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Electrochemical Biosensors for Biomedical and Clinical Applications: A Review

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Abstract: There are strong demands for accurate, fast, and inexpensive devices in the medical diagnostic laboratories, such as biosensors and chemical sensors. Biosensors can provide the reliable and accurate informations on the desired biochemical parameters, which is an essential prerequisite for a patient before going for a treatment. They can be used for continuous measurements of metabolites, blood cations, gases, etc. Of these, electrochemical biosensors play an important role in the improvement of public health, because rapid detection, high sensitivity, small size, and specificity are achievable for clinical diagnostics. In this paper, the clinical applications with electrochemical biosensors are reviewed. An attempt is also made to highlight some of the trends that govern the research and developments of the important biosensors that are associated to clinical diagnosis.

Key words: Electrochemical biosensors, Electrochemical immunosensors, Amperometry, Biomedical and clinical applications, Biological compounds

INTRODUCTION

The control of diseases is essential to improve the quality of our lives, which is greatly dependent on the diagnosis by monitoring of various biochemical compounds in the body fluid. The diagnosis of a disease by measuring the constituents in body fluids first took place in the Middle Ages. For example, diabetes mellitus was then diagnosed by tasting the sweetness of urine [1]. Nowadays, many biochemical compounds, such as salts, sugars, proteins, hormones, DNA etc. are analyzed to assist in diagnosis and to assess disease. The monitoring of the biochemical compounds in the body fluid requires typical analytical methods for biochemical test, experters to run the tests, and time for performing clinical tests. Since the levels of various compounds in a body system are directly related to some diseases, it is possible to monitor the progress of diseases by monitoring the concentration of these compounds.

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To date, samples are often collected batch-wise and subsequently analysed in a laboratory. The results of such analyses are usually available after several hours, and these analyses are generally rather expensive [2].

Continuous analysis offers the advantage of providing instant information of the analyte concentration and facilitating the creation of time-profiles, because these methods can avoid the need for frequent sampling [3]. Therefore, one can expect that the quality of patient care would be improved if batch-wise sampling and analysis were replaced by on-line continuous analysis. Thus, a continuous, fast, and sensitive monitoring is required to measure the body fluid parameters efficiently. Biosensors, those are combining a biological recognition element and a suitable transducer represent various promising tools in this context. This review concentrates on giving examples of electrochemical biosensors, which have great potential in biomedical applications. For more detailed description of the fundamental aspects of biosensors and their applications, many excellent books and reviews are available, and are suggested for further reading [4-7].

BIOSENSOR DESIGNS

A biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is combined with

a transducer (Fig. 1). The transducer translates a biological interaction into an electrical signal. Biosensors can be classified based on the transducer. The most common classification scheme of biosensors is shown in Fig. 2. The biological recognition element of a biosensor interacts selectively with the target analyte(s), assuring the selectivity of the sensors. These elements can be categorized into two main classes: biocatalysts (enzymes, microorganisms, tissue materials) and bioligands (antibodies, nucleic acids, lectins). However, their applications are limited mainly by the existence of efficient preparation methods (e.g. separation, purification, viability of microorganisms or living cells, etc.). Most biosensors described in literature use an enzyme as biological recognition element and the first biosensor, known as the "enzyme electrode" was demonstrated by Clark and Lyons in 1962 for the determination of glucose [8].

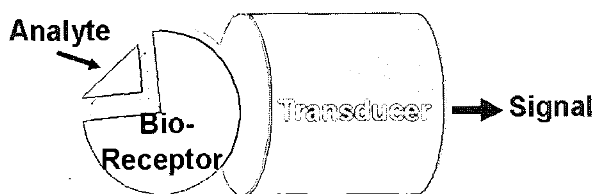

Fig. 1. Different components of Biosensors.

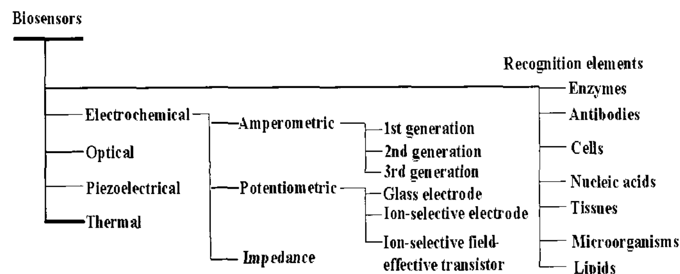

Fig. 2. Classification of Biosensors.

