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Biosensors for clinical diagnostics industry

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Abstract

There is an urgent need in the medical diagnostics laboratories for accurate, fast and inexpensive devices, which can be routinely used. The reliable and accurate information on the desired biochemical parameters is an essential prerequisite for effective healthcare. In this context, biosensors are considered to provide viable solutions to the problems posed by the contemporary healthcare industry. This is because these biosensing devices offer considerable advantages, such as specificity, small size faster response and cost. It is anticipated that these bioanalytical tools can be used for frequent measurements of metabolites, blood cations and gases, etc. In this paper, an attempt has been made to highlight some of the trends that rule the research and developments of some of the important biosensors that are likely to accelerate the growth of clinical diagnostics industry.

Keywords: Biosensors; Immobilization; Cross-linking; Microencapsulation

1. Introduction

Diagnosis and monitoring of various diseases necessitate intensive efforts for routine examination of blood samples and other associated tests. These however require typical analytical techniques, need efficient hands to perform the job and time for collecting the desired samples for performing clinical tests. Clinical laboratory testing enables qualified personnel to diagnose and monitor various diseases. Some of the analytes determined are known to be specific for a given disease and hence can be helpful to monitor its progress. The clinical utility of a biochemical test is determined by its sensitivity, ability to detect the disease with no false negatives and its specificity, i.e. the ability to avoid false positives in a non-diseased person.

The development of biosensors is the result of combined efforts of biologists, physicists, chemists and engineers. Biosensors use the specificity of a biological molecule along with a physicochemical transducer to convert a biological signal into an optical/electrochemical signal [1]. A number of biosensors based on metabolites are available for monitoring clinically important parameters viz. blood glucose, urea, lactate, cholesterol and uric acid, etc. These biosensors offer an advantage to extra laboratory analysis of relevant substances for clinical analysis [2–6].

Enzymes are well-known as biological sensing materials in the development of biosensors due to their specificity. Moreover, role of enzymes in clinical diagnosis has been known for several years. Since enzymes have poor stability in solutions, it is therefore needed to stabilize them by immobilization. In the immobilized phase, they gain excellent stability and can be reused. Numerous techniques of immobilization viz. covalent linkage, physical adsorption, cross-linking, encapsulation and entrapment have been known for stabilization of enzymes for the development of biosensing devices.

The matrix or support to be chosen for immobilization depends on the nature of biomolecule and method of immobilization. A number of matrices, such as polymeric films and membranes [7,8], gels [9–11], LB films [12–14], carbon [15–17], graphite [18,19], diaphorase [20] and conducting polymers [21–24], etc. have been used for the immobilization of biomolecules/enzymes in the development of various types of biosensors.

2. Historical background

Prof. Clark Jr. [25] has been known as the father of the biosensor concept since the publication of his first definitive work on the oxygen electrode in 1956. Later, in 1962 he described an experiment in which glucose oxidase (GOX) was entrapped at a Clark oxygen electrode using dialysis membrane [26]. The decrease in measured oxygen

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concentration was found to be proportional to glucose concentration. However, general interest in biosensors grew considerably since the description by Updike and Hicks [27] who described the first functional enzyme electrode based on glucose oxidase deposited on an oxygen sensor in 1967. This work marked the beginning of a major research effort into biotechnological and environmental applications of biosensors.

The history of amperometric biosensors is linked with that of blood glucose monitoring, which has a world market in excess of US$ 2 billion. Blood glucose is the most common analyte measured after electrolytes and blood gases. The largest growth in blood glucose measurement has been with the home monitoring devices.

Most published work on enzymatic biosensors has targeted blood glucose monitoring and most of them are based on amperometric method of detection. Amperometric biosensors have been divided into three generations. The first-generation biosensors were proposed by Clark and Lyons [26] and implemented by Updike and Hicks [27], who coined the term enzyme electrode. An enzyme electrode, as described by him, consisted of an oxidase enzyme, i.e. GOX, immobilized behind a dialysis membrane at the surface of a platinum electrode. The consumption of O_2, or, as first described by Guilbault and Lubrano [28], the formation of H_2O_2, is subsequently measured at a platinum electrode.

In the 1970s, Yellow Springs Instruments (Yellow Springs, OH) was the first company to successfully market an amperometric biosensor, a bench top glucose analyzer. Today most commercial bench top amperometric biosensors rely on reactions catalyzed by oxidase enzyme and subsequent detection of H_2O_2 on platinum electrodes. The high oxidizing potential (700 mV versus Ag/AgCl) necessary for H_2O_2 oxidation results in substantial interference from the oxidation of other compounds in complex matrices.

