Chapter 1

General introduction

Biosensoren zijn gewoon te moeilijk (J. Koopal, Technisch Weekblad, 9 nov 1994)
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Biomedical analysis

The first diagnosis of a disease by measurement of a constituent in a body fluid took place in the Middle Ages. Diabetes mellitus was diagnosed by tasting the sweetness of urine (Calbreath, 1992). Since then, knowledge of the composition of the body fluids increased, and by the end of the 19th century, many biochemical compounds were isolated and characterized (Calbreath, 1992). Nowadays, salts, sugars, proteins, hormones etc. are analysed to assist in diagnosis and to assess disease. The major metabolite measured today is glucose, mainly for diabetes care (Pickup, 1993). Other examples of the analysis of biochemical compounds can be found in cardiology (e.g. measurement of fatty acid-binding protein for diagnosis of an acute myocardial infarction) (Glatz et al., 1997), hormonal diseases (Cushing syndrome diagnosis by measuring cortisol) (Gosling et al., 1993) and in sports medicine (measuring lactate concentrations for fitness monitoring and to investigate tissue damage after training) (Kearney, 1996). The biochemical parameters for diagnosis of physiological abnormalities are normally measured batch-wise in discrete samples. The (bio)chemical analysis itself is usually performed in a laboratory. An important improvement for rapid clinical intervention or for a follow-up of the progression of a disease would be to apply analysis methods that measure constituents in body fluids without laboratory handling procedures. The ultimate goal would be the development of an on-line, bedside analysis system measuring the body fluid parameters continuously. Biosensors may anticipate in this area, for these can meet such requirements.

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 (biological receptor, selector) which is combined with a transducing (detecting) element.

This chapter concentrates on giving examples of optical and electrochemical techniques, which have been used, or have great potential, in biomedical applications. For more detailed description of fundamental aspects of biosensors and their applications, many excellent books and reviews are available, and are suggested for further reading (Canh, 1991; Aizawa, 1994; Wilkins and Atanasov, 1996; Hansen, 1996; Taylor and Schultz, 1996; Scheller et al., 1997a).

Biosensor concept

A biosensor consists of two elements (see Fig. 1.1):
1) (Bio)selector. The selector recognizes the analyte and reacts or binds to it.
2) Transducer. The transducer is the detector, monitoring the chemical or biochemical reaction initiated by the sample.

The two elements are methodologically closely linked to each other, preferably in a single device. In principle, no additional separation step should be applied and no extra reagents should be added to perform the measurement, though in some literature, devices
need such additional procedures are also called biosensors (Aizawa, 1994). We use this broader understanding of the term “biosensors” in this thesis.

![Biosensor concept diagram]

Table 1.1 Types of biosensors
(modified from Lambrechts and Sansen, 1992a; Scheller et al., 1997b)

<table>
<thead>
<tr>
<th>Bioaffinity sensors</th>
<th>Biocatalytic sensors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Device name</strong></td>
<td><strong>Receptor</strong></td>
</tr>
<tr>
<td>dye</td>
<td>protein</td>
</tr>
<tr>
<td>lectin</td>
<td>glycoprotein</td>
</tr>
<tr>
<td>enzyme</td>
<td>substrate inhibitor</td>
</tr>
<tr>
<td>apoenzyme</td>
<td>hormone</td>
</tr>
<tr>
<td>receptor</td>
<td>hormone</td>
</tr>
<tr>
<td>antibody</td>
<td>antigen</td>
</tr>
<tr>
<td>receptor</td>
<td>hormone</td>
</tr>
</tbody>
</table>

**Selectors**

The selectors of biosensors consist of biomolecules, biorecognition elements or analogues thereof (Table 1.1). The selectors determine the selectivity, so that only the compound which has to be measured leads to a signal. The selection can be based on bioaffinity, in which the bioelement does not change the chemical structure of the analyte...
Chapter 1

(e.g. an antibody), or biocatalysis, in which the bioelement catalyses a biochemical reaction of the analyte (e.g. an enzyme) (see Fig. 1.2).

