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RESEARCH ARTICLE

Biosensor and its Clinical Application

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Abstract

A biosensor is a device for the detection of an analyte that combines a biological component with a physicochemical detector component. The biological component of a biosensor may be the enzyme, whole cells, organelles, tissues, receptor antibodies, nucleic acid, etc. Applications of biosensor are widespread in healthcare, clinical diagnostics, veterinary medicines and chemical industry. Electrochemical biosensors have emerged as the most commonly used biosensor e.g. commercial glucose biosensor which gives the possibility of self testing at home for diabetic patients. Although most of the enzyme based amperometric sensors continue to be developed for glucose, many other compounds of biomedical importance are targeted and they include insulin, lactate, cholesterol, blood urea and ethanol. The sensitivity of the developed biosensor leads to the nanomlor & picomolar detection limit. They are expected to play an increasingly important role in improving the quality of life. This review summary the studies carried on the development of biosensor. The conventional analytical techniques used, although precise, are time consuming and mostly laboratory bound whereas biosensors have the advantages of ease of use, portability and ability to furnish real time signals.

Introduction

Biosensor is an important device that combines a biological component with a physiochemical component and is used for detection of an analyte. Gronow in 1988 defined biosensor as 'An analytical tool or system consisting of an immobilized biological material such as enzyme, antibody, whole cell, organelle or combinations thereof in intimate contact with a physico-chemical transducer device which will convert the biochemical signal into a quantifiable electrical signal'[1]. It is an important tool used for detection of an analyte in industry related to food safety, diagnostics, medical monitors and detection systems for biological warfare agents.

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It offers analytical simplicity both in and outside the analytical laboratory and it is a selective, rapid and sensitive instrument for determination of chemical and biochemical targets**.**

A biosensor consists of 3 parts:

1. The sensitive biological component can be biological material or biologically derived material or a biomimic component e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc or a biologically derived material or biomimic component that interacts or binds with or recognises the analyte under study. The biologically sensitive element can also be created by biological engineering.
2. The transducer or the detector element works in a physicochemical way e.g. optical,

piezoelectric, and electrochemical, etc. and transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transducers) that can be more easily measured and quantified.

3. Biosensor reader device with the associated electronics or signal processors that are primarily responsible for the display of the results in a user-friendly way. This sometimes accounts for the most expensive part of the sensor device; however it is possible to generate a user friendly display that includes transducer and sensitive element (see Holographic Sensor). The readers are usually custom-designed and manufactured to suit the different working principles of biosensors. Known manufacturers of biosensor electronic readers include PalmSens, Gwent Biotechnology Systems and Rapid Labs [2].

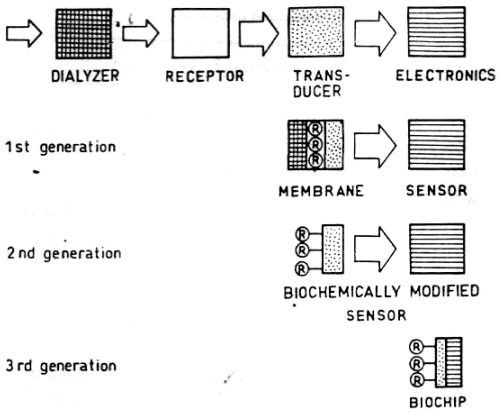
History & Background

Prof. Leland C Clark Jnr. The father of biosensor-gave the concept of biosensor in 1953. He published his definitive paper on the oxygen electrode. Based on his experience and desire to expand the range of analysts that could be measured in the body, he made a landmark address in 1962 in a symposium of New York Academy of Sciences in which he described how to make electrochemical sensors based on pH, polarography, potentiometry or conduct metry, amperometry). Further the idea of commercialization came into reality in 1975 with the successful re-launch of glucose analyzer based on the amperometric detection of hydrogen peroxide (first launch 1973) of the yellow springs Instruments Company (Ohio)[3]. Pfeiffer in 1997 explained the commercialization of biosensor in medical field.Biosensors are represented on the market worldwide by an increasing number of enzyme electrodes working in various areas of medical diagnostics and by a few opto-immunosensor-based analytical systems suited for protein research in pharmaceutical chemistry. An enzyme electrode for metabolites and enzyme activities has been commercially available for about 20 years. Such sensors are successfully applied in laboratory autoanalyzers, point-of-care-systems, patient self-monitoring disposable probes, intensive care analyzers, and for on-line monitoring of diabetics [4].

