Graz University of Technology

Optical sensors for transcutaneous oxygen measurement and enzymatic reaction monitoring

Script for Lab Course 718.019
Biomedical Sensor Systems, Laboratory

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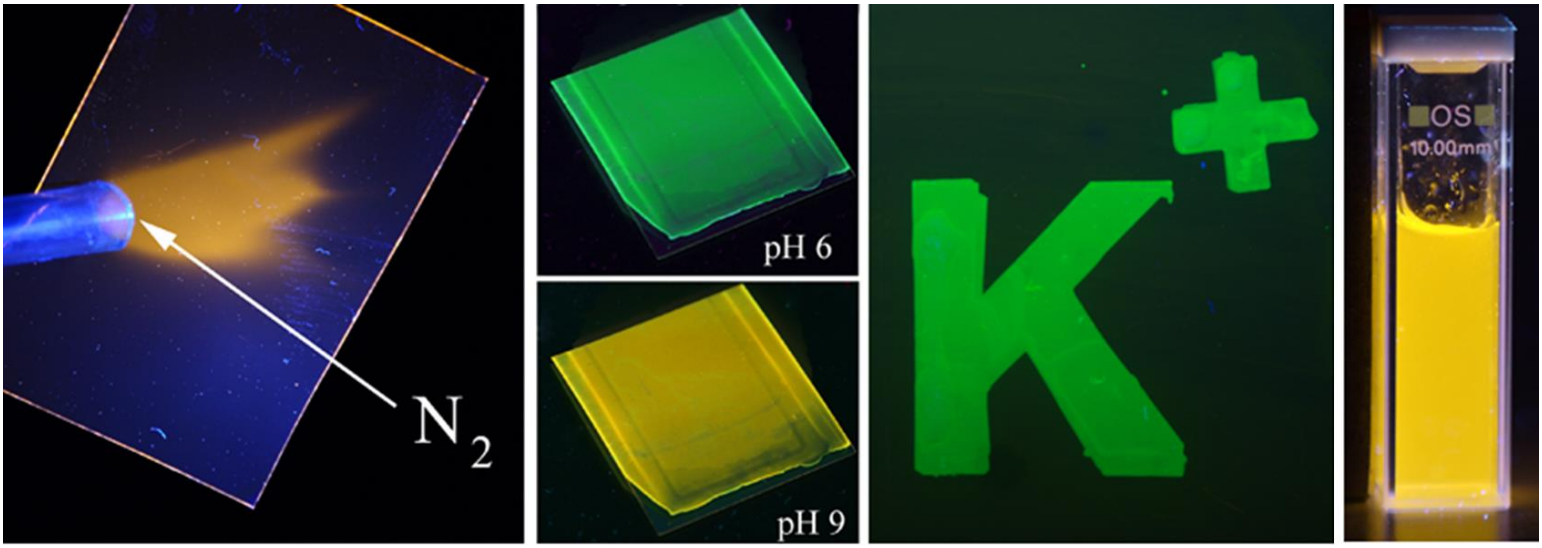

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1 Introduction

Oxygen is an essential agent for life on our planet. Thus, measurements of this chemical species are very important in many fields of our daily life. The use of optical sensors is a convenient possibility to assess the concentration of oxygen. Compared to other methods optical oxygen sensors do not suffer from electrical interferences, exhibit high selectivity, are stable against ambient or scattered light, inexpensive and easy to miniaturize, minimally invasive or non-invasive and can be used in different formats such as films, fibers or even nanoparticles.
The aim of this lab course is to get familiar with preparation and characterization of optical oxygen sensors and their application for glucose detection, transcutaneous oxygen measurements (measuring the oxygen level of the tissue through the skin) and breath analysis.

