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Enzyme Electrodes and its Application

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***Abstract*—Starting from the principles of enzyme electrode. Coupling with structure, methods, and various applications of enzyme electrode .Electrochemical sensor with an enzyme together leads to production of electrode, known as the enzyme electrode. Effect of glucose oxidase with structure of enzyme electrodes were modified by rGO (reduced graphene oxide) sheets. Finalized by the conclusion that applications of bioelectrodes which offer superior sensitivity, selectivity, reagent less detection, and label free fabrication of biosensors. Enzyme electrode is widely used in day to day experiments.**

***Index Terms*—electrochemical sensor, fabrication, glucose oxidase, sensitivity**

I. Introduction

A coenzyme necessarily comprises an oxidoreductase including an electron mediator i.e. a coenzyme which acting as an enzymatic redox reaction. The redox polymer and the oxidoreductase are both of them maintained in the immobilized state and called electron collector. An enzyme electrode system comprises biosensor that includes counter electrode s of considerable the same electrically conducting material and also the same size. The biosensor contains the reagent includes applied potential difference a redox mediator, a buffer, an enzyme, and covers considerably equal surface areas of portions of the working and counter electrodes. The amount of oxidized form of the redox mediator at the applied potential difference and the counter electrode must be sufficient to cause diffusion which were limited electro oxidation at the surface of the working electrode of the reduced form of the redox mediator.

There are many good methods of enzyme electrode, one of the method describes here disclosed which is the contraction of outer membrane with the liquid sample which permits the substance and also permitting the oxygen in the liquid sample, pass through the outer membrane to contact the enzyme layer. In this method, the substance oxidized to generate H_2O_2 (hydrogen peroxide) which pass through the inner membrane, which contact the sensor electrode; and also ensuring that the supply of oxygen in the enzyme layers. The layer is to supply of glucose having sufficient concentration of hydrogen peroxide to produce equilibrium. Immobilization of enzymes in silica gel on oxygen electrode uses glucose oxidase which can influence on the casting solution composition i.e. pH of enzyme solution, gel precursor, buffer ratio (on the investigated response of electrode). The casting solution which is added should be checked i.e. γ-aminopropyltriethoxysilane is the casting solution. The sol gel entrapped method was also used to obtain the electrodes sensitive for disaccharides by co-immobilization of lactase, invertase and maltase with glucose oxidase. This is the simplest and effective method for immobilization of enzyme oxidase.

Application of enzyme electrode by using the structural characteristics and biochemical measurement. Structure had the effect on modified reduced graphene oxide.

II. Construction of Enzyme Electrode on the Basis of Oxygen Electrode

Sol - gel entrapment technique for construction of immobilisation of biosensor. It provides a simplest method to obtain glass material by condensation of metal alkoxides and hydrolysis.

The gel in this technique is obtained from tetrathoxysilane or tetramethoxysilane. For the purpose of the immobilisation of biomolecules, the conditions of precursor hydrolysis and condensation like organic solvent and pH have to be controlled to avoid denaturation of proteins. Silica gel has following modified properties of condensed organosilicon derivatives like ethyltrimethoxysilane, 3-ami- nopropyltriethoxysilane, 3-glycidoxypropyltrimethoxysilane and more others. These properties are capable of giving better gel structure and helpful in porosity and regularity in distribution of immobilised biomaterial as compared with conventional one.

The sol- gel technique is useful in construction of biosensors like amperometric, conductometric, fluorometric and spectrophotometric. A simple enzyme electrode can be obtained by immobilisation of oxidase on the surface of oxygen electrode. The oxygen concentration depletion is proportional to the concentration of oxidase substrate. Local changes in oxygen concentration caused by enzymatic reaction and are measured by oxygen electrode. Only once sol-gel process was used to immobilise glucose oxidase on the tip of Clark oxygen electrode and the resulting biosensor was used in flow injection analyser. The aim of this technique was to optimise the conditions of immobilisation of the model enzyme i.e. glucose oxidase in silica hydro gel on the oxygen electrode, to evaluate some of the properties of the obtained glucose electrode and to check the applicability of the sol gel process to immobilisation of other enzymes.

A. Experiment Involves

The enzymes were not purified, the solid enzyme was

dissolved in phosphate buffer 0.05M, and pH is around 6.