![Diagram of biocatalysis and bioaffinity](image)

**Fig. 1.2. Biocatalysis (A) and bioaffinity (B).** $E_1$, $E_2$ enzymes; $S$: substrate; $P$: product.

Most biosensors described in literature use an enzyme as selecting element (Table 1.2). Purification of enzymes is relatively cheap, because most enzymes can be isolated from micro-organisms, such as fungi or bacteria, which produce these enzymes in excess, naturally or after genetic manipulation (Kopetzki et al., 1994). The enzyme catalyses specifically a conversion of the analyte. In most cases, this reaction only uses the analyte, but sometimes also other compounds (co-substrates). Such a reaction has usually a high specificity and can be followed by measuring the increase of the formed product or the decrease of a co-substrate, which is consumed during the enzyme reaction. Glucose, oxidized by the enzyme glucose oxidase, is an example of an enzymatically detectable metabolite.

When selective enzymes are not available for the detection of an analyte, antibodies can serve as selecting element. Antibodies can bind to analytes very selectively. Biosensors based on monitoring antibody-antigen interaction, are termed immunosensors (e.g. see review Morgan et al. (1996)). A genuine immunosensor essentially comprises an antibody-bearing sensing probe that relies on direct (on-line) measurement of antigen binding by a detecting system (Ekins, 1994). 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. Mass sensitive or optical immunosensors (see below) are based on measuring the change of bound material onto a surface. Antigens (the analyte of interest) or antigen-analogues present in (or added to) the sample associate to surface immobilized antibodies, thus forming a complex which is larger than the antibody alone. Other immunosensors operate following the ELISA-principle, i.e. one immuno compound is labelled, e.g. with a fluorophore or enzyme. This labelled compound can then compete with an unlabelled immuno compound (the analyte) or form a sandwich complex. If an enzyme is used, the signal can be amplified by increasing the incubation time.
Table 1.2. Enzyme biosensors
(modified from Lambrechts and Sansen, 1992; Pfeiffer et al., 1996)

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Substrate</th>
<th>Enzyme</th>
<th>Indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>saccharides</td>
<td>glucose</td>
<td>glucose oxidase</td>
<td>O₂, H₂O₂</td>
</tr>
<tr>
<td>alcohols</td>
<td>lactate</td>
<td>lactate oxidase</td>
<td>O₂, H₂O₂</td>
</tr>
<tr>
<td>aminoacids</td>
<td>ethanol</td>
<td>ethanol oxidase</td>
<td>O₂</td>
</tr>
<tr>
<td>acids</td>
<td>glutamate</td>
<td>glutamate dehydrogenase</td>
<td>NH₄⁺</td>
</tr>
<tr>
<td></td>
<td>acetic acid</td>
<td>alcohol oxidase</td>
<td>O₂</td>
</tr>
<tr>
<td>lipids</td>
<td>cholesterol</td>
<td>cholesterol oxidase</td>
<td>H₂O₂</td>
</tr>
<tr>
<td>antibiotics</td>
<td>penicillin</td>
<td>penicillinase</td>
<td>pH electrode</td>
</tr>
<tr>
<td>other substrates</td>
<td>urea</td>
<td>urease</td>
<td>NH₄⁺</td>
</tr>
<tr>
<td></td>
<td>nitrate</td>
<td>nitrate reductase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>nitrite</td>
<td>nitrite reductase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>creatine</td>
<td>Creatinase</td>
<td>NH₄⁺</td>
</tr>
<tr>
<td></td>
<td>inorganic phosphorus</td>
<td>Alkaline phosphatase</td>
<td>H₂O₂</td>
</tr>
</tbody>
</table>

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 enzyme biosensors, has physiological concentrations in the millimolar range. Analytes that are usually detected with immunosensors, such as proteins and hormones, normally have to be detected in nano- and picomolar concentrations or even below that. This requires a more specific, more sensitive detection system.

As illustrated in Table 1.1, other selective elements can be applied in biosensors. However, these are not applied as frequently as enzymes and antibodies, especially in the medical field. Therefore, they will not be discussed in this chapter.

Transducers

The transducer (detector) translates the recognition of the selector into a digital or analogue (preferably quantitative) signal. Possible transducer technologies are optical, electrochemical and acoustical/mechanical or calorimmetrical (see Table 1.3).