Programme:

- Preparation of Sensor Material
- Enzymatic reaction monitoring
- Transcutaneous oxygen measurement
- Breath analysis

2 Theoretical Background

2.1 Optical Sensors

In 1991, IUPAC defined chemical sensors as follows: "A chemical sensor is a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal. The chemical information, mentioned above, may originate from a chemical reaction of the analyte or from a physical property of the system investigated." An ideal sensor should be a small, robust and cheap device, able to measure an analyte selectively in a broad dynamic range with a fast response time. For continuous measurements, reversibility and absence of drifts are crucial. To enable use by untrained personnel, simple handling and easy calibration or even calibrationless measurement are desirable.
A sensor is usually composed of a filter, a receptor (the recognition element interacting with sample components) and a transducer that converts the physicochemical signal generated by the receptor into an electrical signal like current or voltage, which can be measured.

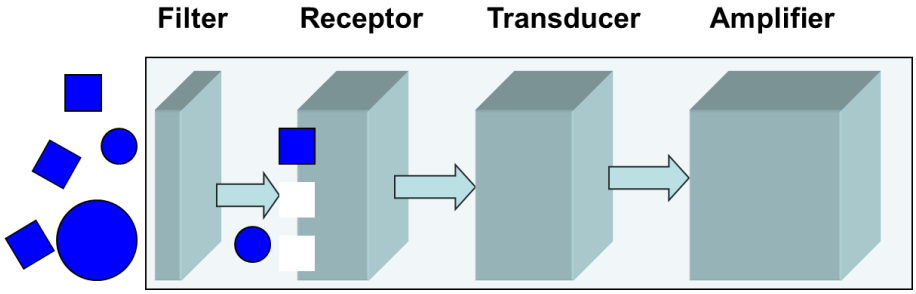

Figure 1. Typical components of a chemo- or a biosensor

Optical sensors are classified according to their transducer principle – they are based on materials that change their optical properties upon interaction with the analyte and an optical transducer is used to convert the optical signal into electronic information. Absorption, fluorescence intensity and luminescence decay time rank among the most commonly measured properties, but also reflectance, refractive index, light scattering and polarization can be used to obtain analytical information.
Optical chemical sensing has gained increasing interest throughout the last decades. The rise of the research field, but also of commercially available sensing systems is not only due to the beneficial features of optical sensors, such as their applicability for non-invasive measurements, remote and online-sensing, high sensitivity and versatility, but also due to advances in optoelectronics, which enabled the production of low-cost and miniaturized light sources and photodetectors, and availability of high quality optical fibers.

2.2 Optical oxygen sensing

Oxygen is a very important parameter to be measured in a range of applications – many of them dealing with biological or biochemical issues. This is due to the fact that oxygen is a key metabolite for almost all living organisms. Thus it is no surprise that a range of methods to determine oxygen concentration exist. Classical methods include methods such as the Winkler titration, amperometric measurement, measurement via thermoluminescence or via chemiluminescence.
The Clark electrode, for example, is robust and commonly used. Nevertheless it has some disadvantages. It is bulky and limited by oxygen consumption during the utilization time,

comparatively long response times and electrical interferences. The electrode can also be poisoned by sample constituents.
An alternative way to measure oxygen concentration is to use optical oxygen sensors. These sensors do not suffer from electrical interferences, exhibit a high selectivity, are mostly inexpensive and easy to miniaturize, minimally invasive or non-invasive and can be used as films, fibers and even nanoparticles.

2.2.1 Sensing principle

Luminescent oxygen sensors are based on the so-called dynamic quenching process, where the luminescence intensity of a luminescent dye is quenched by molecular oxygen. In presence of oxygen excited-state energy from a phosphorescent indicator molecule is transferred to an oxygen molecule upon collision (Figure 2 A). That means that a higher concentration (cO_2) or partial pressure of oxygen (pO_2) leads to a decreased luminescence intensity and luminescence lifetime, which can be measured by a typical sensor set-up, shown in Figure 2 B. Light is used to excite a luminescent indicator, which is dissolved in a polymer matrix and immobilized on the tip of an optical fiber. The oxygen-dependent emission of the indicator is guided back to the photodetector through the optical fiber.