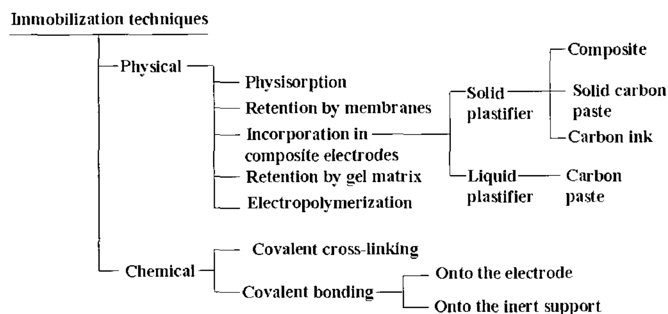

Fig. 3. Classification of the immobilization techniques.

CLASSIFICATION OF BIOSENSORS BASED ON BIOLOGICAL RECOGNITION ELEMENTS

The usual aim of a biosensor is to produce either discrete or continuous electrical signals, which are proportional to a single analyte or a related group of analytes. The biological recognition element interacts selectively with the target analyte(s), assuring the selectivity of the sensors [9]. With biosensors, it is possible to measure specific analytes in complex matrices considerably reducing the need for sample preparation and it, therefore, results in a fast response time with great accuracy. One characteristic of biosensors that distinguishes them from other bioanalytical methods is that the analyte tracers or catalytic products can be directly and instantaneously measured. The biological recognition elements used in the biosensor construction is shown in Fig. 4 and can be catagorized into two types. The first category uses enzymes, microorganisms, and tissue materials as biocomponents. Thus, a continuous consumption of analyte(s) is achieved by the immobilized biocatalyst incorporated into the sensor: transient or steady-state responses are monitored by integrated detector [10].

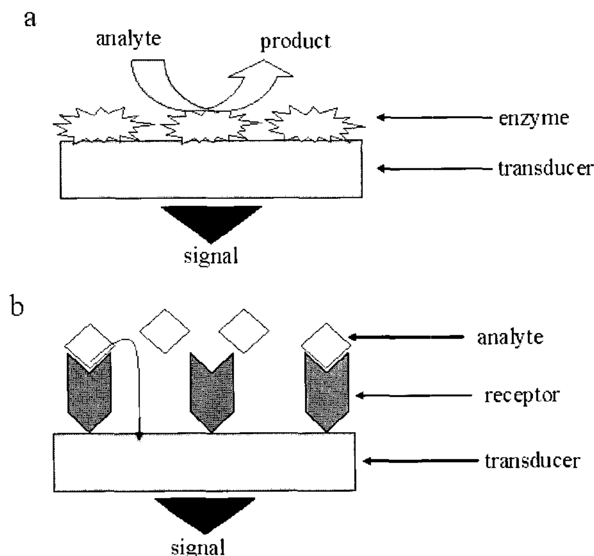

Fig. 4. Schematic representation of biosensors based on (a) biocatalysis and (b) bioaffinity.

Secondly, when selective enzymes are not available for the detection of an analyte, antibodies, nucleic acids, and lectins can serve as selecting element as bioligands or bioaffinity recognition elements. The sensor operation is based on the interaction of the analyte with macromolecules or organized molecular

assemblies that have either been isolated from their original biological environment or engineered. Thus, equilibrium is usually reached and there is no further net consumption of the analyte by the immobilized biocomplexing agent. These equilibrium responses are monitored by the integrated detector. In some cases, this biocomplexing reaction is itself monitored using a complementary biocatalytic reaction. Steady-state or transient signals are then monitored by the integrated detector.