Second-generation biosensors have been commercialized, mostly in single-use testing format. MediSense (Waltham, MA) was the first company to launch a second-generation product. Again the application was blood glucose monitoring, but this device was for home use. The mediation was provided by a ferrocene species. In March 1996, Abbott Laboratories (Abbott Park, IL) acquired MediSense for US$ 876 million. Other second-generation amperometric biosensors have subsequently come into the market.

Third-generation sensors are marked by the progression from the use of a freely diffusing mediator (O_2 or artificial) to a system where enzyme and mediator are co-immobilized at an electrode surface, making the biorecognition component an integral part of the electrode transducer. Co-immobilization of enzyme and mediator can be accomplished by redox mediator labeling of the enzyme followed by enzyme immobilization, enzyme immobilization in a redox polymer, or enzyme and mediator immobilization in a conducting polymer. There are even reported cases of direct electrical contact of enzyme to electrode. Whether this is direct electrical connection or mediation by surface functionalities is a matter of debate.

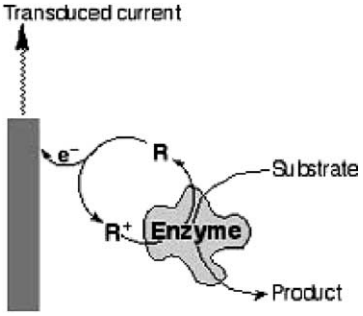

Fig. 1. The electrical 'wiring' of an oxidative redox-enzyme via a diffusional electron-transfer mediator.

Third-generation biosensors offer all the benefits of second-generation sensors, and some new ones as well. These biosensors are self-contained in the sense that there is no need to add either mediator or enzyme. Therefore, this design facilitates repeated measurements. Sensor used for multiple analyses minimizes cost pressures on sensor design. It also follows that such a sensor could allow for continuous analyte monitoring. TheraSense, Inc. (Alameda, CA), is researching continuous blood glucose monitoring using wired enzyme technology (Fig. 1).

Recently, use of the ion selective electrodes has been gaining interest for fabrication of effective devices for clinical applications.

3. Technical development of biosensing devices

Biosensors are developed by immobilization of desired sensing biomolecules to a support and coupling with a physicochemical transducer. The most commonly used techniques of immobilization are adsorption of inert carriers, cross-linking by bifuctional reagents into the macroscopic particles, physical entrapment in gel lattices, covalent binding of water insoluble matrices, microencapsulation within the wall spheres and electrochemical entrapment, etc. All the methods can be broadly divided into two parts: attachment and entrapment. The entrapped enzymes are isolated from the large molecules, which cannot diffuse into the matrix. The attached enzyme can be exposed to the molecules of all sizes. Thus different form of kinetics is observed during the reaction.

Optimization of the electron transfer between immobilized redox enzymes and an electrode surface is a presupposition for the development of novel fast-responding and reagentless biosensors. Based on conducting polymers, mainly polypyrrole and its derivatives bearing functional side-chains, a secure (covalent) technique of immobilization of the biological recognition elements should be attained. However, the electron-transfer rate should be increased using simultaneously immobilized redox relays (Os-complexes). Additionally, in the deposition of conducting polymer films in two and three dimensions using potentiometric

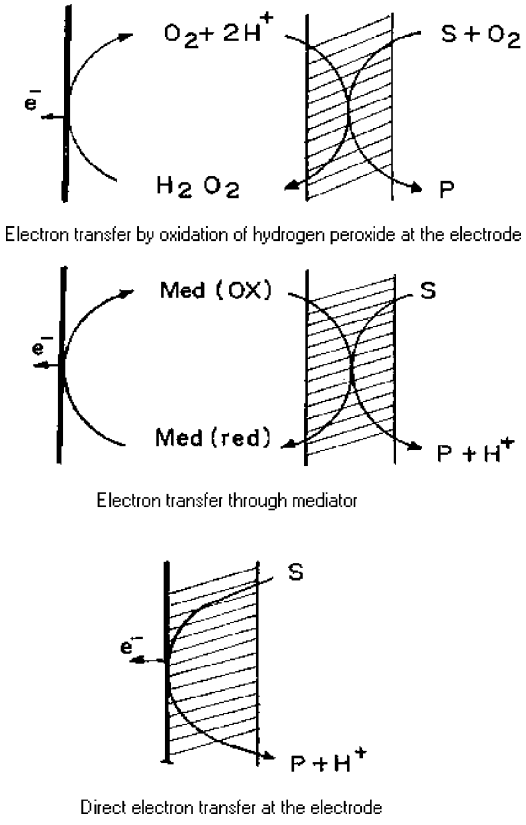

Fig. 2. Mechanism of electron transfer in amperometric biosensing devices.

and amperometric sensor signals, and the individual modification of sensor arrays has also been widely investigated.