Generations of biosensors

There are three generations of biosensors categorized as first, second and third generation based on the degree of intimacy between the biocomponent and the transducer as shown in Fig. 1. In the first generation biosensor the biocomponent and the transducer may be easily separated and both may remain functional in the absence of the other. In the second generation biosensor the two components interact in a more intimate fashion and the removal of one of the two components affects the functioning of the other. In the third generation biosensor the level of intimacy between the biocomponent and the transducer is very high and the two components cannot function without each other [5].

Fig. 1 Generation of Biosensor



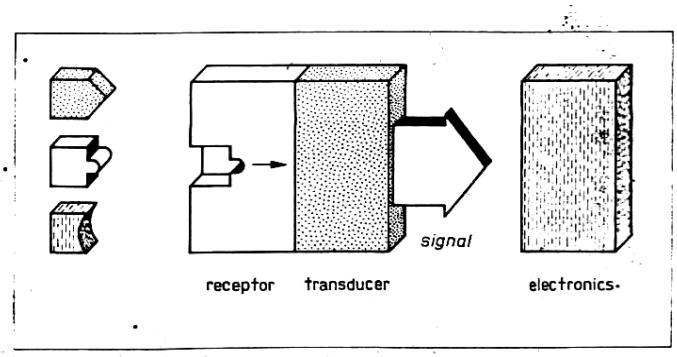
Biosensor Design and Development

Biocomponent

The biologic component used in a biosensor can be enzymes, whole cells (bacterial, fungal, animal or plant), organelles, membranes, tissues, receptors, antibodies, nucleic acids ,etc [6, 7, 8, 9, and 10] .

Varieties of enzymes have found use in many biosensors [10]. Fig.2 depicts the diagrammatic representation of enzyme being used as a biosensor.

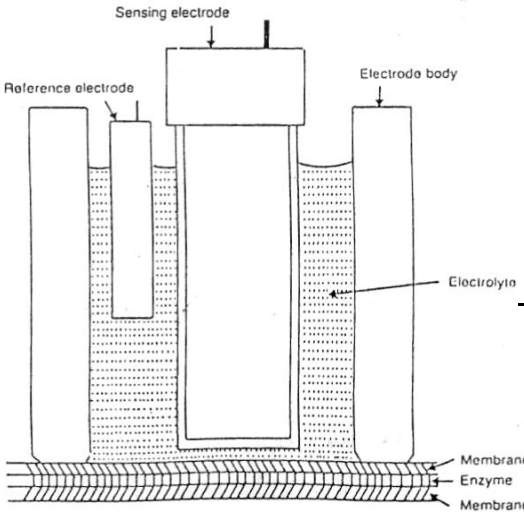
Fig. 2 Diagrammatic Representation of a Biosensor



The concept of enzyme being used as biosensor was pioneered by Clark and Lyons in 1962 and proposed that enzyme could be immobilized as electrical detectors to form enzyme electrodes as shown in

Fig.3

Fig. 3 First enzyme electrode developed by Clark and Lyons



In this first enzyme electrode, an oxido-reductase enzyme, glucose oxidase, was held next to a platinum electrode in a membrane sandwich. The platinum anode polarized at +0.6 V responded to the peroxide produced by the enzyme reaction with substrate. The primary target substrate for this system was glucose.

Glucose oxidase

glucose + O_2 ⟶ gluconic acid + H_2O_2

This led to the development of the first enzyme based analyzer for the measurement of glucose in whole blood. This Yellow Springs instrument (Model 23 YSI) appeared in the market in 1974[11]. The enzymes are widely used as biosensor due to their specificity, moreover, then widespread role in clinical diagnosis. Since enzymes have poor stability in solutions, therefore they are stabilized by immobilization. In the immobilized phase, they gain excellent stability and can be reused [12].

In this device, either immobilized whole cell of microorganisms or their organelles are used. These react with a large number of substrates and show generally slow response. Immobilized Azotobacter vinelandii coupled with ammonia electrode shows sensitivity range between 10^{-5} and 8 x 10 mol dm^{-3}. It measures the concentration of nitrate within 5-10 min^{-2}. Examples of microbial biosensors are given in Table 1 [14].