Of the bioligands, antibodies can bind to analytes very selectively. Biosensors based on monitoring antibody-antigen interaction, are termed immunosensors [11]. A genuine immunosensor essentially comprises an antibody-bearing sensing probe that relies on direct (on-line) measurement of antigen binding by a detecting system [12]. Immunosensors are often more complex than enzyme biosensors, because there are hardly any (sensitive) detection methods that can measure the interaction between antibody and antigen directly. Besides the problem that the binding of an antigen to an antibody can rarely be detected itself, there is another reason why creating immunosensors is more difficult than creating enzyme biosensors. The analytes measured by immunosensors, e.g. proteins and hormones, are often present in the body fluids in a much lower concentration than the analytes detected with an enzyme biosensor. For instance, glucose, a typical analyte for an enzyme biosensor, has physiological concentration in the millimolar range. Analytes that are usually detected with immunosensors, such as proteins and hormones, normally have to be detected in nano- and pico-molar concentrations or even below that. This requires a more specific and more sensitive detection system. Other selective elements such as organelle, microbe, tissue, ionophore etc. can be applied in biosensors. However, these are not applied as frequently as enzymes and antibodies, especially in the clinical and biomedical fields. Therefore, they will not be discussed in this review.

IMMOBILIZATION OF BIOLOGICAL RECOGNITION ELEMENTS

The most important part in the fabrication of a biosensor is the successful immobilization of an enzyme or other biological components. The biological component has to be intimately connected to the transducer by various immobilization methods. The most common immobilization techniques are presented in Fig. 3. The general immobilization methods are physical adsorption, physical entrapment, crosslinking, and covalent coupling. Physical immobilization occurs through electrostatic or hydrophobic interaction, which keeps the enzyme chemically untouched, while chemical techniques bind the enzyme via covalent binding to the matrix [13,14]. Although adsorption is the simplest method for immobilizing receptor biomolecules, the disadvantage is a weak immobilization leading to the possible leak of biocatalyst [15]. Chemical immobilization (crosslinking or covalent binding) utilizes chemical reactions applicable to organic functionalities present in the biomolecule protein shell, such as amino groups, the phenol ring of tyrosine, carboxylic groups, the sulfhydryl group of cysteine, and imidazole group of histidine. Crosslinking of biomolecules to a support or other enzymes using intermolecular covalent linkages can increase sensor stability and improve electron transfer. Covalent coupling leads to strong binding of the biomolecules and desorption is practically not observed. This is of special interest in flow systems, where the mechanical stress is high. Functional groups on the support (the electrode) can be treated with suitable reagents (e.g. carbodiimide) allowing the activated surface to bind with amine, thiol, and hydroxyl groups present in the amino side chains of the enzymes or biomolecules.

DETECTION METHODS

The choice of the detection method is not only determined by the sensitivity of the detection method, but also by the interfering compounds present in the matrix. Most biosensors used so far optical or electrochemical detection methods. The detection of an analyte (e.g. as a result of an enzymatic reaction of the analyte) by measuring the change of the light absorption at a certain wavelength is normally not so very sensitive. Electrochemical methods are usually amperometric (measuring changes of current), potentiometric (measuring a difference in potential), impedometry, or conductometric (measuring changes in conductance on the electrode) [16]. An advantage of electrochemical detection methods is a high sensitive, compared to most optical detection methods [17]. Furthermore, the materials that are required are cheap, compared to fluorometric (or the older radiochemical) detection methods. Other detection methods, such as SPR (surface plasmon resonance) [18,19] and piezo-immunosensors [20,21] are also available. However, this review is concerned with only the electrochemical detection methods because of their simplicity and high sensitivity.

ELECTROCHEMICAL BIOSENSORS

Electrochemical biosensors are devices capable of retrieving analytical informations from the operational environment by utilizing biological components as part of the sensor based on electrochemical techniques. The biocomponent provides the selective molecular

recognition capabilities required in sensors and may play a role in producing a readily detectable product if the analyte itself is not electroactive. The principle of the electrochemical biosensor is presented in Fig. 5. Electrochemical biosensors have four major advantages over traditional analytical methods, which will certainly lead to their even more pronounced use in the biomedical or environmental field:

- Miniaturization of device
- Substrate detection without prior separation
- Short response time
- asy to fabricate and use

The principle of electron transfer is the basis for separating amperometric biosensors in three main groups (named generations). The first generation biosensors depend on the detection of natural co-substrates and products. The second generation biosensors utilize redox mediators for the electron transfer. The third generation biosensors depend on direct electron transfer between the prosthetic group of the enzyme and the electrode.