A transducer converts the biochemical signal to an electronic signal. The biochemical transducer or biocomponent gives the biosensor selectivity or specificity. The transducer of an electrical device responds in such a way that a signal can be electronically amplified and displayed. The physical transducers vary from electrochemical, spectroscopic, thermal, piezoelectric and surface acoustic wave technology [29–33]. The most common electrochemical transducers being utilized are based on amperometric and potentiometric techniques [34–37]. Depending on the type of transducers, biosensors can be classified into optical, calorimetric, peizoelectric and electrochemical biosensors. Fig. 2 shows different schemes of electron transfer in amperometric biosensors for fabrication of devices.

4. Clinical laboratory testing verses biosensing devices

As a patient reaches a clinician for medical examination, it is useful to first develop a problem list based on the history, physical examination and basic laboratory studies. Synthesis and analysis result in differential diagnosis with associated disease probabilities. A selective use of diagnostic tests is then used to rule in or rule out these possibilities, e.g., a patient having symptoms of jaundice anorexia, fever and abdominal pain, the relative increased level of liver function tests will help to guide the subsequent evaluation. The type of disease is decided by the biochemical parameter and the level of increase. Clinical laboratories in the hospitals have attained a significant growth during the past few years. However, it is sometimes realized that these facilities are inadequate due to long assay procedures and time consuming sensitive procedures. This is particularly true for critical care at the emergency, which requires accurate and quick results. But delay in the results may affect the timely commencement of proper treatment. A point of care system has the practical utility to the physician only if it provides analysis for a wide range of analytes with the same accuracy as that obtained by the central clinical laboratories.

Biosensors promise low-cost, rapid, and simple-to-operate analytical tools for applications in various fields such as medical diagnosis, environmental monitoring, food industries, etc. They therefore represent a broad area of emerging technology ideally suited for point-of-care analysis. Biosensors are analytical tools combining a biochemical recognition component with a physical transducer. The biochemical component serves to selectively catalyze a reaction or facilitate a binding event. The selectivity of the biochemical recognition event allows the operation of biosensors in a complex sample matrix, i.e. a body fluid. The transducer converts the biochemical event into a measurable signal, thus providing the means for detecting it. Measurable events range from spectral changes, which are due to production or consumption of an enzymatic reaction product/substrate (optical), to mass change upon biochemical reaction (piezoelectric). The problem is that the enzyme is very sensitive to the effects of moisture and light, which can change its effectiveness and the accuracy of the measurement. The challenge is, therefore, to design a system that may totally protect the tiny and delicate test strips until a measurement is made and yet allows the test strip to be removed from the packaging and put into the measurement position using a easy single-handed movement.

4.1. Current diagnostics market

While most of the diagnostic market remains stagnant, the point-of-care testing market is growing at an annual rate of about 25%, from US$ 600 millions in 1994 to US$ 692 millions in 1998. It is expected that the medical biosensor market shall reach US$ 1.5 billions by 2003 [38].

The nature of the biosensor limits the opportunities for commercial success. Affinity biosensors will have a difficult time competing with techniques, such as standard enzyme-linked immunosorbent assays, which can be fully automated and operated in multiplexed batches of 96 and even 384 samples. Biosensors seem best suited for limited-use and point-of-care applications.

The Technology Partnership plc (TTP) developed a unique, cartridge-based system in which ten test strips are

stored in a protective cartridge within the meter [39]. One simple one-handed movement is all that is needed to make a test strip ready to take a blood sample, which it does automatically. Only 3–4 μl of blood are required, which is about 30% of the volume used by other systems. A measurement can be made dramatically faster than with other systems, in about half the time.

Currently used automated machines are usually capable of meeting the general requirements of clinical analysis. These are either based on the ELISA technique, clinical chemistry and spectro-photometric assays. Therefore, biosensors developed for clinical purposes are to be compared with established methods in terms of assay time, accuracy, sensitivity and costs.