Table 1 Microbial biosensors containing oxygen electrodes

Microorganism	Sensoring for	Response time(min)	Range
Brevibacterium lactofermentum	Assimilase sugars	1-10	Linear above 1 m mole dm^{-3}
Bacillus subtilis	Mutagen screening	90-100	1-6m g cm^{-3}
Methylomonas flagellata	Methane	1	upto 6.6m mol dm^{-3}
Trichosporon brassicae	Acetate	6-10	Linear upto 22.5 mg dm^{-3}
T. brassicae	Ethanol	10	below 22.5 mg dm^{-3}

Tranducers

The second part of biosensor is a physico-chemical transducer. Transducer is a device that converts one form of energy into another form of energy. Measurement transducers or input transducers may exploit a wide range of physical, chemical or biological effects to achieve transduction and their design principles usually revolve around high sensitivity and minimum disturbance to the quantity to be measured. The signal from the transducer can be further amplified, processed or stored for later analysis. Different types of transducers used to construct a biosensor are listed in Table 2 [10, 13].

Table 2 Different types of transducers

Potentiometeric	Piezoelectriccrystal
Amperometric	Voltammetric
Conductometric	FieldEffectTransistors
Calorimetric(Thermistor)	Optical (Fiberoptics)

Methods of immobilization

Various immobilization procedures have been used in biosensor construction. In general, the choice of procedure depends on the nature of the biological element, the type of transducer used, the physicochemical properties of the analyte and the operation conditions in which the biosensor is to function. There are in principle four methods of whole cell immobilization i.e. entrapment and encapsulation, covalent binding, cross-linking and adsorption.The advantages and disadvantages of these methods have been given in Table 3[12].

Table 3 Methods of Biological component immobilization

Method	Advantages	Disadvantages
Entrapment and encapsulation	Gentle treatment of biocatalyst No direct chemical modification of biocatalyst. Specific of biocatalyst and analyte interaction retained.	Applicable only for small analyte detection. High diffusion barrier to both substrate and product transport Continuous loss of biocatalyst.
Covalent binding	Low diffusional resistance Strong binding force between biocatalyst and matrix System not severely affected by adverse conditions of pH, ionic strength	May involve harsh/toxic chemicals Matrix not regenerable. Frequently occurring loss of activity
Cross-linking	Used in conjunction with entrapment to reduce loss of biocomponent.	Harsh treatment of biocatalyst with toxic chemicals. Covalent links formed between protein molecules rather than matrix and protein.
Adsorption	Gentle treatment of biocatalyst No modification of biological component Matrix can be regenerated Maximal retention of activity	Very weak bonds Susceptible to changes in pH, temperature, ionic strength

Entrapment is the most widely used technique for immobilization of whole cells with much success. Alginate entrapment is the most frequently used technique offering extremely mild conditions. Polycrylamide gels are also often used; however, these are toxic to viable cells. Some other natural polymers used for whole cell immobilization are collagen, gelatin, agarose/agar, carrageenan, etc. The immobilization of whole cells in support through covalent binding produces a system not limited by diffusional limitations. Covalent coupling leads to a reactive binding which links the biocatalyst to the carrier. Various coupling agents are used for this purpose; however, most coupling agents are toxic and damage the cells. Cross linking has not been employed to any great extent for immobilization of whole cells. The method is more frequently used in combination with entrapment techniques. Adsorption of whole cells on solid surfaces is a simple and quick technique. A suitable adsorbent should display high affinity towards the biocatalyst and yet cause minimal denaturation. The simplicity with which cells are immobilized by adsorption has led to a large number of applications [13].

Types of Biosensors

Electro-chemical biosensor

This type of biosensor has been developed by using electronic devices such as field effect transmitters or light emitting diode; the former measures charge accumulation on their surface and the later photoresponse generated in silica based chip as an alternating current. As shown in Figure 4. Hence, the field effect transmitor measures a biochemical reaction at the surface and induce into current [1]. Moreover, the field effect transmitors can be modified to ion sensitive, enzyme sensitive or antibody sensitive ones by using selective ions, enzymes or antibodies respectively.