The distilled water is not enough for the double distilled water is used throughout the construction. At lower pH than 6 i.e. 5.5 pH, 0.05 M acetate buffer were used.

TABLE I
EXPERIMENT

Name of reagent	Composition
Invertase	baker's yeast solid, 500 U/mg, Sigma
bovine liver	solution, 155 000 U/ml, Serva
β-glucosidase	
Aspergillus oryzae	solid, 3.8 U/mg, Sigma
Escherichia coli	solid, 388 U/mg, Sigma
β-galactosidase	Lactase
Aglucosidase	Maltase
almonds	solid, 6.6 U/mg, Sigma
butyryl Pseudocholinesterase	
Phenolase	
ascorbate oxidase from cucurbita species	solid, 146 U/mg, Sigma
laccase	solid, 180 U/mg, Sigma
tyrosinase	solid, 3 000 U/mg, Sigma
choline oxidase from Alcaligenes species cholinesterase	solid, 14 U/mg, Sigma
galactose oxidase	solid, 16 U/mg, Sigma
horse serum	solid, 8.7 U/mg, Sigma

B. Apparatus

Galvanic silver-zinc oxygen electrode CTN-920.S is used to measure the dissolved oxygen. The results of measurements are expressed as the percentage of oxygen concentration in saturated solution from air at given conditions i.e. pressure and temperature.

C. Immobilisation of the Enzymes and Electrode Preparation

TABLE II
SOL- GEL TECHNIQUE'S STOCK SOLUTION

SOL I	SOL II
4.5 ml TEOS	5 ml of TMOS
0.1 ml 0.1 M HCl	0.05 ml 0.1 M HCl
Stiring at room temp [3h]	Stiring at room temp[15 min]
1.4 ml H_2O	1.0 ml H_2O

The casting solution was prepared by mixing 200 µl of the enzyme solution with sol solution and buffer of pH ranging from 5 to 8.9. 20 µl of this mixture was dropped immediately after mixing on the surface of oxygen electrode covered with nylon mesh. The mesh was fixed on the electrode by rubber O-ring and was used as a mechanical holder of gel layer because of the poor adhesion to the Teflon® membrane of oxygen electrode.

As a result for example, Glucose electrode is a model enzyme glucose oxidase was used because of its high stability, activity, selectivity and very well-known properties is constructed and immobilized by this technique.

These enzyme catalyses the reaction:

2 2 GOD β-D-glucose + O2 →gluconic acid + H O

The depletion of oxygen concentration is the measure of glucose concentration.

The technique of immobilisation by sol-gel transition on the tip of oxygen electrode can be applied to different enzymes to obtain enzyme electrodes.

To avoid the denaturation of the enzyme by ethanol, the ratio (water to sol ratio) should be high, thus to obtain electrode with high sensitivity.

III. METHOD OF USING ENZYME ELECTRODE

Disclosed is a method of assaying a high concentration of a substance in a liquid sample with a polarographic cell. The cell contains an electrode assembly which includes:

- A reference electrode
- A hydrogen peroxide sensor electrode which contains a laminated membrane covering the liquid sample contacting face of sensor electrode.

The laminating membrane has:

- An outer membrane which have the permeability for substances and oxygen.
- An inner membrane which is permeable to hydrogen peroxide and located adjacent the face of sensor electrode
- An enzyme layer in between these two membranes, the enzyme in the enzyme layer can oxidize the substance to generate hydrogen peroxide.

A. What is involved in the method?

Method includes the contact of the outer membrane with the liquid sample, which allows the substance and oxygen to pass through the outer membrane to contact the enzyme layer. In enzyme layer, substance is oxidized to generate hydrogen peroxide. generated hydrogen peroxide passes from enzyme layer to inner membrane to contact the sensor electrode; and ensuring that the supply of oxygen in the enzyme layer relative to the supply of oxygen in the enzyme layer relative to the supply of glucose is sufficient to produce an equilibrium concentration of hydrogen peroxide, which generated a steady state response at the sensor electrode proportional to the concentration of the substance in the liquid sample.

IV. APPLICATIONS OF ENZYME ELECTRODE

Enzymes applications are wider and its use has increased in analytical tools in clinical diagnosis, food analysis and to control pollution. In the analytical application of immobilized biocatalysts, enzyme electrodes are at the leading edge. Before this time biosensors are described in many papers to determine many substrates such as inhibitors, prosthetic groups, activators, enzyme activities, antigens, haptens, and microorganisms.