5. Clinical applications of biosensors

There has been a great demand for rapid and reliable methods which can be used in biochemical laboratories for determination of substances in biological fluids such as blood, serum and urine, etc. There is also a demand to move clinical analysis from centralized laboratories to a doctor's clinic and patients self-testing at home. Most of the methods available in the market for rapid detection are based on enzyme electrodes. They provide for a negligible enzyme consumption of <1 μg per sample. The Glucometer GKM 01 was the first commercial enzyme electrode based glucose analyzer developed in Europe. It was introduced in 1980 at the Centre of Scientific Equipment of the Academy of Sciences of the GDR. The glucometer is being adapted to the quantification of uric acid, lactate and the activity of acetylcholine esterase. The lipid analyzer ICA-LG 400 from the Japanese company Toyo Jozo is capable of measuring whole group of analytes, namely cholesterol, triglycerides and phospholipids by using enzyme electrodes [40]. Although biosensors have found immense applications in various fields, their use in health care monitoring is of utmost importance.

Recently, measurement of metabolites in media other than blood has a great demand. Such types of measurements are important. Where there is a need of continuous monitoring of analytes, such as glucose, urea, etc. therefore invasive biosensing sometimes proves to be very painful for the patients undergoing self-testing. Therefore, the concept of non-invasive testing in sweat, saliva or skin has become popular. Guilbault et al. [41] have discussed the work carried out at labs in New Orleans and Rome for development of non-invasive sensors. Recently work on near infra red (NIR) method which is a reagentless system and non-invasive has started gaining interest.

5.1. Ex-vivo monitoring

A few instruments, which are of great help in the treatment of continuous monitoring of diabetes and other metabolites, such as lactate, pyruvate, glucose, etc. have been used for ex vivo monitoring. An artificial pancreas ''betalike'' (EsaOte Biomedica, Geneoa, Italy) has been known for continuous measurement of glucose. It takes the blood from a patient vein, dilutes it, dialyzes it and re-infuses blood cells into the blood stream and analysis of glucose concentration. Similarly this ex vivo monitoring is used in sports medicine for measurement of lactate concentration. Another glucose sensor (Unitech, Uln) has been introduced as a commercial portable sensor for continuous glucose monitoring. This instrument is helpful for obtaining long-term glucograms. The instrument utilizes a enzyme electrode connected to a wick implanting to equilibrate with subcutaneous fluid. Lipid analyzer (ICA-LG 400) utilizes the enzyme electrode and is useful for determination of triglycerides, cholesterol and phospholipids. An amperometric urea sensor based on pH dependence of the anodic oxidation of hydrazine has been utilized in the glucometer GKM 02. The first enzyme electrode based lactate analyser was developed in 1976 by LaRosch (Switzerland) which used Cyt b_2 on platinum electrode.

5.2. In vivo monitoring

Several approaches are known for in vivo measurements. One of the approaches consists of an assembly of needles comprising a glucose electrode [42] for subcutaneous use and the other approach is microdialysis. Needle sensors have been implanted subcutaneously for several days and results are tele-transmitted to the receiver. The transmitter converts current signal generated by the glucose needle biosensor to a very high frequency audio signal and the receiver demodulates back to a voltage. Microdialysis is a more recent approach to an implantable biosensor and works on the principle of mimicking the function of a blood vessel by implanting a microdialysis probe into the tissue. The probe essentially consists of a thin dialysis tube perfused with a sample (blood). The substances, which are in higher concentration in the extra cellular fluid outside the probe, defuse in. As soon as the substances are carried out of the body by the perfusion liquid, the concentration can be determined by coupling it with a biosensor. Other approaches, which have been proposed for in vivo monitoring include enzyme based electrochemical, enzyme based field effect transistor (ENFET), enzyme based thermoelectric, electrochemical and optical approach.

6. Biosensors for health care

6.1. Glucose biosensor

Detection of glucose has been the most studied analyte in diabetic patients. The level of the glucose can be monitored either in vivo or in vitro. The first approach for in vitro study was pioneered by Shichiri et al. [42]. Mascini et al. [43]

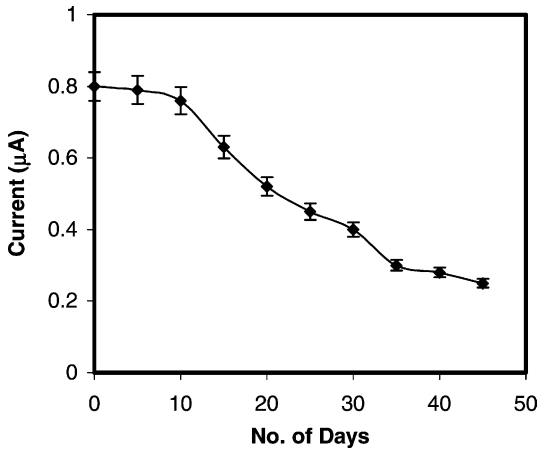

Fig. 3. Response of P3DT/SA/GOX electrodes as a function of storage time in the presence of glucose (100 mg/dl) in phosphate buffer (pH 7).