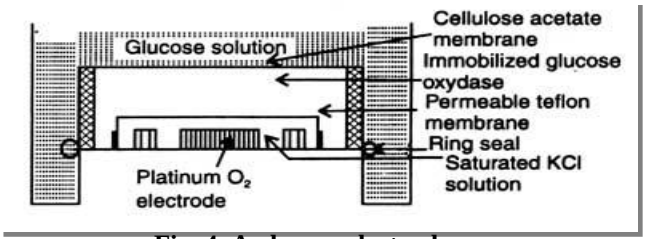


Fig. 4. A glucose electrode

Amperometric biosensor

Amperometric biosensors are those which measure the reaction of anylate with enzyme and generate electrons directly or through a mediator. The amperometric biosensors contain either enzyme-electrode with or without a mediator, or chemically modified electrodes. The oxygen and peroxide based biosensor and others (Table 4) are enzyme -electrode biosensor.

Table 4 Typical enzymes based biosensors.

Substance	Enzymes	Response time	Range
Amines (for meat freshness)	Monoamine oxidase	4 min	50-200 m mol dm^{-3}
Cholesterol	Cholesterol oxidase	2 min	10^{-2} - $3x10^{-5}$ mol dm^{-3}
Carbon monoxide	CO : acceptor	15 sec	0-65 m mol dm^{-3}
Glucose	Glucose oxidase	20 sec	2 x 10^{-3} -$3x10^{-6}$ mol dm^{-3}
Penicillin	Penicillinases	25 sec	1-10 m mol dm^{-3}
Sucrose	Invertase	6 min	10^{-2} -$2x10^{-3}$ mol dm^{-3}
Uric acid	Uricase	30 min	$5x10^{-3}$-$5x10^{-5}$ mol dm^{-3}

Source : Gronow *et al.* (1988)

Some advancement has been brought into this type of biosensor by using a mediator. In addition, more advanced types are the direct electron transfer systems. Principle of a mediated biosensor is shown in Fig.5.

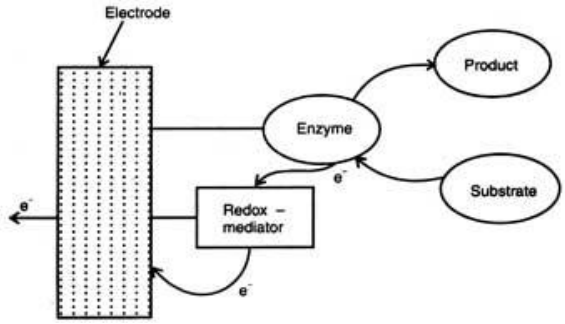


Fig. 5. Mediated biosensor.

In this biosensor, a redox reaction catalyzed by an enzyme is directly coupled to an electrode where enzyme is presented with the oxidizable substrate. The electrons are transferred from the substrate to the electrode via enzyme and redox mediator. In this biosensor the oxidase replaces the oxygen requirement of the enzymes.

Thermistor containing biosensor

Thermistor is used to record even a small temperature changes (between 0.1-0.001°C) during biochemical reactions. By immobilizing enzymes like cholesterol oxidase, glucose oxidase, invertase, tyrosinase, etc. thermistors have been developed [1]. Moreover, thermistors are also employed for the study of antigen- antibody with very high sensitivity (10^{-13} mol dm^{-3}) in case of thermometric Enzyme Linked Immunoabsorbant Assay (ELISA).

Bioaffinity sensor

Bioaffinity sensors are developed recently. It measures the concentration of the determinants, *i.e.* substrates based on equilibrium binding. This shows a high degree of selectivity. These are of diverse nature because of the use of radiolabelled, enzyme labeled or fluorescence-labeled substance as shown in Fig. 6. [15]

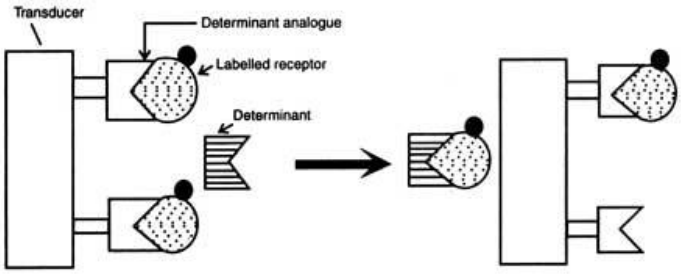


Fig. 6. Bioaffinity sensor.