The choice of the detection method is not only determined by the sensitivity of the detection method, but also by the contaminants present in the matrix. Most biosensors used so far apply optical or electrochemical detection. 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 very sensitive. For analytes present at sufficiently high concentrations, this is not a problem. An advantage of this optical detection system is that there are not many compounds, which might interfere in measurement, therefore the selectivity is high.
Table 1.3. Transducer devices
(modified from Schultz and Taylor, 1996; Wang, 1996; Hock, 1997)

<table>
<thead>
<tr>
<th>Device</th>
<th>Output change</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electronic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amperometric</td>
<td>applied current</td>
<td>immunosensor</td>
</tr>
<tr>
<td>potentiometric</td>
<td>voltage (potential)</td>
<td>ISFET</td>
</tr>
<tr>
<td>capacitance/impedance</td>
<td>impedance (modulation)</td>
<td>conductiometers</td>
</tr>
<tr>
<td><strong>Optical/Photometric</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>light adsorption or scattering; refractive index</td>
<td>light intensity, colour, emission</td>
<td>ellipsometry, internal reflectometry, laser light scattering</td>
</tr>
<tr>
<td>fluorescence or luminescence activation, quenching, polarization</td>
<td>fluorescence or chemiluminescence</td>
<td>surface plasmon resonance, fiber optic wave guides, fluorescence polarization</td>
</tr>
<tr>
<td><strong>Acoustical/mechanical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acoustical</td>
<td>amplitude, phase or frequency (acoustic wave)</td>
<td>surface Acoustic Wave Devices</td>
</tr>
<tr>
<td>mass/density</td>
<td>weight</td>
<td>piezoelectrometric devices</td>
</tr>
<tr>
<td><strong>Calorimetric</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thermistor</td>
<td>temperature</td>
<td>enzyme sensors</td>
</tr>
</tbody>
</table>

Electrochemical detection can be amperometric (measuring changes of current) or potentiometric (measuring a difference in potential) or conductiometric (measuring changes in conductance on the electrode) (Wang, 1996). An example of an electrochemical sensor is an ISFET (ion-sensitive field-effect transistor). These ISFET systems opened possibilities for measuring ions and miniaturization of the transduction system, and have been used for example for the detection of heparin in blood samples (Van Kerkhof et al., 1995). An advantage of electrochemical detection (ECD) devices is that the sensitivity is high, compared to most optical detection methods (Pfeiffer et al., 1996). Furthermore, the materials that are required are cheap, compared to fluorimetric (or the older radiochemical) detection devices. Disadvantages of ECD-based sensors are that their signal may drift and that unspecific reduction or oxidation of compounds present in the sample may occur. Thus, ECD devices have high sensitivity but relatively low specificity.

An alternative optical detection method is measuring fluorimetric changes (Hemmila, 1985; Morgan et al., 1996). Fluorescent signals can be created by probes carrying fluorescent dyes, or by probes containing enzymes, catalysing reactions forming fluorescent products. The sensitivity of electrochemical and fluorescent detection is often comparable, but fluorescent measurements encounter less interference. However, the apparatus required is large and both the equipment and the fluorescent dyes or substrates are expensive. Application of fiber optics may improve this technique considerably (Abel et al., 1996).

During the last decades some new detection methods for immunosensors were developed. Examples of these methods are sensors based on measurement of SPR (surface plasmon resonance) (Jonsson et al., 1991; Brecht and Gauglitz, 1997) and piezo immunosensors (Kricka, 1994; Joracek and Skaladal, 1997), detecting antigen-antibody
interactions without additional labels and in real time. However, the antigens should have high molecular weight, and only increases of antigen amounts can normally be detected.

Clinical biosensors

In 1962, Clark et al. (1962) introduced the first biosensor for glucose. This enzyme biosensor was based on detecting the decrease of oxygen, 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 (McNeil et al., 1997).

Of all biosensors, the glucose biosensor has been studied most. In 1994, almost 2000 articles describing glucose electrodes and glucose sensors have been published (Fig. 1.3) (Lundi, 1997). Glucose dipsticks (e.g. the Glucocard or Medisense Glucopen) became available in the eighties. Since then, diabetic patients are able to monitor their blood glucose themselves, pricking a blood sample and using dry strip chemistry analysis (see Table 1.4) (Pfeiffer et al., 1996). However, the patients experience this as troublesome and painful. Furthermore, the measurement only gives the glucose concentration at the time the fingerprick was performed and the measurement is still rather expensive (Pfeiffer, 1997).