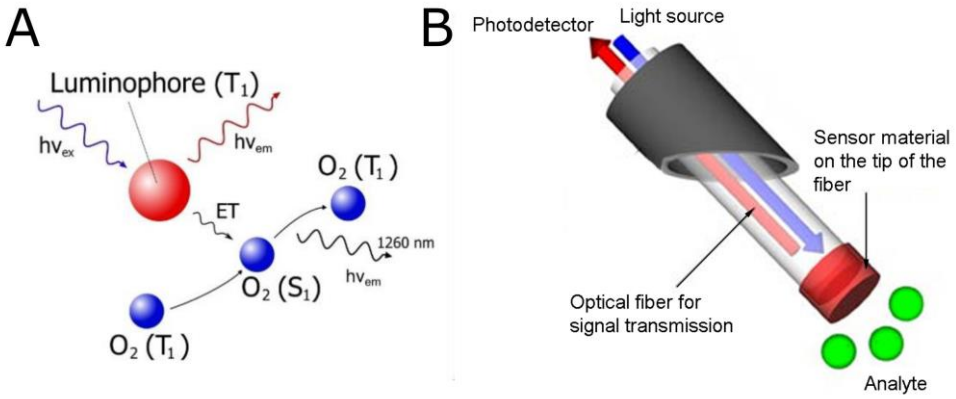

Figure 2. Basic principle of luminescent oxygen sensing. (A) Scheme of the dynamic quenching process and (B) typical sensor set-up.

The impact of collisional quenching on luminescence intensity and luminescence lifetime is described by the linear Stern-Volmer equation

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot pO_2$$

where I_0 and I are the luminescence intensities in absence and presence of the quenching oxygen, τ_0 and τ are the luminescence lifetimes in absence and presence of the quencher, K_{SV} is the Stern-Volmer quenching constant, which characterizes the quenching efficiency and therefore the sensitivity of the sensor and pO_2 is the partial pressure of oxygen. The Stern-Volmer curve slightly deviates from linearity, when the oxygen indicator is dissolved in a polymer. Figure 3 shows a typical calibration plot of an oxygen sensor.

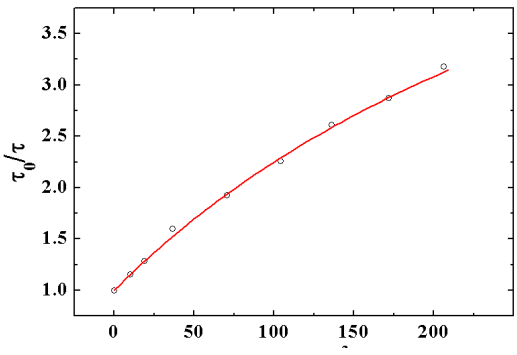

Figure 3: Typical Stern-Volmer calibration plot

It is important to note that the calibration of oxygen optodes is temperature dependent. The luminescence intensity as well as the luminescence lifetime are more easily quenched at higher temperatures. That is why temperature must be recorded during optical oxygen measurements.

2.2.2 Optical oxygen sensing methods

Luminescence quenching by molecular oxygen affects luminescence intensity and luminescence lifetime as described in the preceding chapter. Thus these are the parameters, which can be used to determine the pO_2.

Different methods to detect oxygen are depicted in Figure 4. The measurement of luminescence intensity is the most simple method for sensing oxygen. This parameter, however, is influenced by a variety of factors such as light source intensity, indicator concentration in the polymer matrix and thickness of the sensor layer, photobleaching or leaching of the indicator, scattering, ambient light and detection efficiency. Several methods exist to overcome these potential sources of error for intensity-based measurements: ratiometric methods rely on the use of an oxygen-sensitive and a reference dye, which are excited by the same light source, but have different emission spectra. The presence of oxygen only influences the emission of the indicator dye, while the emission of the reference dye remains stable. Consequentially, oxygen levels can be determined by the ratio between indicator and reference emission at two different wavelengths. The use of the ratio as oxygen-sensitive parameter helps to improve oxygen measurements in terms of stability to light source variations, scattering, detection efficieny and layer thickness. However, signal variations due to photobleaching or leaching of the luminophore, ambient light or wavelength-dependent scattering still influence the apparent oxygen concentration.