Opto-electronic biosensor

In these biosensors either enzymes or antibodies are immobilized on the surface of a membrane. For measuring color, biosensor with an enzyme and dye is immobilized to a membrane. When a substrate is catalyzed to yield product, changes in pH of the medium occur. This results in changes in dye - membrane complex. These changes in color are measured by using a light emitting diode and a photodiode [1].

Application of Biosensor

Biosensors have are widespread application in healthcare, clinical diagnostics, veterinary medicines, the food ,agriculture and chemical industry, environment monitoring and the defense and security industry [3,15]. The potentiometric sensor, voltametric sensor, electrochemical gas sensor consume less time for analysis and very low detection limit. The main unique thing in these sensors is that they are specific. Weetal in 1999 and Eric Bakkar in 2004 highlighted the status of the actual developments of electrochemical sensing

principles and covers potentiometric sensors, references electrodes, voltammetric sensors, electrochemical biosensors (enzyme electrodes and affinity-based sensing principle), and electrochemical gas sensors[16,17]. Babkina and his co- worker Ulakhovich developed amperometric biosensor based on denatured DNA for the study of heavy metals complexing with DNA and their determination in biological,water and food samples[18]. Further there was introduction of the mediator which was used in the fabrication of amperometric biosensors, shuttles redox equivalents between the recognition element and transducer. This supplementary step in biosensing chain usually results in the increase of the biosensors sensitivity and selectivity.Castillo et al., in 2004 described in detail about the benefit of biosensor and its application in medicine, food quality and safety control and environment pollution monitoring as shown in Table 5. [15].

Table 5 Biosensors with potential use in food and/or beverage industries, (Castillo *et al.*, 2004)

Name of substrate to be detected	Enzyme to be used as biosensor	Principle	Linear range and detection range	Product in which analyte was detected
Glucose	Glucose oxidase	Electrochemical Luminescence	LR: 0.050-10 mM DL: 26 µM	Soft drinks
Fructose	Fructose dehydrogenase	Amperometric	LR: 0.01-1 mM DL: 2.4 µM	Honey
Ethanol	Alcohol dehydrogenase	Amperometric	LR: 0 - 7 mM	Wine, beer
Glycerol	Glycerol dehydrogenase	Spectrofluorometric	LR: 30-300 mg/L DL: 8 mg/L	Wine
Acetate	Acetate kinase Pyruvate kinase Pyruvate oxidase	Amperometric	LR: 0.05-20 mM DL: 50 µM	Wine
Cholestrol	Cholestrol oxidase	Spectrofluorometric	LR: 0.07-7.5 mM[3] DL: 70 µM	Butter
Methanol	Alcohol oxidase Horseradish peroxidase	Amperometric	LR: 100-800 mM DL: 20 µM	Beer, wine, liquor
Lactose	Glucose oxidase	Manometric	LR: 0-5 mM[3] DL: <1 µM[3]	Milk
Sulfite	Sulfite oxidase	Amperometric	LR: 0.4-750 ppm DL: 4ppm	Water
Hypoxanthine	Xanhine oxidase	Amperometric	LR: 1-400 mM DL: 800 µM	Fish
Histamine	Monoamino oxidase	Amperometric	LR: H, 10-200; T, 10-200; P, 5-100 mg/kg.	Fish, meat, sauerkraut, beer, dairy products, wine
Tyramine Putrescine	Tyramine oxidase Diamine oxidase		DL: H, 10; T, 10; P, 5 mg/kg.	
Pesticides: organo phosphate carbamate	Acetly Cholinesterase Butyryl Cholinesterase	Photothermometric	DL: 0.2 ng/ml	Salad, onion
Penicillin G	Protein with carboxy peptidase activity	Surface plasma resonance	DL: 5.2 µg/kg	Milk

Extrapolated and/or calculated values; LR: linear range; DL: detection limit; H: histamine; T: Tyramine; P: putrescine.

Wang in 2006 did a remarkable work in the field of electrochemical biosensor. With the widespread application for day-to-day routine diagnosis, electrochemical sensors are playing a significant role in the transition towards point of care diagnostic devices. Such electrical devices are extremely useful for delivering the diagnostic information in a fast, simple and low cost effective manner. Further study is in the progress for development of biosensor which can help in diagnosis of cancer. Such a sensor shall be easy to access at patient's bed side or in physician's office [19].