There are analyzers such as Roche Lactate Analyzer 640 which are based on enzyme electrodes, and these are commercialized for the determination of following metabolites of the sucrose, lactose, and alpha-amylase activity.

- Galactose
- Glucose
- Uric acid

- Choline
- Lysine
- Ethanol
- Lysine
- Lactate

A. Potential of Enzyme Electrodes

1) Easy to operate

When we get something new method then the main thing to accept that method is its operation. If it is simple then we will accept it. Urea concentration in serum is an important diagnostic parameter in kidney disease and for dialysis control of artificial kidneys so for this urease-based potentiometric sensors are established. Ampherometric urea sensor are developed which are based on the pH-dependent anodic oxidation of hydrazine and advantage of this is

- Good reproducibility
- Calibration curve is linear
- In an hour throughout of around 40 samples
- Urea monitoring of dialysis patients

There is a great combination of urea sensor with the glucometer which is applied in urea monitoring of dialysis patients. This method has a lower cost and it also reduces the discomfort of the patient.

- To measure the activity of alanine aminopeptidase

Hydrazine oxidation which is a simple electrode reaction is used to measure it. Hydrazide is used as a substrate, and the formed hydrazine is then coupled to a color-forming reaction.

2) Optimal sample frequency

Enzyme-electrode-based analyzers are limited to a maximum of about 100 samples per hour.

When there is a loading of the high enzyme, the membrane-covered electrodes depends both on the following:

- Characteristic diffusion time, i.e. the internal diffusion
- Time profile in the cell compartment

3) Extension of new substances

Monoenzyme electrode determined a limited number of substances because the co-substrate and product formed are electrochemically inactive. Therefore, readily measurable substances have to be formed in enzyme reaction coupled sequentially or in parallel to the analyte conversion. There can be two outcomes:

Either the sequentially acting enzyme are co-immobilized in the membrane system in front of the electrode

The preceding enzyme reaction is established in a reactor upstream of the enzyme electrode.

For (glucose oxidase) GOD sandwich membrane, a characteristic diffusion time of half a minute was determined with the enzyme- loading factor of about a thousand.

4) Adapted sensitivity

By using substrate amplification, the sensitivity of the enzyme electrodes can be enhanced if substrate concentration in the nanomolar range is to be determined. Conditions should be operated in such a manner that one enzyme catalyzes the regeneration of the substrate of the second enzyme. To achieve this, we can couple the respective oxidase and dehydrogenase, and by kinases.

5) Improved specificity

Inhibitors and some alternative substrates may interfere with the enzymatic analyte conversion and may cause a serious problem in working of enzyme sensor. Loss of specificity of the biosensor may be also introduced at the level of the electrochemical indicator reaction.

We can eliminate interfering substances by enzyme-catalyzed reactions

Some examples:

1) Coal electrode
Interference by ascorbic acid
Eliminating enzyme: ascorbate oxidase
Analyte: catecholamines

2) Invertase-GOD-H_20_2 amyloglucosidase-GOD H_20_2
Interference by glucose
Eliminating enzyme: GOD- catalase
Analyte: sucrose and maltose

3) Invertase-GOD-O_2
Interference by glucose
Eliminating enzyme: hexokinase
Analyte: sucrose

4) LOD-LDH-O_2
Interference by lactate
Eliminating enzyme: LMO
Analyte: pyruvate

5) GOD-H_20_2
Interference by ascorbic acid and uric acid
Eliminating enzyme: laccase
Analyte: glucose

6) HDME
Interference by O_2
Eliminating enzyme: GOD- catalase
Analyte: NAD^+, pyruvate

V. Conclusion

- Sol-gel transition technique of immobilization on the tip of oxygen electrode can be applied to different enzymes to obtain enzyme electrodes i.e. construction of enzyme electrode.
- To obtain high sensitivity the ratio of water to sol during gel formation must be high to avoid the denaturation of the

enzyme by ethanol.

- Disclosed, the method which involve high concentration of a substance in a liquid sample with a polarographic cell.
- Enzymes applications are increased in analytical tools in clinical diagnosis, food analysis and to control pollution.

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