Biosensors, continuously monitoring the glucose concentration of blood, would enable the patient to keep the glucose concentration in the blood more constant, and avoid the painful fingerprick. 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 (Reach and Wilson, 1992). In the first glucose biosensors, the enzyme glucose oxidase was immobilized on electrodes. The glucose sensors were improved over the years, for example by the utilization of mediators. Mediators, electroactive chemical compounds which can reoxidize the enzyme and itself reoxidizes at the electrode, create a better link between the selection and the detection part.
and turn the measurements independent on oxygen (Boutelle et al., 1996; Parellada et al., 1997). Prototypes of glucose sensors are reported to measure up to ten days now (Wilkins et al., 1995). Needle-type biosensors, in which the sensor system is perfused with an exogenous fluid have also been tested in volunteers (Poitout et al., 1993; Hashiguchi et al., 1994). Despite all research, in vivo glucose sensors have not yet been introduced into clinical practice (Wilkins and Atanasov, 1996).

<table>
<thead>
<tr>
<th>Model</th>
<th>Company</th>
<th>Analyte</th>
<th>Measuring range (mM)</th>
<th>Functional stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>ExactTech glucose</td>
<td>MediSence (USA)</td>
<td>glucose</td>
<td>1.1-33.3</td>
<td>disposables</td>
</tr>
<tr>
<td>Satillite G glucose</td>
<td>Bayer Diagnostics (Germany)</td>
<td>glucose</td>
<td>2.0-33.3</td>
<td>disposables</td>
</tr>
<tr>
<td>Glucometer Elite glucose</td>
<td>Kyoto Daiichi Kagaku Co (Japan)</td>
<td>glucose</td>
<td>2.2-27.8</td>
<td>disposables</td>
</tr>
<tr>
<td>Glucocard glucose</td>
<td>i-STAT Corp. Princeton (USA)</td>
<td>glucose</td>
<td>2.9-23.6</td>
<td>disposables</td>
</tr>
<tr>
<td>i-STAT PCA urea</td>
<td>i-STAT Corp. Princeton (USA)</td>
<td>urea</td>
<td>1.0-43.0</td>
<td>disposables</td>
</tr>
<tr>
<td>BSE 5500 glucose</td>
<td>Orion Anal. Technol. Inc. (USA)/Dosivit (France)</td>
<td>glucose</td>
<td>1.6-16.0</td>
<td>disposables</td>
</tr>
<tr>
<td>BSE 5500 sucrose</td>
<td>Orion Anal. Technol. Inc. (USA)/Dosivit (France)</td>
<td>sucrose</td>
<td>1.6-16.0</td>
<td>disposables</td>
</tr>
<tr>
<td>BSE 5500 lactose</td>
<td>Orion Anal. Technol. Inc. (USA)/Dosivit (France)</td>
<td>lactose</td>
<td>1.6-16.0</td>
<td>disposables</td>
</tr>
<tr>
<td>Biosen 6020 G glucose</td>
<td>EKF Industrial Electronics (Germany)</td>
<td>glucose</td>
<td>0.5-20.0</td>
<td>24 days</td>
</tr>
<tr>
<td>Biosen 5020 L lactate</td>
<td>EKF Industrial Electronics (Germany)</td>
<td>lactate</td>
<td>0.5-20.0</td>
<td>10 days</td>
</tr>
</tbody>
</table>

*Fig. 1.4. In vivo immunosensor Cook (adapted from Turner, 1997).*
Although many model systems have been described for (electrochemical) immunosensors, there are no commercial sensor devices available yet (McNeil et al., 1997). A first in vivo immunosensor device has been recently described (Cook, 1997). He demonstrated an electrochemical immunosensor for cortisol, placing an electrochemical sensor in a needle type microdialysis probe (see Fig. 1.4). With this, he detected cortisol and corticosterone in a competition immunoassay. This approach was posed as the next generation of immunosensors (Turner, 1997). 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.

**Main chemical and physiological characteristics studied compounds**

**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 medicine, 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, in vivo and ex vivo applications in the intensive care, the operation theatre and in the field of bedside analysis (Urban and Jobst, 1997).

**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 (Lamont, 1987; Pilardeau et al., 1988; Scheller et al., 1997). In clinical medicine, several pathological conditions cause increased lactate production, for example vascular occlusion and lacto-acidosis caused by shock or mitochondrial enzyme deficiencies (De Boer et al., 1991).