The measurement of luminescence lifetime is a possibility to overcome the remaining limitations of intensity-based methods. Luminescence lifetime can be detected using two different methods: the time-domain or the frequency-domain approach, both relying on a modulation of the light source. For the time-domain approach a square-shaped light pulse is used for excitation of the luminophore and the emission intensity is detected during the luminescence decay of the excited luminophore.

The frequency-domain method is the second method to determine the luminescence lifetime. This method involves excitation of the luminophore with sinusoidally modulated light and detection of the phase-shifted emission signal. The phase-shift φ between excitation and emission is oxygen dependent and can be used to calculate luminescence lifetime τ according to

$$\tau = \frac{\tan \varphi}{2 \cdot \pi \cdot f}$$

where f is the modulation frequency.

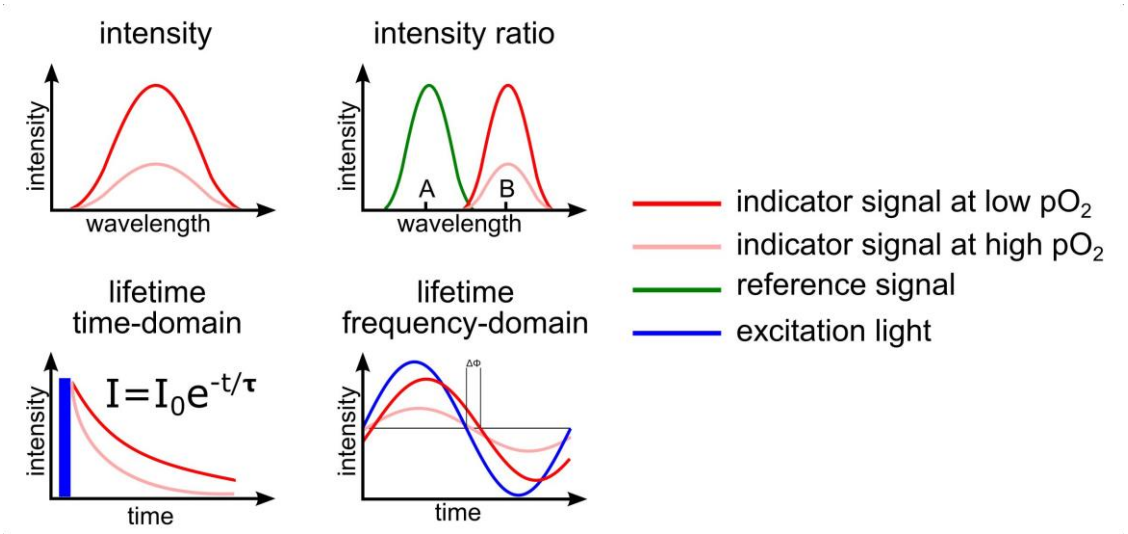

Figure 4. Measurement methods for luminescent oxygen sensors

2.2.3 Optical Sensor Formats

The possibility to use different sensor formats makes optical sensors be a very versatile platform for a variety of applications. The format can be chosen – coordinated with the detection method - according to the respective measurements requirements, e.g. the requirement for single-point measurements or laterally resolved information.

2.2.3.1 Fiber-optic sensors

Fiber-optic sensors represent the most frequently used sensor format as they enable remote sensing at poorly accessible sites or in harsh or hazardous environments. They consist of the sensing element (matrix material and indicator) attached to an optical fiber, which serves as waveguide and carries excitation and emission light from the light source to the sensing element and from the sensing element to the detector, respectively. The sensing element is usually attached to the fiber via dip-coating from a sensor cocktail and is sometimes covered by layers of black silicone providing optical isolation from ambient light.