reported an "artificial pancreas" for continuous measurement of glucose. A number of glucose biosensors have been reported which are based on conducting polymers [13,44–47]. Ramanathan et al. [47] covalently attached glucose oxidase on poly (*o*-amino benzoic acid) and fabricated the screen printed electrodes made of this material. These electrodes have been shown to be useful for glucose estimation from 1 to 40 mM and stability of about 6 days. The i-STAT portable clinical analyser which is a significant commercially available biosensor, can measure a range of parameters: sodium, chloride, potassium, glucose, blood urea nitrogen (BUN) and haematocrit. The NPL Glucosense developed at the National Physical Laboratory, India is based on the screen printed graphite electrode having a mediator incorporated in the working electrode. The product is available with the Indian markets for the consumers. The sensors are fabricated using thin film microfabrication technology on a disposable cartridge [48]. Recently, Singhal et al. [13] reported that poly (3-dodecylthiophene)/stearic acid/glucose oxidase (P3DT/SA/GOX) Langmuir–Blodgett films based glucose biosensor can be used for at least 35 measurements and was found to be stable upto 40 days. Fig. 3 shows the amperometric response of the P3DT/SA/GOX LB electrodes show almost same response for about 10 days after which there is a gradual decrease in response upto about 40 days. The half-life of these electrodes has been determined as about 25 days (Tables 1 and 2).

6.2. Lactate biosensor

Lactate measurement is helpful in respiratory insufficiencies, shocks, heart failure, metabolic disorder and monitoring the physical condition of athletes. Many biosensors have been reported to date [49–55]. Two different technologies have been approached for the development of miniaturized systems. Thin film electrodes have been developed, which can be used as either implantable catheter type devices or for in vivo monitoring in combination with microdialysis system [51,56]. Secondly, disposable type sensors were developed for the purpose of on-line analysis [57,58]. Our group at the National Physical Laboratory, India has recently developed a screen printed electrode based lactate biosensor. Fig. 4 shows the comparative results of the lactate biosensor with those obtained with Sigma Kit (correlation coefficient 0.88).

Li and coworkers [59] have recently reported the sol–gel encapsulation of lactate dehydrogenase for optical sensing of L-lactate. Such a disposable lactate sensor has a linear dynamic range from 0.2 to 1 mM of lactate and stability of about 3 weeks. The sensor was found to have a diminished enzyme activity (about 10%) and leaching of the enzyme from the matrix.

6.3. Urea and creatinine biosensors

Urea estimation is of utmost importance in monitoring kidney functions and disorders associated with it. Most of the urea biosensors available in literature are based on detection of NH_4^+ or HCO_3^- sensitive electrodes [14,60–65]. Osaka et al. [66] constructed a highly sensitive and rapid flow injection system for urea analysis with a composite film of electropolymerized inactive polypyrrole and a polyion complex. Gambhir et al. [64] have recently co-immobilized urease and glutamate dehydrogenase on electrochemically prepared polypyrrole/polyvinyl sulphonate for the fabrication of urea biosensor. Singhal et al. [14] have recently immobilized urease on poly(*N*-vinyl carbazole/stearic acid)

Table 1
Various biosensor transducers, principles and applications

Transducer system	Principle	Applications
Enzyme electrode	Amperometric	Enzyme substrate and immunological system
Conductrometer	Conductance	Enzyme substrate
Piezoelectric crystal	Mass change	Volatile gases and vapors
Thermistor	Calorimetric	Enzyme, organelle, whole cell or tissue sensors for substrates, products, gases, pollutants, antibiotics, vitamins, etc.
Optoelectronic/wave guide and fiber optic device	Optical	pH, enzyme substrates and immunological systems
Ion sensitive electrode (ISE)	Potentiometric	Ions in biological media, enzyme electrodes, enzyme immunosensors
Field effect transistor (FET)	Potentiometric	Ions, gases, enzyme substrates and immunological analytes

