia. Glucose measurements involve the interaction of enzymes like hexokinase, glucose oxidase (GOD), and glucose-1-dehydrogenase (GDH). GOD (immobilized on a polyacrylamide gel), used in biosensors, catalyzes the oxidation of α-D-glucose to gluconic acid using molecular oxygen with simultaneous production of H_2O_2.

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{Catalase}} 1/2\ O_2 + H_2O$$

Glucose concentration can be measured through O_2 consumption or H_2O_2 production, and temperature rise due to energy released in reactions.

Biosensors for the measurement of urea in blood or urine: Researchers have developed sensors based on urease enzyme to accurately measure urea in urine or blood samples for diagnosing renal and liver disorders, as urease converts urea to hydrogen carbonate and ammonium ions.

$$(NH_2)_2CO + 2H_2O + H^+ \rightarrow 2NH_4^+ + HCO_3^-$$

The urease biosensor has been developed by immobilizing urease in a bovine serum albumin membrane on the surface of an ion-sensitive field-effect transistor (ISFET).

Biosensors for lactic acid: Lactate sensors, based on lactic acid dehydrogenase, detect oxygen deficits in muscles and liver, indicating tissue oxygenation and warning of ischemic conditions. Surgical procedures, intensive therapy, sports medicine, and spatial medicine utilize these biosensors. Different biosensors use immobilized lactate monooxygenase (LMO) and lactate oxidase (LOD) for monitoring lactate levels. Two other types of lactate biosensors are

- *Ex vivo biosensors,* equipped with microdialysis or ultrafiltration probes that measure blood lactic acid by diffusing it into the dialysate/filtrate for external measurement.
- *In vivo biosensors* measure blood lactate concentration directly, responding quickly to lactate level changes, either through skin placement or subcutaneous implantation.

The rapid response to lactate concentration changes is comparable to that of ex vivo biosensors, but biocompatibility requirements are more stringent.

Biosensor for Creatinine: These are based on creatine amidinohydrolase, sarcosine oxidase, and creatine amidohydrolase. Creatinine estimation is crucial for diagnosing renal, thyroid, muscle function, acute myocardial infarction, and hemodialysis therapy. Creatinine biosensors use creatinine deaminase, which catalyzes a reaction, making them essential for diagnosing acute myocardial infarction and for hemodialysis therapy. Creatinine biosensors generally employ creatinine deaminase, which catalyses the following reaction.

$$\text{Creatinine} + H_2O \longrightarrow \text{N-Methylhyadantoin} + NH_4^+$$

Allosteric enzyme based biosensor: Allosteric enzymes regulate catalytic activities by binding effector molecules to different receptor sites, forming easily detectable products and serving as biosensors.

Modular engineering can integrate allosteric enzymes into biosensors, using their receptor site as the recognition element and their active site as the transducer. However, most of their modulators are not analytically relevant, making them unsuitable for direct use. Some of the biosensors are based on allosteric enzyme such as

- α-galactosidase
- Alkaline phosphatase
- L-Lactamase
- Neutral protease
- Ribozymes

A summary of some recent applications of enzyme-based sensors in clinical diagnosis is presented in the Table 11.9.

Table 11.9: Examples of enzyme- based biosensors

Sensor	Immobilized enzyme
Acetyl choline and Choline	Acetyl choline esterase, Choline oxidase
Amino acid	Amino acid oxidase
Arginine	Arginase
Asparagine	Asparaginase
Bilirubin	Hemoglobin, Glucose oxidase
Carnitine	Carnitine dehydrogenase and diaphorase
Cholesterol	Cholesterol oxidase
Creatine and Creatinine	Creatinase, Creatininase, Sarcosine oxidase
Ethanol	Alcohol Dehydrogenase
Glucose	Glucose oxidase/Glucose dehydrogenase
Glutamate	Glutamate oxidase/Glutaminase
Lactate	Lactate oxidase/Lactate dehydrogenase
Oxalic acid	Oxalate oxidase
Penicillin	Penicillinase
Theophylline	Theophylline oxidase
Triglycerides	Glycerol kinase and glycerol phosphate oxidase
Urea	Urease
Uric acid	Uricase
γ-Amino butyric acid	Catalase, Glutamate oxidase

11.10.4 Advantages of Enzyme Biosensors

Enzyme biosensors offer a number of advantages:

- Negligible enzyme consumption, which is in the range of a few milliunits per sample;
- Ability to quantify the diagnostically important metabolites without purifying them from other impurities that may otherwise interfere with the diagnosis;
- Advantage of measuring labile metabolites without degradation as it happens with chemical methods;
- Highly specific as compared to cell-based sensors;
- Short reaction time, or faster response.

11.10.5 Disadvantages of Enzyme Biosensors

- The high cost of purified enzymes may prohibit the use of diagnostic methods compared to other diagnostic tools,
- Inactivation of enzymes during the enzymatic process by inhibitors present in the sample or other ingredients.
- They are too expensive to produce as an additional step in isolating the enzyme.
- The stability of enzymes is hindered during isolation.
- Cofactors are required for the detection of substances.

11.10.6 Limitations of Enzyme Electrodes

Enzyme electrodes are simple to operate, allowing for simple sample pre-treatment and analysis, but they have several limitations.