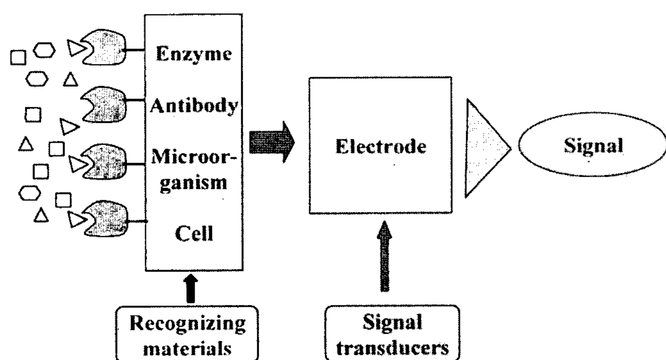

Fig. 5. Schematic configuration of the principles of electrochemical biosensors.

Electrochemical immunosensors combine the analytical power of electrochemical techniques with the specificity of biological recognition processes, which constitute an evident alternative to the already existing traditional methods [22]. The antigen-antibody interaction occurs through multiple non-covalent bonds, such as hydrogen bonds, electrostatic, hydrophobic, and Vander Walls interactions. The antigen-antibody complex formation can be detected either directly (without using any labeled compound) by certain physical (potential, capacitance, conductivity) measurements or indirect approaches in which one of the immuno-compounds is conjugated with an indicator molecule (label) [23]. For example, enzyme catalyzing redox reactions are used as label and the signal measures the electron transfer during enzyme activity by electrochemical means [24-27]. Thus, it required additional chemical synthesis steps. This detection technique utilizes two immunoassay formats: competitive and non-competitive immunoassay and is shown in Fig. 6. Although many model systems have been described for (electrochemical) immunosensors, there are no commercial sensor devices available yet [28].

Due to the tremendously growing variety of developments, this review is not intended to be comprehensive. The main focus will be the description of the reported clinical applications of immunosensors. For a more through understanding, the readers are suggested to read the several excellent reviews on the technical aspects and the applications of immunosensors [29-32].

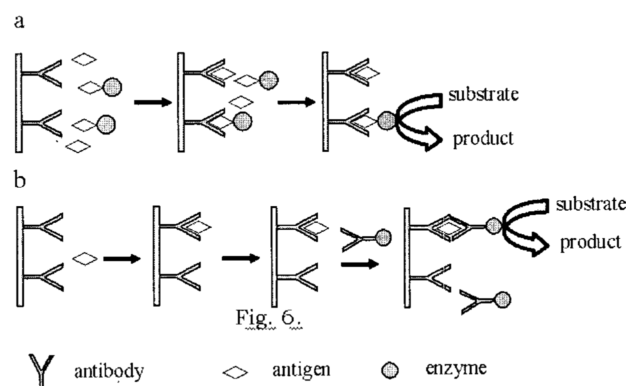

Fig. 6. Configuration of a (a) competitive and (b) non-competitive immunosensor principles using immobilized antibody.

CONDUCTING POLYMER BASED ELECTROCHEMICAL BIOSENSORS

Conductive polymers [33] have attracted much interests in the development of biosensors [34]. Polymers have been used to immobilize biomolecules onto the electrode surface and recently they have been used to fulfill a number of diverse roles. A large number of polymers have been used in the production of enzyme sensor, but by far the most attractive group is conducting polymers, which are capable of electropolymerization. This kind of device can leads to develop so called reagentless 'third-generation biosensor', where redox probe is not required [34]. This technique confers many advantages over the more traditional dip-coating procedures, such as greater reproducibility, complete coverage of the active surface, greater control over film thickness, and the most important fact is to provide a means for the

entrapment of biomolecules. However, in cases where no electroactive species are present, the biomolecular interaction of interest may give rise to a change in electronic properties (conductivity, capacitance) of the polymer and/or the electrochemical switching properties of the polymer. Some of the electropolymers can also serve as a mediator, decreasing the working potential and thereby avoiding interference from other species.