Table 2
Commercially available biosensors and their characteristics

Company	Model	Analyte	Measuring range (mM)	Stability
Yellow Springs Instruments	23A	Glucose	1–45	300
	23L	Lactate	0–15	
	27	Ethanol	0–60	
		Lactose	0–55	
		Galactose		
		Sucrose		
Zentrum fur Wissenschaftlichen Geratebau (ZWG), Berlin, GDR	Glucometer	Glucose	0.5–50	>1000 samples
		Uric acid	0.1–1.2	10 days
Fuji Electric, Tokyo, Japan	Gluco 20	Glucose α-amylase	0–27	>500 samples
Pulsatum Health Care Ltd., India	Glucometer	Glucose	0–600 mg/dl	6 months
Abott Laboratories, Abott Park, USA (formerly Medisense)	Exatech Glucose sensor	Glucose	–	–
EKF Industrie-Elktronik GmbH	Biosens 040	Glucose	–	–
Daiichi, Kyoto, Japan	Autostat GA–1120	Glucose	1–40	–
LaRoche, Basle, Switzerland	LA 640	Lactate	0.5–12	40 days
Eppendorf, FRG	ADM 300	Glucose	1–100	>2000 samples
	ECA 20 (ESAT 6660)	Glucose	0.6–60	10 days
		Lactate	1–30	14 days
		Uric acid	0.1–1.2	10 days
	ACC	Lacate	1.2–18.7	
Accusport® lacate analyser (ACC)	–	Total cholestrol	–	
	–	Alcohol	0.1%	
Lifestream cholesterol monitor				
Alcosan™ saliva alcohol dipstick	i-STAT	Glucose		
	PCA	Urea nitrogen		
		Cl^-		
		K^+		
		Na^+		
		Hematocrit blood gases		
	HHxx series	Water; urine; blood	0–25000 ± 6.1 PA	–
TFS biosensor fluorometers	–	HIV whole blood	–	
Ultra compact analyser (Akers Bioscience Incl.)	BIAcore 2000	Biomolecular interactions	0^{-3}–10^{-10}	
BIAcore AB SPR instrumentation	–	Glucose		
DEX blood glucose meter	Bioscanner 2000	Glucose; cholesterol; HDL; blood ketone; triglycerides		–
Bioscanner 2000 Home blood testing kit and glucose monitor	–	Haemoglobin		
HemoCue B haemoglobin analyser	Glucometer Elite XL	Glucose	20–600 mg/dl	–
Elite Glucometer	Lactate Pro LT-1710	Lactate	0.8–23	–
KDK lacate analyzer				
HemoCue		Glucose	–	–
GEM®		Blood gas	–	–

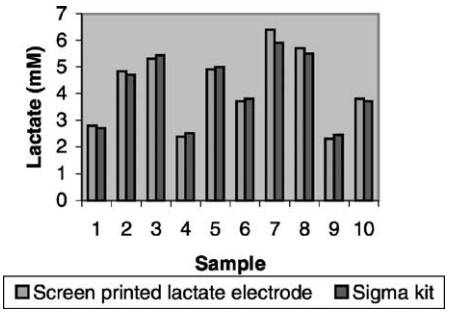

Fig. 4. Results obtained for graphite based lactate biosensor and Sigma kit.

(PNVK/SA) Langmuir–Blodgett films and observed a potentiometric response. Two linear ranges were obtained viz. 0.5–10 and 10–68 mM as shown in Fig. 5. It has been shown that such a urease electrode can be used for about 10 times.

Creatinine is an analyte used for the determination of renal and muscular dysfunction. The attempts to fabricate a potentiometric device began in 1976 with Meyerhoff and Rechnitz [67]. Later, systems with improved operational and storage capability were developed [43,68,69]. Recently, an impedimetric device has been reported to assay urea and creatinine in serum using poly(methylvinyl ether)/maleic anhydride modified screen printed electrodes [70].

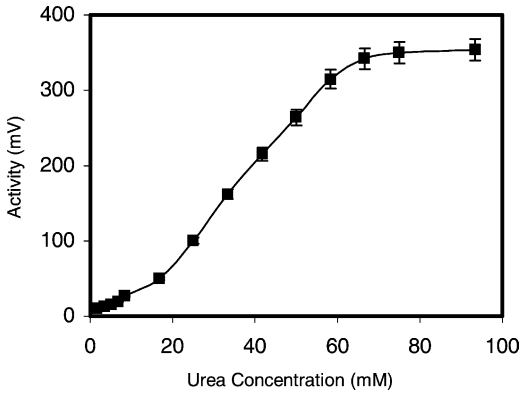

Fig. 5. Response curve of PNVK/SA/urease LB films.

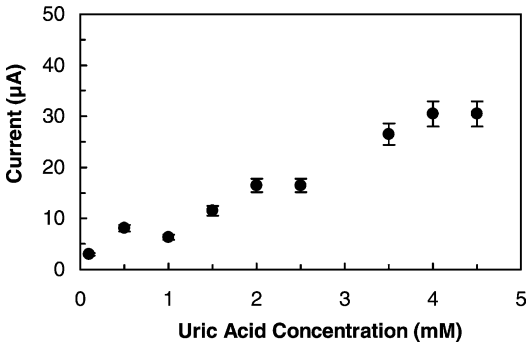

Fig. 7. Amperometric response of the polyaniline/uricase electrode to the various concentration of uric acid at 0.4 V.