2.2.3.2 Sensor layers

Sensor layers represent another frequently used format of optical sensors. They can be fabricated from a so-called "sensor cocktail" (matrix material, indicator and additives dissolved in a suitable solvent) onto a substrate by spin-coating, screen printing, spray coating, knife coating, inkjet or pin-printing. Subsequently the applied wet film is allowed to dry, polymerize or cure. Due to their 2D nature, sensor layers are frequently used for imaging applications, where cameras are used for 2D mapping of oxygen.

2.2.3.3 Micro/nanoparticles

Sensor particles represent a simple and versatile tool as they can simply be added to the fluid, in which oxygen should be measured. They can also be equipped with magnetic properties, allowing to generate in-situ sensor spots in any desirable vessel.

2.3 Application of optical oxygen sensors

Within this course optical oxygen sensors are going to be used for different applications, which are described in the next chapter.

2.3.1 Enzymatic reaction monitoring

Optical oxygen sensors can be used to investigate any enzymatic reaction, during which oxygen is consumed or produced. In the lab course, we will investigate the glucose concentration of an unknown solution by monitoring the reaction kinetics of the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone in presence of Glucose oxidase (GOx).

$$\beta - D - Glu\cos e + O_2 + H_2O \xrightarrow{GOx} \beta - D - Gluconolactone + H_2O_2$$

The same principle is also used in optical enzymatic glucose sensors. Such glucose sensors consist of an enzyme layer and an oxygen-sensitive layer. The enzyme layer consumes oxygen dependent on the glucose concentration, while the oxygen-sensitive layer acts as a transducer for the rate of oxygen consumption. Often a diffusion layer is added, slowing the diffusion of glucose, hence reducing the enzymatic conversion and shifting the dynamic range of the glucose sensor to higher glucose concentration. It ensures, that the sensor response is limited by mass transfer rather than by the enzymatic reaction and prevents additional local oxygen depletion by oxygen consumption of the sensor. An additional reference oxygen sensor is usually used for compensation of the oxygen cross-talk of the glucose biosensor. The

obtained signal is the difference between the oxygen levels (ΔpO_2) measured by the two sensor elements. The oxygen level resulting from oxygen consumption by the enzymatic reaction (glucose sensor) is subtracted from the local oxygen partial pressure (reference oxygen sensor).

2.3.2 Transcutaneous oxygen measurement

Transcutaneous oximetry, $tcpO_2$ or TCOM, is a local, non-invasive method measuring the amount of O_2 that has diffused from the capillaries through the epidermis. Therefore, it provides information about the body's ability to deliver oxygen to the tissue. Since tissue oxygenation is an important parameter for wound healing, $tcpO_2$ is often used to evaluate the ability of tissue to effectively heal. $TcpO_2$ is dependent on oxygen uptake in the respiratory system, the oxygen transport/capacity of the blood and the general status of the circulatory system. Any impairment of the body's ability to deliver oxygen to the tissue will be revealed immediately since the skin is ranked very low in the body's system of oxygenation priority.
During a measurement the investigated tissue is heated to create a local hyperaemia, which intensifies the blood perfusion, increasing the oxygen pressure. In addition, the heat will dissolve the lipid structure of the dead, keratinized cells in the epidermal layer making the skin permeable to gas diffusion. On its way the oxygen may be consumed by the cells if the metabolism is high.
Note that transcutaneous oxygen is not the same as the arterial oxygen pressure measured using standard pulse oximeters.