Furthermore, two major immobilization methods are frequently used in fabrication of conducting polymer-based biosensors. They are: 1. direct electropolymerization-deposition to include the biological component. 2. immobilizing bioactive materials subsequent to polymerization. However, the entrapment of proteins during electropolymerization in organic solution may cause damage to activities of proteins. To overcome this problem conducting polymer that has –COOH or $-NH_2$ functional groups is advantageous [35, 36]. Most importantly, biomolecules can be covalently immobilized by exposed lysine residue onto the conducting polymer having functional groups. A lot of works on the conducting polymer based biosensors, immunosensors, and DNA sensors have been carried out in our laboratory. Amperometric enzymatic biosensors [37-39] for phosphate, choline, and glutamate have been fabricated by the covalent immobilization of enzymes onto the poly-5,2′:5′,2″-terthiophene-3′-carboxylic acid, poly-TTCA (poly-CP) layers on a glassy carbon electrode. The electrochemically prepared conducting polymer layer oxidized the enzymatically generated H_2O_2 at a lower potential and was the basis of detection of phosphate, choline, and glutamate. The biosensors showed good selectivity towards other interfering anions. The long-term storage stability of the phosphate biosensor has been studied and the sensor was applied in a human serum sample for the phosphate ions detection.

A disposable and mediatorless immnunosensor based on a conducting polymer coated screen-printed carbon electrode has been developed using a separation-free homogeneous technique for the detection of rabbit IgG as a model analyte [40]. In an another work, a simple and direct immunosensor for the determination of carp (*carassius auratus*) vitellogenin (Vtg), a female specific protein, has been proposed based on an antibody captured conducting polymer coated electrode [41]. A disposable amperometric immunosensor has been studied for the rapid detection of carp (*carassius auratus*) Vitellogenin (Vtg) [42]. To develop a general method for the detection of histidine-tagged protein, the interactions of the histidine epitope tag of MutH and MutL proteins with the epitope specific monoclonal anti-His_6 antibody has been monitored by a label-free direct method using impedance spectroscopy [43].

Electrochemical methods for DNA hybridization detection have many advantages that are very fast to detect and can be directly applied for a potable DNA sensor. An electrochemical method to directly detect DNA hybridization has been developed on the basis of a conductive polymer, which was polymerized on the glassy carbon electrode with a terthiophene monomer having a carboxyl group [44]. Detection of protein-DNA interaction with a conducting polymer based DNA probe has also been carried out [45].

BIOMEDICAL APPLICATIONS OF ENZYME BASED AMPEROMETRIC BIOSENSORS

The enzyme-based amperometric biosensors are successfully applied in the biomedical field. These include in-vivo continuous monitoring of glucose in human volunteers [46] and monitoring biochemical reactions at a single cell level [47], two areas thought to be difficult to achieve not long time ago. As mentioned before, biosensors have many advantages, such as miniaturization, fast response time, and easy to fabrication. These advantages will certainly lead to their even more pronounced use in the biomedical field in the near future. However, many enzyme-based amperometric biosensors still suffer from a low stability and interference effect [48]. These two problems should be overcome due to the complex sample matrices in the biomedical field. Biocompatibility and biofouling are critical issues in case of in-vivo measurements.

The amperometic enzyme-based biosensors have already been utilized in the biomedical applications. Glucose biosensors are now commercially available. Diabetes affects a large number of persons, and the best way to manage this disorder involves a careful monitoring of the blood glucose level and insulin administration, if necessary. Amperometric glucose biosensors are today widely a fatal outcome [49]. The lactate blood concentration is also important data for athletes, since elevated levels of blood lactate decrease the blood pH resulting in muscular fatigue or cramps. Another analyte with biomedical importance is cholesterol, since excess of cholesterol is associated with problems in the blood circulatory system. The blood urea level is also an important index due to the disorders associated with it such as gout, Lesch–Nyhan syndrome, and Fanconi syndrome. Certain alterations in the level of different neurotransmitters follow a number of brain disorders. Glutamate, for example, is associated with a range of brain disorders including schizophrenia, Parkinson's disease, stroke and epilepsy. Creatinine is a protein produced by muscle and released into the blood. It is a key analyte for diagnosis of renal and muscular dysfunctions. Detection of ethanol in human breath, saliva or blood serum is an important tool in clinical forensic [50]. Enzyme-based amperometric sensors developed in laboratories around the world have targeted all these substrates, and many more. The list of biosensors in biomedical applications is given in Table 1