Medical Application of Biosensors

Glucose Biosensor

Biosensors have tremendous potential for its application in the field of medical science. In 1979, the first glucose analyzer using biomolecule for the detection of blood glucose was commercialized by Yellow Springs Instruments Co., USA. A device, a minipump filled with insulin, had been constructed to deliver insulin to diabetics based on glucose levels of blood. When biosensor provides information, the device delivers accurate amount of insulin required by the diabetics. Mitomycin, an aflatoxin, causes cancer in inborn infants. Therefore, mutagenicity of such chemicals can be detected by using the biosensor. Similarly, any other abnormal toxic substance produced in body due to infectious disease can also be detected. Several biosensors have been reported in the literature for analysis of various biochemical analytes.[11].Mascini in 1992 and Hart et al, in 2003, clinical chemistry, glucose biosensor was available commercially and in future these will be available for detection of alcohol, cholesterol, progesterone, hemoglobin, lactate. [20, 21].

Maximum work has been done on glucose biosensor therefore it has undergone maximum modifications with passage of time. These modifications improved the detection limit of glucose i.e. from molar conc. to mili molar and then further to micro molar conc. The picoamperometric detection of glucose was reported by Luong *et al.,* 2003[22]. They fabricated a sensing electrode of platinum and immobilized (GOX) on the tip with the extra insulation of phenol and 2 allyphenol was electropolymerized. The detection limit of glucose by glucose oxidase was 20 µM and the picoamperometric current response was within 2 seconds. The glucose biosensor developed was based on subcutaneous, a continuous monitoring technology. It was based on the principle of coupling between micro dialysis and biosensor and it had the detection limit up to nano molar . Malhotra et al., in 2003 reviewed various biosensors which had biomedical importance[3]. Glucose Biosensor is shown in Fig. 7.

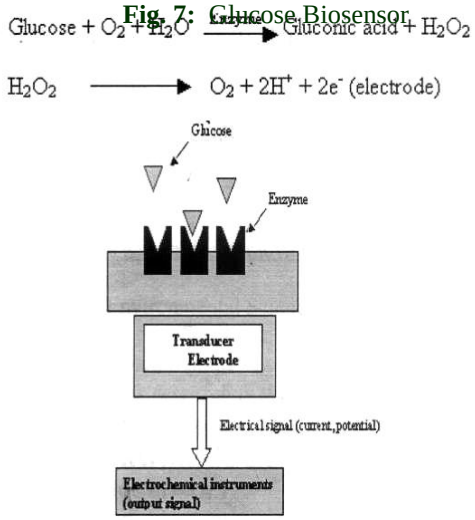


Fig. 7: Glucose Biosensor

Xian et al. in 2006 fabricated a novel biosensor based on composite of gold nanoparticles. It ultimately measured electrochemical oxidation of H2O2 and it had good stability [23]. Liu and Sun exploited the enzymes glucose oxidase and hexokinase and developed amperometric biosensor for estimation of glucose. They immobilized these enzymes in silica hybrid sol-gel film. The silica hybrid film was fabricated by hydrolysis of the mixture of tetraethylorthosilicate and 3-(-trimethoxysiyl) propyl methacrylate. This combination provided the sensor a good stability and the sol-gel matrix were further suggested for the study of immobilization and electrochemistry of proteins [24].

Urea and Creatinine Biosensor

Urea and Creatinine are very important parameters for monitoring renal function, so biosensor were developed for these substrates as well. In 1976, the Creatinine biosensor was first fabricated on potentiometeric principle by Meychoff and Rechmitz. The response of the urea and Creatinine biosensor's which were developed in due course was reported by impedimetric device as the modified screen printed electrode [25]. Gambhir and his coworkers fabricated the urea biosensor by immobilizing the enzyme like unease and glutamate dehydrogenase. The enzyme was immobilized on electrochemically prepared polypyrrole/polyvinyl sulphonate[26].Premanode and Toumazou in 2006 developed a novel low power biosensor for real time monitoring of creatinine and urea in peritoneal dialysis. Creatininase,creatinase and urease enzyme were immobilized .The results had a linear relationship of urea and creatinine at the range of 0-200 and 0-20 mM [27] .A similar

approach for development of urea biosensor was adopted by Minni and her research team exploiting immobilization technique. The difference was instead of enzyme, cell membrane was expoited for urea biosensor construction [28].