6.4. Cholesterol biosensor

Determination of cholesterol is clinically very important because abnormal concentrations of cholesterol are related with hypertension, hyperthyroidism, anemia and coronary artery diseases. Determination based on the inherent specificity of an enzymatic reaction provides the most accurate means for obtaining true blood cholesterol concentration. Reports on the development of cholesterol biosensors are available [11,36,71–75]. Recently, Vengatajalabathy and Mizutani [76] demonstrated an amperometric biosensor for cholesterol determination by a layer-by-layer self-assembly using ChOx and poly(styrenesulfonate) on a monolayer of microperoxidase covalently-immobilised on Au-alkanethiolate electrodes. The sensor was found to be responsive even in the presence of potential electrical interferents, L-ascorbic acid, pyruvic acid and uric acid.

Kumar et al. [11] presented a cholesterol biosensor by co-immobilization of cholesterol oxidase and peroxidase on sol–gel films and utilized these films for estimations of cholesterol. Fig. 6 shows amperometric response obtained for enzyme electrodes prepared from polypyrrole films doped with dodecylbenzene sulphonic acid at different cholesterol concentrations.

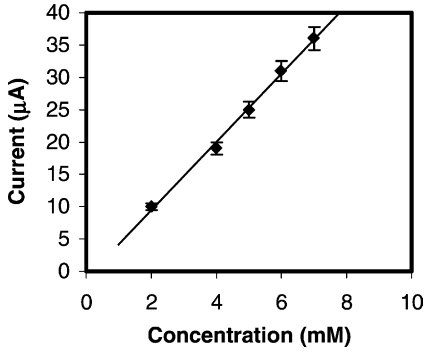

Fig. 6. Amperometric response obtained with ChOx/DBS-PPY electrode in phosphate buffer (pH 7.0) as a function of cholesterol concentration.

6.5. Uric acid biosensor

Uric acid is one of the major product of purine breakdown in humans and therefore its determination serves as a market for the detection of range of diosorders associated with altered purine metabolism, notably gout, hyperuricaemia and Lesch–Nyhan Syndrome. Elevated levels of uric acid are observed in a wide range of conditions such as leukaemia, pneumonia, kidney injury, hypertension, ischemia, etc. Additionally, as a reducing agent uric acid scavenges free oxygen radicals, preventing their destructive action towards tissue and cells. Various attempts have been made to develop a biosensor for the estimation of uric acid [77–83]. Fig. 7 represents the response of polyaniline/uricase electrodes for different urea concentrations.

6.6. DNA biosensor

DNA biosensors have an enormous application in clinical diagnostics for inherited diseases, rapid detection of pathogenic infections, and screening of cDNA colonies required in molecular biology. Conventional methods for the analysis of specific gene sequences are based on either direct sequencing or DNA hybridization [84]. Because of its simplicity, most of the traditional techniques in molecular biology are based on hybridization. Several immobilization techniques such as adsorption [85], covalent attachment [86], or immobilization involving avidin–biotin complexation [87] were adopted for a DNA probe to the surface of an electrochemical transducer. The transducer was made from carbon [88]; gold [89–91]; or conducting polymer [92,93]. In the case of a common sandwich assay the signal generating species is an enzyme, such as horseradish peroxidase [94]. Lund et al. [95] linked the tagged DNA to the surface of the microsphere using a suitable reagent. Another effort is the use of microfabrication system and micro mechanical technology to the preparation of DNA samples and their analysis, e.g., DNA chip. Gambhir et al. [93] have recently attempted to immobilize DNA on conducting polypyrrole/polyvinyl sulphonate films and demonstrated the adsorption characteristics. They believed that anion doped polypyrrole

undergoes ion exchange with PO_4^- of DNA to facilitating the adsorption. Presently, DNA probes and biosensors have widely attracted attention for diagnosis of various disorders [96–99].

6.7. Immuno-sensors

Immuno-sensors are small, portable instruments for analysis of complex fluids and are designed for the ease of use by un-trained personnel, rapid assay and sensitivity comparable to that of ELISA. During the past decade, a number of methods for immunoassay by specific interactions between antibodies and antigens to analyze microorganisms, viruses, pesticides and industrial pollutants have been developed [100–102]. Immuno-sensors are the analytical systems based on immuno-chemical principles that can automatically carry out estimation of desired parameter. Barnett et al. have detected thaumatin using antibody containing polypyrrole electrodes [103]. In the recent past, immunoassays have relied on complex indirect enzyme methods in which the resultant product of the enzyme immuno reaction can be measured. Recently, antibodies have been raised against the conducting polymer, carbazole as a hapten, which may react to modulate the polymer electrochemistry. It has been observed by cyclic voltammetry that the reaction of the antiserum influences the polymer matrix electrochemistry by an amperometric response.