Table 1. *Biosensors for biomedical applications (9)*

Analyte	Enzyme	Characteristics	Application
Glucose	Glucose oxidase	LR: up to 25mM	Measurements with the sensor implanted subcutaneously in dog
	Glucose oxidase	LR: up to 25mM DL: 0.5mM	Detection during glucose tolerance tests on volunteers
	Glucose oxidase	LR: up to 30mM	Determination of glucose in whole blood
Lactate	Lactate oxidase	Duel electrode system	Detection in human saliva
	Lactate oxisade	LR: 0.2–10mM	Simultaneous detection of glucose and lactate in rat brain and in human serum
Cholestorel	Cholesterol oxidase	LR: 0.5–15mM DL: 120 μM RT: 30 s	Serum samples from hospitalized patients
Urea	Urease	LR: up to 350 μM DL: 3 μM More than 3 months stability	Measurements in fresh blood and urine from healthy persons
	Urea amidolyase	LR: up to 350 μM DL: 5 μM RT: 30 s	In serum and urine samples
l-Glutamate	Glutamate oxidase	DL: 0.5 μM Multisite detection	Glutamate release and uptake in rat prefrontal cortex
	Glutamate oxidase	DL: 6.4 nM	Monitoring release from cultured rat nerve cell
Choline	Choline oxidase	LR: up to 200 μM DL: 0.4 μM	Measurements in rat brain
Creatinine	Creatinine Amidohydrolase	LR: up to 2mM RT: 1.0 min	Measurements in control and hospital human serum
	Creatinine Amidohydrolase	DL: 10–20 μM LR: 0.01–1.7 mg/dl	Measurements in effluent dialysate

LR: linear range; DL: detection limit; RT: response time.

ELECTROCHEMICAL BIOSENSORS IN CLINICAL APPLICATIONS

In 1962, Clark et al. [8] introduced the first biosensor for glucose. This enzyme biosensor was based on detecting the decrease of oxygen concentration, which was the co-substrate for the conversion of glucose by the enzyme glucose oxidase. Later, the oxidation of glucose was also followed by the increase of hydrogen peroxide [28]. Of all biosensors, the glucose biosensor has been studied most intensively. Now, it is possible for a diabetic patient to monitor their blood glucose level by themselves just pricking a blood sample and on a strip of biosensor [17]. However, the patients experience this as troublesome and painful.

Furthermore, the measurement only gives the glucose concentration at the time the finger prick was performed and the measurement is still expensive [17]. Biosensors, continuously monitoring the glucose concentration of blood, would enable the patient to keep the glucose concentration in the blood more constant without trouble and pain. Research to develop such a sensor has started a long time ago. When such a sensor is linked to an insulin pump, being a kind of "artificial pancreas", discomfort and complications will be reduced [51]. The glucose sensors were improved over the years, for example by the utilization of mediators. Prototypes of glucose sensors [52] and needle-type glucose biosensors [53, 54] are tested in volunteers. An acetaminophen sensor has been fabricated and tested to measure acetaminophen in blood [55].

MAIN COMPOUNDS IN CHEMICAL AND PHYSIOLOGICAL CHARACTERISTICS

Glucose

Glucose is a sugar that is an important source of energy in the body and the sole source of energy for the brain. In clinical diagnostics, its determination is a routine clinical test to measure glucose plasma levels in several diseases or during hospitalization. One of the main reasons to monitor the glucose concentrations is to achieve adequate metabolic control of diabetics. For this, frequent analysis is required (several times a day). Besides diabetology, there is a strong demand for miniaturized integrated glucose biosensors for in vitro and in vivo applications in the intensive care, the operation theatre, and in the field of bedside analysis [56].

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. [57]. Mascini et al. [58] reported an "artificial pancreas" for continuous measurement of glucose. A number of glucose biosensors have been reported which are based on conducting polymers [59-61]. These electrodes have been shown to be useful for glucose estimation from 1 to 40 mM and stability of about 6 days.