Lactate Biosensor

As the biosensors were on the developing stages, the approach towards the miniaturization was also progressing. National Physical Laboratory, India developed a lactate biosensor based on screen printed electrode

Cholesterol Biosensor

Cholesterol Biosensor was fabricated by immobilizing the enzymes in the sol gel films and then utilized these films on amperometer instrument [14].

DNA Biosensor

DNA biosensor is of utmost importance in diagnosis of inherited diseases, screening of c-DNA colonies required in molecular biology. The technique here is same as of immobilization of DNA on conducting polypyrrole and these were adopted for response on electrochemical sensor.

Immuno-Sensors

The immunosensors are small portable instruments which have great benefits over ELISA. Various antibodies have been raised against the conducting polymer, carbazole as a hapten, which might react to modulate the polymer electrochemistry. The response was seen on cyclic voltametry [29].

Insulin Biosensor

Heding(1996) developed the technique for measurement of the binding of insulin like growth factor I and II and their analogues to the insulin like growth factor – binding protein -3, using a BIAcoretrade mark instrument (Pharmacia Biosensor AB)[30]. Sutaria and Sadana (1997) immobilized antigen (or antibody) in solution to antibody (or antigen) on a biosensor and other surfaces were analyzed with a fractal analysis i.e. a change in the reaction mechanism on the surface was noted [29]. Wong and his coworkers (1999) proposed the theory for biosensor measurement of the interaction kinetics between insulin like growth factors and the binding proteins (IGFBP) [31]. Vorwerk et al. studied the antigen antibody interaction of the insulin growth factors binding I GF-I and IGF-II to recombinant human –N-terminal and C-terminal fragments and structurally related proteins mac25 and connective tissue growth factor measured using a biosensor[32].Piquepaille in 2006 developed implantable chip or insulin pump. They are developing a future implantable wireless biosensor or a living chip [33].Qu and his coworkers developed a protocol for amperometric determination of insulin. He fabricated a glassy carbon electrode by solubilization of carbon nanotubes in chitosan together with cobalt hexacyano ferrate nanoparticles. It exhibited a linear response in range of (0.1 – 3 µM)[34].

In 2008,Amandeep et al, developed electrochemical insulin biosensor by immobilizing biocomponent in carbon paste electrode (CPE) which contain enzyme hexokinase and glucose-6-phosphate dehydrogenase ,adeniosine triphosphate(ATP)and insulin receptor with concomitant generation of NADPH where oxidation was measured amperiometrically at 0.32 V. It covered the calibration range 0.006-0.09 nM with stability of two weeks and its detection limit is 0.006 nM which is significantly lower than the existing methods [35].

Alcohol Biosensor

Yao et al. (2000)fabricated a carbon paste enzyme electrode for monitoring alcohol . The enzyme, alcohol dehydrogenase was immobilized in carbon paste .The current response for the electrode was proportional to the ethanol concentration up to 1 Mm, and the response time was calculated 1 min and lower detection limit of ethanol was 50µ M.The enzyme electrode was applied to the ethanol determination in food samples [36].

Ampicilin

Ampicilin electrochemical sensor was developed by Khalilzadeh et al in 2009.It has a linear range of 2.34 – 30 µ mol/L with a detection limit of 0.67 µ mol/L. This sensor had been applied for detection of drugs in urine samples [37].

Fructosyl Valine

Chun and Chou developed a elctrochemical sensor for estimation of Fructosyl Valine.Its measurement is far better and sensitive than Glucose for diabetes management. The minimum detection limit is <0.05mM [38].

NADH

The electrochemical dihydronicotinamide (NADH) sensor was developed by L. Zheng et al in 2011.This sensor attracted intensive interest because it behaves as an essential cofactor in enzymatic reactions. It covered a reference range from 0.8 – 500 µM with a detection limit of 0.10 µM [39].