7. Commercialization of biosensors

In the last few decades, efforts have been directed towards the development of practical biosensors both by academic and commercial sectors. Increased understanding of the concept of immobilized bioreagents, improved techniques for immobilization and technological advances in the microelectronics has significantly contributed to this potential field of research. Yellow Springs Instruments introduced the first biosensor product in the market for estimation of glucose, lactate and alcohol, etc. A pen style device was launched in 1987 by MediSense, developed by Turner's group at Cranfield in collaboration with researchers at the University of Oxford for glucose monitoring. In 1993, Erickson and Wilding [48] introduced the i-STAT portable clinical analyzer, which can measure a range of parameters—glucose, BUN sodium, potassium and haematocrit. National Physical Laboratory, India has also patented a glucose biosensor based on amperometric method. Fig. 8 exhibits the model of the NPL Glucosense which is available in Indian markets. The technology of this first Indian amperometric biosensor has been transferred to three companies in India.

It is anticipated that the clinical diagnostics market represents a unique opportunity for the introduction of biosensors for commercial applications. However, only a few have been successfully launched in the markets. The reason is that

Fig. 8. The commercially available glucose biosensor developed at NPL, New Delhi, India.

many critical parameters, such as cost per test, regulatory requirements, quality control, instrumentation design and test parameter selection are to be taken care of. There is a great competition in the clinical diagnostics market as a large number of highly automated diagnostic instruments are available in the centralized hospital laboratories and have been used in clinical diagnostics. Therefore, it is essential that the projected biosensor meets the need that has not been met by the automated analyzers and provide some distinct advantage for the patient care and assessment. Among these are the potential ease in using, self-testing, fast response and portability, etc. Therefore, proper marketing of the product is essential for attracting the attention of the users.

The personal blood glucose monitoring business is the prime example of a market requiring immediate on-site analysis without requiring high throughputs. To successfully commercialize affinity biosensors, a similar niche market will have to be identified. Likely targets include infectious disease detection, military applications for immediate detection of hazardous chemicals/microbes, food safety monitoring for bacteria, and possibly genome testing.

One hurdle to tackling the limited markets is the difficulty in recovering product development costs. The blood glucose market is several billion dollars strong and can sustain major R&D efforts. The market for an affinity biosensor is only a fraction of this market. A biosensor strategy that is adaptable to multiple analytes will have the distinct advantage of spreading development costs over several products.

8. Present status of the biosensor markets

The earliest success for the commercial biosensor goes to Clark and Lyons [26]. This development led to widespread introduction of laboratory glucose analyzer by Yellow Springs Instruments Co., Inc. Whereas portable instrument

produced by MediSense Inc. has been the most popular glucose sensor. This was based on the printed electrode technology and employs mediated glucose oxidase. It is now believed that the glucose monitoring market is gradually reaching stagnation. However, there is a great demand for monitoring of other parameters, e.g., cholesterol, lactate, urea, creatinine, uric acid, hemoglobin, etc. Where the point of critical care of patient comes in mind, multisensors have been found useful for such a purpose. A great example of such a system is the i-STAT portable Clinical Analyzer that is capable of measuring sodium, chloride, potassium, glucose, urea and haematocrit. The sensor is based on thin film microfabrication technology on a disposable cartridge and use ion selective potentiometry, amperometry and conductometry to make the required measurements [48]. Similarly HemoCue® provides hand-held instruments for glucose and haemoglobin testing whereas GEM® presents blood gas and electrolyte analyzers.

Blood tests that are generally required to be done include blood gases, electrolytes such as sodium, potassium, chloride, calcium, BUN, hematocrit, magnesium, creatinine, lactate, bilirubin, hemoglobin, total white blood cells count, liver function tests, cardiac markers, etc.

9. Conclusions

It has been shown that biosensors are advantageous due to their specificity, sensitivity, fast response, small size and convenience for self-testing. Among various fields, commercial development of biosensors for health care has attracted the greatest attention. This is because the patients suffering from common diseases require routine track of the biochemical profile before going for a treatment. Therefore, biosensors for clinical diagnostics industry provide excellent devices for self-testing at home and critical care at bed side in emergency.

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