Lactate

Lactate is an intermediate metabolite of the anaerobic glycolysis. It has been of interest in physiology, for example because of its relation to anaerobic metabolism during muscle contraction [5]. In clinical medicine, several pathological conditions cause increased lactate production. 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 for lactate measurements [62,63]. Li and coworkers [64] 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.

Cholesterol

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 [65-69]. Recently, Vengatajalabathy and Mizutani [70] demonstrated an amperometric biosensor for cholesterol determination by a layer-by-layer self-assembly using cholesterol oxidase (ChOx) and poly(styrenesulfonate) on a monolayer of microperoxidase covalently immobilized on Au-alkanethiolate electrodes. The sensor was found to be responsive even in the presence of potential interfering compounds, L-ascorbic acid, pyruvic acid, and uric acid.

Uric Acid

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 disorders 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 [71-73].

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 [9, 74].

Creatinine is an analyte used for the determination of renal and muscular dysfunction. By using creatinine amidohydrolase, a craetinine biosesnor have been fabricated and tested in control and hospital human serum. The linear range is up to 2 mM and the detection limit is about 10-20 µM. The response time is about 1 min [9].

Development of In Vivo or In Vitro On-line Biosensors

To develop an on-line clinical biosensor device for in vivo or in vitro applications, there are physiological and technical aspects to be considered. The physiological aspects are merely concerned with the choice of analyte and, subsequently, the site in the body where

this analyte has to be measured. The technical aspects of an on-line biosensor can be divided into three parts: Firstly, contact with the body fluid has to be made. Secondly, the analyte in the complex matrix has to be recognized specifically. Thirdly, the recognition should be translated into a quantitative signal, e.g. a number or an analogue signal. The occurrence and "behaviour" of the analyte in a certain body fluid dictate the place of sampling. Most analytes have to be monitored in blood, but sometimes subcutaneous (under the skin) or transcutaneous (on top of the skin) measurements can be performed, even to estimate analyte concentrations in blood.

The configuration of a biosensor has to be adjusted to the sampling site. When intravenous measurements are necessary, a small contact area between the device and the body is essential, because it has to be inserted into a blood vessel. For transcutaneous analysis, a large contact surface may be preferred to establish a larger signal. For the first requirement, continuous contact with the matrix is needed. To enable this, there are two options: measurement directly inside the body or a combination of a sampling and an analysis system. The direct measurement inside the body with biosensors often leads to problems, such as instability of the sensor signal and biocompatibility. Problems that might occur in these sensors are calibration (because the response *in vivo* can be rather different compared to the *in vitro* response) and the stability of the sensor [75]. An amperometric glutamate microbiosensor based on conducting polymer for in vivo measurements has been reported. This glutamate microbiosensor was successfully used in the rat brain for in vivo monitoring of the extra cellular glutamate released by cocaine stimulation [39]

IMMUNOSENSORS IN CLINICAL APPLICATIONS

The development of immunosensor technology is a promising alternate to traditional immunoassay especially for the clinically laboratory because of continuous monitoring. However, there are only a few commercial applications of immunosensors in clinical diagnostics. Applications to the real clinical samples are also still rare. An interesting application is the determination of human chorionic gonadotrophin (hCG) by an amperometric immunosensor, described by Chetcuti et al. [76]. The authors used Nafion, a polyanionic perfluorosulfonated ionomer to immobilize anti-hCG monoclonal antibodies onto a glassy carbon electrode. Santandreu et al. [77] analyzed b-hCG using an amperometric sensor device based on a conducting immunocomposite. Kelly et al. [78] described an immunosensor for lactate dehydrogenase (LDH) isoenzyme 1 in serum. The LDH activity was measured amperometrically by the oxidation of NADH at the electrode surface. Reproducibility, as well as selectivity of the sensor, however, was insufficient for clinical use. Liu et al. [79] used a similar approach with Nafion and a protein A coupled polyaniline on an amperometric sensor surface. A prototype amperometric immunosensor for rapid detection of plasma-heart-type fatty acid-binding protein H-FABP as early marker for myocardial damage, tested under routine conditions, was presented by Key et al. [80, 81]. The authors used a monoclonal anti-FABP antibody, linked to aP, the enzyme catalyzing the hydrolysis of 4-APP to the electrochemically active phenol derivative. A first in vivo immunosensor device has been recently described [82]. The author demonstrated an electrochemical immunosensor for cortisol, placing an electrochemical sensor in a needle type microdialysis probe. With this, cortisol and corticosterone was detected in a competition immunoassay. This approach was posed as the next generation of immunosensors [83]. However, the implementation of this method may harm the surrounding tissue, e.g. because HCl-solutions are perfused through the probe and may therefore penetrate into the tissue. Furthermore, the applied cortisol-horseradish-peroxidase-complex may (partially) leak into the tissue because of small irregularities in the membrane. Above all, the proposed device still requires complex pumping and valve connections. Some more applications of amperometric immunosensors in real sample matrices are summarized in Table 2.