**Cortisol**

Cortisol (hydrocortisone) is a steroid hormone of the adrenal cortex. The cortisol concentration in blood is strongly influenced by stress. It has relatively high concentrations in blood and it exhibits a strong circadian rhythm (see Fig. 1.5).
Chapter 1

**Surge after meal**

<table>
<thead>
<tr>
<th>Time of day</th>
<th>cortisol concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>midnight</td>
<td></td>
</tr>
<tr>
<td>4 a.m.</td>
<td></td>
</tr>
<tr>
<td>8 a.m.</td>
<td></td>
</tr>
<tr>
<td>noon</td>
<td></td>
</tr>
<tr>
<td>4 p.m.</td>
<td></td>
</tr>
<tr>
<td>8 p.m.</td>
<td></td>
</tr>
<tr>
<td>midnight</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1.5. Cortisol circadian rhythm.**

**Fatty acid-binding protein**

Fatty acid-binding protein (FABP) is a 15 kD protein. It is a novel plasma marker protein for acute myocardial infarction (AMI), which has a high sensitivity and specificity for early detection of AMI (see Fig. 1.6) (Glatz et al., 1997).

**A**

**B**

**Fig 1.6. FABP after infarction (A) and reinfarction (B).** FABP (○); myoglobin ( ); creatine kinase ( ); hydroxybutyrate dehydrogenase ( ); lactate dehydrogenase ( ). (adapted from Wodzig et al., 1997a, Glatz et al., 1997).

Other so-called biochemical markers for myocardial tissue injury are released later and/or have lower specificity (Apple, 1992). Because of the small size of FABP, its concentration is returned to normal values within 12 to 24 hours (Tsuji et al., 1993b; Van Nieuwenhoven et al., 1995; Wodzig et al., 1997b). Because of this fast raise and fall, the infarct size and recurrent infarctions can be monitored when FABP is determined on a regular base (Kleine et al., 1992a; Glatz et al., 1994a; Wodzig et al., 1997a; Ishii et al., 1997a).
General introduction

Development of *in vivo* or *ex vivo* biosensors

To develop an on-line clinical biosensor device for *in vivo* or *ex vivo* 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 (Arner and Bolinder, 1991; Fischer et al., 1994, 1995; Fischer, 1995).

An alternative for measuring parameters *in vivo* can be found in combining an analysis system with a continuous sampling system, such as microdialysis or ultrafiltration sampling (Fig. 1.7) (Ungerstedt, 1991; Moscone et al., 1996; Ballerstadt and Schultz, 1996; Elmquist and Sawchuk, 1997).

![Fig. 1.7. Schematic representation of microdialysis (A) and ultrafiltration (B).](image-url)
Microdialysis (MD) is a dynamic sampling method based on analyte diffusion across a semi-permeable membrane, due to a concentration gradient (Palmisano et al., 1997). The membrane forms the contact area with the body fluid, thus determining the biocompatibility. Furthermore, the membrane may serve as a selector, because it prevents diffusion of large molecules or cells to the analysis system. Accordingly, the sensing surface remains rather clean and, when membranes with the right characteristics are chosen, interference of many substances is reduced. MD probes are not only useful in intravenous sampling, they also provide the possibility for subcutaneous sampling. This is because the invasive part, the probe, can be very small and the sampling does not withdraw the scarce extracellular fluid (Arner and Bolinder, 1991). A problem encountered when using MD is that the absolute amounts of analyte are difficult to estimate (Justice, Jr. 1993). Moreover, MD can cause depletion of the analyte at the sampling site (Rosdahl et al., 1993).

Recently, ultrafiltration (UF) has been proposed as alternative for on-line sampling (Moscone et al., 1996). The UF technique consists of a sampling probe withdrawing fluid from the surrounding tissue by means of an underpressure. This technique has been tested in rats and humans. It may become (under certain circumstances) a suitable alternative for MD, because depletion is prevented and absolute concentrations can be measured. When the sampling is performed at very low flow rates (e.g. 100 nl min\(^{-1}\), as proposed by Moscone et al. (1996)), tissue damage as a result of the withdrawal of fluid is expected to be minimal.

For the second requirement of the biosensor (the selection), a continuous selection method is necessary. For metabolites like glucose, lactate and glutamate, specific enzymes (oxidases) are used to produce electrochemically active species. As these species are continuously produced by the enzymes, they can also be continuously monitored. For immuno detection, e.g. of hormones or proteins, one of the immuno compounds (the antigen or antibody) is labelled. However, the conventional competitive or sandwich immunoassays do not allow continuous detection. The only immunological selection system allowing an on-line measurement is called displacement (see Fig. 1.8).

\[ \text{Diagram of immunoassays: A. Competition; B. sandwich; C. displacement.} \]
Selection based on displacement utilizes the replacement of a previously associated antigen (or its analogue) from the antibody binding site of an antibody by an antigen of the sample. In displacement detection either antibodies or antigens are immobilized, and the respective interacting antigen (analogue) or antibody is labelled. After the antibody binding sites are saturated with antigens, the actual displacement consists of the release of labelled molecules from the immobilized ones as a result of the binding of the antigen from the sample to the antibody binding site. When these antigen-antibody complexes are placed in a flow system, the amount of displaced molecules will be dependent on the concentration of the analyte. When the complexes are immobilized in excess, the displaced fraction after a certain time interval can be neglected, and continuous samples can be analysed in a constant displacement system.