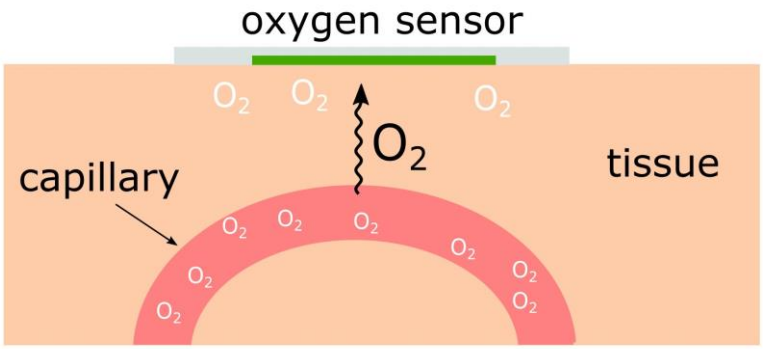

Figure 5: Scheme of transcutaneous oxygen monitoring

Transcutaneous monitoring of oxygen and carbon dioxide, originally developed for neonatal use, has become a routine measurement in several clinical areas including:

- Determination of peripheral vascular oxygenation
- Quantification of the degree of peripheral vascular disease
- Determination of the optimum level of amputation
- Evaluation of revascularization procedures
- Selecting candidates for hyperbaric oxygen therapy and predicting non-responders to treatment

$TcpO_2$ measurements usually require at least two or three sites to provide a good picture. The more sites assessed, the better the oxygenation picture.
In the lab course we will study the effect of applying heat, the effect of ointments, which increase the blood circulation, and the effect of physical exertion on oxygenation of the tissue by applying transcutaneous oxygen monitoring using an improvised setup with optical oxygen sensors.

2.3.3 Breath analysis

Breathing is the process moving air in and out of the lungs. It allows oxygen and carbon dioxide to diffuse from the external environment into and out of the blood. At the end of each exhalation the adult human lungs still contain 2.5 – 3.0 liters of air, termed the functional residual capacity (FRC). Breathing replaces only about 15% of this volume of gas with moistened ambient air with each breath. This ensures that the composition of the FRC changes very little during the breathing cycle, and remains significantly different from the composition of the ambient air. The partial pressures of the gases in the blood flowing through the alveolar capillaries equilibrate with the partial pressures of the gases in the FRC, ensuring that the pCO_2 and pO_2 of the arterial blood, and therefore its pH, remain constant. The equilibration of the gases in the alveolar blood with those in the alveolar air (i.e. the gas exchange between the two) occurs by passive diffusion.

3 Experimental procedure

3.1 Preparation of sensor materials

A sensor cocktail, containing polystyrene and the oxygen indicator Platinum(II) tetraphenyltetrabenzoporphyrin PtTPTBP, is knife-coated onto a flexible PEN foil according to the instructions of the supervisor. After drying of the sensor cocktail sensor spots are punched out of the sensor foil to be ready for application.
The same sensor cocktail is used to fabricate a fiber-optic oxygen sensor by dipcoating according to the instructions of the supervisor.

3.2 Enzymatic reaction monitoring

Fiber-optic oxygen sensors are used to investigate the glucose concentration of an unknown solution by monitoring the reaction kinetics of the oxidation of glucose to D-glucono-δ-lactone in presence of Glucose oxidase (GOx).

$$\beta - D - Glu\cos e + O_2 + H_2O \xrightarrow{GOx} \beta - D - Gluconolactone + H_2O_2$$

Experimental procedure:
The oxygen sensor (Pyro Science, Germany) has to be calibrated before the measurement by applying a two-point calibration procedure using air-saturated water and deoxygenated water containing 1% Na_2SO_3. Sensor signals are obtained using the miniaturized phase fluorimeter GO_2 (Pyro Science, Germany). After calibration the sensor is carefully rinsed with deionised water.
A 5 mL Supelco vial is filled with 4 mL of a 0.5 g/L glucose solution (in 0.10 M phosphate buffer, pH 6). The solution is stirred continuously at a slow rotation speed using a magnetic stirrer and the calibrated sensor is placed inside the vial. 100 µL of a GOx stock solution (1 mg/mL in 0.10 M phosphate buffer, pH 6) are quickly added to the glucose solution. The measured dissolved oxygen concentration is logged using the GO_2 until the oxygen curve deviates from linearity.
The experiment is repeated with different glucose concentrations of 0.3 g/L, 0.2 g/L, 0.1 g/L 0.05 g/L and 0 g/L and with a sample of unknown glucose concentration (triple determination). The same rotation speed of the magnetic stirrer must be ensured for all glucose concentrations.