CONCLUSIONS

Biosensors are firmly established for applications in clinical analyses. Biosensors for measurement of blood metabolites such as glucose, lactate, urea, and creatinine using electrochemical transduction are commercially developed. They are routinely used in the laboratory, in point-of-care settings, and in the case for self-testing of glucose.

Clinical diagnostics represent a huge, well-established, and important analytical field. The electrochemical immunosensor have great opportunities in this field. However, the design of an immunosensor must meet the special demands of clinical chemistry. Several issues, such as cost, regulatory requirements, quality control panel etc. need to be taken into consideration when evaluating immunosensor projects. The technical developments of immunosensors are ongoing efforts. In particular, electrochemical immunosensors have proven to be potent and future directed analytical tools. Their sensitivities of are better or comparable to immunoassay methods. It is conceivable that in clinical diagnostics, immunosensors will also be able to occupy an analytical niche for online monitoring. Finally, we expect that biosensors coupled with ubiquitous method will give promising technology to medical diagnostics in near future.

Table2. Practical applications of amperometric immunosensors (26)

Analyte	Range	Time (min)	Format	Label	Transducer	Design
hAlbumin (urine)	0.5-100 mg/L	30	Compet.	HSA-GOD	amp. H_2O_2	Disposable membrane
Apoliprot.E (serum)	50-1000 Microg/L	120	Sandwich	AB-ALP	amp. PAP	Disposable membrane
hCG (serum)	20-10^5 IU/L	75	Compet.	hCG-Catalase	amp. O_2	Disposable membrane
hCG (urine)	150-2500 IU/L	20	Sandwich	MAb2-GOD	amp. H_2O_2	Magnetic particle
hCG(blood)	2.5-70 IU/L	30	Sandwich	MAb2-ALP	amp. PAP	2-compartment dev.
Cortisol (serum)	30-700 microg/L	20	Compet.	Cortisol-ALP	amp.	Kinetic
αFetoproteine (serum)	0.01-10 microg/L	120	Compet.	Fetoproteine-Catalase	Amp. O_2	Disposable membrane
αFetoproteine (serum)	0.5-120 microg/L	70	Sandwich	MAb2-Catalase	amp.O_2	Regenerable surface
Hepatitis B (serum)	0.1-100 microg/L	90	Sandwich	AB2-GOD	amp. O_2	
HFABP (serum)	5-80 microg/L	27	Sanwich	AB2-GOD	amp.O_2	
hIgE(serum)	2-340 microg/L	10	Sandwich	Ab-POD	amp. I_2	Automated apparatus
hLH(serum)	1-200 microg/L	5	Sandwich	GOD-POD	amp. I_2	Channelling assay
Prostate antigen	0.4-30 microg/L	30	Sandwich	MAb2-ALP	amp. PAP	Homogeneous
Thyroxin BG (serum)	30-1000 microg/L	30	Sandwich	Ab2-ALP	amp.PAP	
TSH(serum)	0.2-100 mIU/L	30	Compet.	AlcDH/ NADH OD	amp. H_2O_2	Applification
TSH(Serum)	0.0018-3 mIU/L	90	Sandwich	MAb2-β-GAL	amp.O_2	Amplify. ODH/Lacc

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