- *Optimal sample frequency*: Enzyme-electrode-based analysis has a maximum sample throughput of 100 samples per hour, with response based on cell compartment concentration-time profile and internal diffusion time at high loading.
- *Extension of new substances*: The number of substances directly determined with a monoenzyme electrode is limited due to electrochemical inactivity in many enzyme-catalyzed reactions, necessitating readily measurable substances to be formed sequentially or parallel to analyte conversion.
- *Adapted sensitivity*:The sensitivity of an enzyme electrode can be improved by substrate amplification, which involves adjusting operational conditions to enable one enzyme to regenerate the substrate of the other, using oxidase and dehydrogenase couplings.

SAQ 8

Do as Directed:

a) Which of the following is not a characteristics feature of biosensor?

i) The biocatalyst used in the biosensor must be highly specific for the purpose of the analyses.

ii) They are simple to operate.

iii) Active site is mainly constituted by non-polar amino acids for catalysis to take place.

iv) The response from the biosensors should be accurate, precise, reproducible and linear.

b) Immobilized enzymes are more preferred over free enzymes in producing biosensors. (True/False)

c) When the physical change produces in a biosensor is due to the movement of electrons produced in the redox reaction, the biosensor is referred to as …… (Fill in the Blanks).

d) In the biosensor, …….. processor is one which involves subtracting a 'reference' baseline from the sample signal (Fill in the Blanks).

11.11 SUMMARY

Let us summarize:

- Enzymology includes the diagnostics of enzymes as a subfield. The methodology encompasses two primary objectives: firstly, the utilization of enzymes as reagents to identify normal and pathological components in biological samples (e.g., gastric juice, urine, and serum), thereby functioning as indicators of cellular damage; and secondly, enzymatic activity measurement in biological material.
- These days there is an increasing trend towards the use of enzymes in clinical diagnosis, and in enzyme therapy. These trends are due to increased availability of the sophisticated automated equipment in clinical laboratories.
- A wide variety of purified enzymes are becoming commercially available in larger quantities due to recombinant DNA technology using bacterial and other expression systems, and many of these have been successfully immobilized to support materials. Therefore assay of metabolites and enzyme replacement therapy is becoming more economical.
- Immobilized enzymes are being used increasingly in the industry and research due to their ability to be used continuously and reused without a difficult recovery process.

- Diagnostic enzymes are the enzymes that are used for disease detection, diagnosis, or prognosis. They are used to determine normal and pathological components in substances like serum, urine, and gastric juice. These enzymes are often used in assays and tests to diagnose diseases, monitoring of health conditions, or evaluation of the effectiveness of treatments.
- Agricultural enzymes are bioactive proteins that maintain soil health by balancing biological, chemical, and physical components. They break down plant residues and organic matter, providing nutrients and aiding in seed development.
- Enzyme immunoassay methods are sensitive, versatile, and are used to determine protein and hormone levels. Two common examples of enzyme immunoassay (EIA) procedures are Enzyme- linked immunosorbent assay (ELISA) and Enzyme multiplied immunoassay test (EMIT).
- Enzyme therapy involves the usage of enzymes to treat medical conditions such as enzyme deficiencies and other health issues. In the enzyme therapy, enzymes are added to change the existing abnormal or diseased state owing to the lack or short supply of same enzyme in the body.
- Biosensors, or enzyme electrodes, are electronic devices that sense and analyse biological information using enzyme specificity and immobilization techniques. Biosensors have found an extensive usage in the analytical chemistry. They are used by autoanalysers to estimate biochemical and pathological quantities in biological samples like plasma, serum, urine, and cerebrospinal fluid by converting the biological response to an electronic signal which is amplified, processed and displayed.

11.12 TERMINAL QUESTIONS

1. How enzymes help in the diagnosis of a disease? Give examples.
2. Discuss the therapeutic applications of enzymes.
3. How do enzymes help in the growth and development of plants?
4. What are diagnostic enzymes? Discuss with examples.
5. Write a note on ELISA.
6. What is meant by enzyme therapy? Give examples.
7. Discuss the characteristics of Taq polymerase.
8. Explain the components of a biosensor.

11.13 ANSWERS

1. a) (ii), b) (iv), c) Lactate dehydrogenase, d) DNA diagnostics
2. a) (ii), b) (i), c) Ribonuclease, d) Chymotrypsin
3. a) (ii), b) (i), c) phosphates, d) (i)
4. a) (iii), b) (iii), c) Alpha amylase, d) myocardial infarction
5. a) (iii), b) (iv), c) antigen, d) Enzyme-linked immunosorbent assay
6. a) (iv), b) (iii), c) streptokinase
7. a) (i), b) Thermus aquaticus, c) DNA or RNA
8. a) (iii), b) True, c) amperometric biosensor, d) signal

Terminal Answers

1. Discussed in Section 11.2
2. Enzymes have been widely utilized in the therapeutic industry of the present day and are essential for the detection, prevention, and biochemical analysis of severe diseases. The treatment facility has been significantly enhanced through the use of enzymes, which also aid in the acceleration of biochemical reactions within living organisms and the improvement of the human body's emulative system. Examples are discussed in Section 11.3.
3. Refer to Section 11.4.
4. Refer to Section 11.6.
5. Refer to Section 11.7.1.
6. Discussed in Section 11.8.
7. Discussed in Section 11.9.1.
8. Discussed in Section 11.10.1.

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