Dopamine

A new type of porphyrin-functionalized graphene was synthesized and used for highly selective and sensitive detection of dopamine (DA). The detection limit of DA can be as low as 0.01µM. With good sensitivity and selectivity, the present method was applied to the determination of DA in real hydrochloride injection sample, human urine and serum samples, respectively, and the results were

satisfactory[40].On similar footsteps, Sheng etal in 2012, developed electrochemical sensor based on nitrogen doped graphene: simultaneously which was able to determine ascorbic acid, dopamine and uric acid. Nitrogen doped graphene (NG) was prepared by thermally annealing graphite oxide and melamine mixture. This electrochemical sensor showed a wide linear response for AA, DA and UA in the concentration range of 5.0×10−6 to 1.3×10−3M, 5.0×10−7 to 1.7×10−4M and 1.0×10−7 to 2.0×10−5M with detection limit of 2.2×10−6M, 2.5×10−7M and 4.5×10−8M at S/N=3, respectively [41].

Other metabolite Sensor

Verma and Dhillon, (2003) had reported the detection of various insecticides i.e. organophosphorous, carbamates and herbicides by integration of the various biocomponents with different transducers-potentiometer, amperometer, thermistor[42]. Valentini and his coworkers in 2004 prepared and characterized carbon nanotube paste (CNTP) modified electrodes . These were found to be promising tools for the detection of significant catacholamines which belong to the family of the excitatory chemical neurotramitters, such as dopamine and several different biological molecules, as catechol and guanine, and also inorganic electroactive substrates, as ferricynaide[43]. A new sensor based on a porous silicon oxide microcavity was developed by Rocchia etal in 2007 for the determination of the alcoholic strength of white and red wines. The results demonstrated the advantage of working in evaporation mode and possibility to determine the alcoholic strength of wine with good reproducibility and selectivity [44].Shimomura etal in 2009 successfully prepared acetylcholine sensor by using immobilized enzymesi.e. , acetylcholinesterase and choline oxidase within separate hybrid mesoporous silica membrane. The determination range and the response time were 6-800 μM and within approximately 3 mins, respectively [45].Andree et al in 2010 explored novel doxycycline derivative for analyzing low concentrations of tetracycline in biological matrices and food in competitive assays [46]. Kuila and his research team in 2011 did extensive work in grapheme based biosensors for the detection of glucose, Cyt-c, NADH, Hb, cholesterol, AA, UA, DA, and H2O2. In all these cases, the biosensors performed well with low working potentials, high sensitivities, low detection limits, and long-term stabilities [47].

2. Uses in pollution control

Biosensors are very helpful in environmental minitoring and pollution control, since they can be miniaturized and automated. As far as quality control of drinking water is concerned, the minitoring biosensors are successful in monitoring of pesticides in water. In Japan, a biosensor coupled with oxygen electrode and immobilized Trichosporon cutaneum is used for measuring biological oxygen demand (BOD)[1].The whole cell biosensor developed by immobilizing Salmonella typhimurium and Bacillus subtilis in conjugation with oxygen electrode can be used to measure mutagenicity and carcinogenecity of several chemical compounds.

3. Uses in industry

Generally, spectrophotometer and autoanalyzer are used to estimate the substrates utilized and the products formed in the fermented broth. In addition, there are a lot of problems associated with these. So the biosensors can be designed to measure the fermentation products to improve the feed back control, to carry out rapid sampling and rejection of below standard raw materials to improve the efficiency of workers. Isaokarube and coworkers of Tokyo University Research Centre of Advance Science & Technology have recently developed an ion sensitive field effect transistor (ISFET). This device is highly sensitive to the change in the ion concentration. Using this biosensor, it is possible to measure the odor, freshness and taste of food. Various enzymes like ATPase, aminoxidase or putrescine oxidase can be used to determine the freshness of fish. ATPase detects the presence of ATP in fish muscle. Recently, a biosensor has been developed at Cransfield Institute of Technology, UK which measures cholesterol levels in butter. The enzyme cholesterol oxidase, when immobilized on the electrodes, reacts with cholesterol of food.

4. Biosensor in military

The darker side of biosensor application is to provide support to military with such a biosensor that can detect toxic gases including chemical warfare agents. Such biosensors have advantages over the traditional methods of sensing of chemicals.

Conclusion

The topic of electrochemical sensors is quite vast and continues to grow and broaden. The field of potentiometric sensors, as a mature technology, has experienced important change in the past few years. The principal developments in this area focusssed on reducing the detection limit to true trace levels, down to the low parts per trillion concentration range, and there are important advances in the areas of materials and active components design.

However, the step from research to production development and commercial availability is still

significant and requires an active collaboration between universities/research institutes, small and medium enterprises, stakeholders and investers.

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