**Scope of the thesis**

The aim of the studies described in this thesis is to develop sampling and bioselective techniques, which can be used in continuous *in vivo* or *ex vivo* clinical biosensors. There are three elements required for the on-line clinical biosensor devices: contact with the analysis site, specific recognition of the analyte of interest, and translation of this recognition into a signal. The present thesis emphasizes on the creation of a contact with the analysis site (the body fluids) and a selection of the analyte, rather than on signal translation (see Fig. 1.9).

*Chapter 2* introduces the techniques for continuous *in vivo* sampling. The techniques available are, apart from direct, undiluted blood sampling, microdialysis (MD) and ultrafiltration (UF). These techniques, their merits and their disadvantages are discussed. Furthermore, sampling in various body fluids is reviewed, and the physiological implications of the analysis of samples from these body fluids, are shortly addressed. Special attention is given to measurements performed to estimate concentrations of an analyte in blood, which can not only be done by sampling in blood itself, but also in other body compartments, e.g. in subcutaneous tissue, because all body compartments are connected to each other.

*Chapter 3* describes UF sampling experiments in subcutaneous tissue as well as in the jugular vein. Very low flow rates have been obtained with an underpressure driven, homemade pump. The homemade UF probe consists of a semi-permeable dialysis tube. In this study the performance of the sampling system was analysed. Furthermore, the subcutaneous and intravenous glucose concentrations were compared.
Biomedical sensing device

Contact with sampling site

Specific biorecognition

Selection by antibodies

Selection by enzymes

Glucose

Glucose and lactate

Glucocorticoid

Fatty acid-binding protein

Colored product

Off-line optical

On-line electrochemical

HRP & GOD based detection

HRP & LOD & GOD based detection

GOD detection with glucose and ferrocene

Chapter 6

Sampling site

in vitro

i.v. and s.c. UF

s.c. UF and usMD

Translation into signal

Fig. 1.9. Overview research articles thesis.
Chapter 4 describes on-line sampling experiments in the subcutaneous tissue of the rat. The sampling was performed with UF and with a new ultraslow microdialysis (usMD) design. The aim of this study was to investigate the differences between these two sampling techniques at different flow rates. To test both sampling systems, the glucose and lactate concentrations of the samples were analysed simultaneously, using a dual bienzymatic reactor system (for selection) and electrochemical detection (for transduction).

Chapter 5 describes the chosen continuous immuno selective method: displacement. Displacement in non-flow and flow systems, and for discrete or continuous sample is reviewed. Also, examples of displacement of labelled antigen and labelled antibodies are given. The displacement is addressed both from a theoretical and a practical point of view.

Chapter 6 describes the displacement of cortisol labelled with horseradish peroxidase from immobilized antibodies initiated by the cortisol hormone present in the matrix. The aim of the study is to investigate whether displacement occurs when the displacement unit is continuously perfused with buffer containing cortisol at flow rates of a microliter scale. The selectivity and sensitivity of the displacement reaction was tested under different conditions for two different monoclonal antibodies.

Chapter 7 investigates the displacement for fatty acid-binding protein (FABP), a 15 kDa protein. FABP is a novel plasma marker protein for acute myocardial infarction (AMI), which has a high sensitivity and specificity for early detection of AMI. FABP is detected by the displacement of labelled antibodies from immobilized FABP. Unique in this experiment is the displacement in a flow system of labelled antibodies, whereas this is normally done with labelled antigens. Also, displacement has never been demonstrated for proteins before. In this chapter, the sensitivity and specificity of the displacement have been investigated, as well as different FABP-immobilization methods. The relative displacement signal for different FABP concentration has been studied.

The final chapter, chapter 8 will summarize, discuss and interpret the main results of the previous chapters and discuss the merits of the research for future biosensor development.

References
Chapter 1


General introduction


Chapter 1