Data analysis:
Excel (or any other suitable software) is used to yield the intitial reaction rates from raw kinetic data. Oxygen concentration is plotted versus time for each run to verify that each set of data is generating a plot that appears to be roughly linear (Figure 6 (left)). If there is significant and clear deviation from linearity in the data at longer times, it may be necessary to omit some of the data prior to obtaining the initial rate of the reaction. Then a linear-least-squares fit is performed to obtain the slope, which should be the initial rate of the reaction, for each run.
Initial rates are plotted as a function of the concentration of glucose (Figure 6 (right)) and a linear-least-squares fit is performed to these data. From this plot, the concentration of the unknown glucose samples is obtained. The best estimates for the unknown glucose concentrations should be reported and its associated uncertainty (standard deviation) that can be estimated. A plot containing the kinetic runs and a calibration curve should be included in the final report.

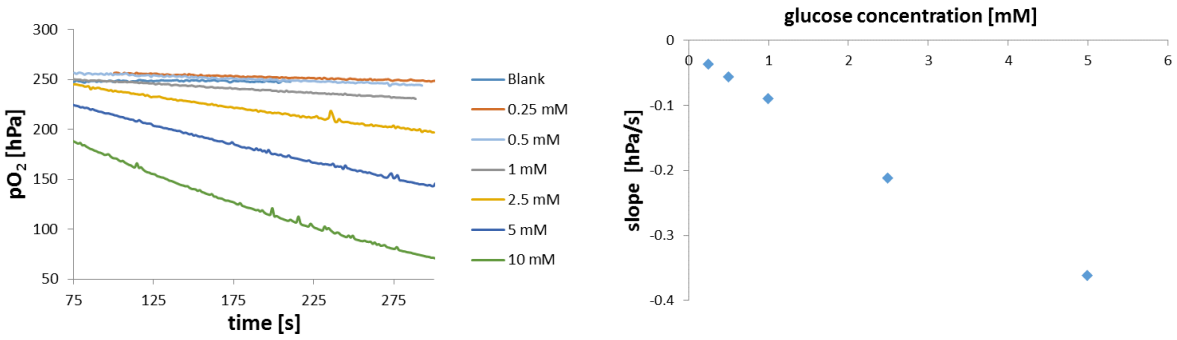

Figure 6: (left) Measured O_2 depletion of glucose calibration standards and (right) calibration curve for calculation of the glucose concentration.

3.3 Breath analysis

In this part of the lab course the oxygen concentration in exhaled breath will be investigated. Therefore, capillaries with integrated oxygen sensors are provided.
Again it is necessary to calibrate the sensors before the measurements. This is done by using a two-point calibration with ambient air and nitrogen. Oxygen values are recorded under different conditions:

- Exhaled breath under normal conditions
- Exhaled breath after breath holding
- Exhaled breath after breath holding with previous hyperventilation
- Exhaled breath during excercise (for example squats)

The results of these oxygen measurements should be listed and discussed in the report of the lab course.

3.4 Transcutaneous oxygen measurement

The sensor spots derived from 3.1 are first calibrated by using a two-point calibration with ambient air and nitrogen and fixed on a flexible and oxygen-impermeable support. These sensor patches are transferred onto the skin of a volunteer (one per group) by using spray-on plaster. Then the backside has to be hermetically sealed by using a provided poly(vinyl

alcohol) solution. Oxygen values are recorded under different conditions:

- Without any additional treatment
- Shortly after irradiation of the skin by an IR lamp
- After treatment with an ointment, which should increase the blood circulation
- Effect after 5 min physical exertion

The results of these oxygen measurements should be listed and discussed in the report of the lab course.