OPTOCHEMICAL SENSORS IN WATER MONITORING

W. TRETTNAK and F. REININGER, Joanneum Research, Institute for Chemical and Optical Sensors, Steyrergasse 17, A-8010 Graz, Austria

1. Abstract

Optochemical sensors or optodes have shown to be a suitable tool for the detection of a large number of chemical parameters. They may offer advantages over already existing and established measurement methods, and new analytes have become accessable. Despite these facts, only a small number of optochemical sensors is already available on the market or close to the point of commercialization. In this review, a state-of-art and outlook on the developments in optical sensor instrumentation and the problems associated with it will be presented mainly on the basis of the results obtained at Joanneum Research, and with respect to the application of the sensors to water monitoring.
Optochemical sensors for the parameters oxygen, pH, carbon dioxide and ammonia will be discussed with an outlook on the determination of new species. Some of the critical points in sensor development and some needs of research institutions will be identified aswell.

2. Introduction

A considerable increase in research activity in the field of the development of optochemical sensors (or "optodes") could be observed in the last two decades. This fact is supported by an annually and steady increasing number of publications and conferences on this subject. The number of research groups all over the world seems to be augmented, new small companies have been founded, and many large companies are known to have been experimenting or at least "playing" with optochemical sensors. It has been demonstrated that optochemical sensors can measure numerous of parameters and are capable of offering a true alternative over already existing and established methods. For example, in contrast to electrochemical sensors, optochemical sensors do not require an electrolyte solution, do not consume the analyte and are not affected by electromagnetical fields.

However, there is a large discrepancy between the number of optical sensors, which have been reported and investigated in the laboratories, and the number of sensors, which are already available on the market. Only a few optochemical sensors are commercially available, mainly sensors for basic analytes, such as oxygen, pH and carbon dioxide. These sensors have been developed with a special view on medical application in blood gas analysis or blood gas monitoring. Only recently, optochemical sensors have also been offered for water monitoring and environmental analysis. One of the reasons may be that optical sensor development is an interdisciplinary field which needs inputs from, for example, chemistry, optics and electronics.

This requires heterogeneous research groups or well-established cooperation networks, which are both still in an early stage of development.

Joanneum Research, which is Austrias second-largest research institution, has been active in the field of optical sensor development for several years. Optochemical sensors have been investigated and developed for various parameters (e.g. pH, carbon dioxide, ammonia) with the main focus on oxygen measurement and its application to various fields. In this review, a summary will be given on the developments and experiences obtained in optochemical sensors on the background of our results and with respect to their application in water monitoring. The aim is to provide a better understanding in sensor development, to identify critical points and the needs for future sensor development coming from the research institutions, the industry (which should provide the boundary conditions) and, finally, the end user.

3. Optochemical sensors for oxygen

Most optochemical oxygen sensors are based on the quenching of the luminescence of an oxygen-sensitive dye by molecular oxygen. A variety of dyes and measurement schemes have been reported to date .
Typically, the dye is incorporated into a gas-permeable membrane, and the luminescence intensity or the luminescence decay time, which are both dependent on the oxygen concentration, are determined [1].
The relationship between the oxygen concentration and the luminescence intensity or luminescence decay time can (in an ideal case) be described by the Stern-Volmer equation [1] :

$$\frac{\tau_0}{\tau} = \frac{I_0}{I} = 1 + K_{sv} \cdot [O_2] \qquad \text{Eq. 1}$$

where I_0 and I are the luminescence intensities and τ_0 and τ are the luminescence decay times in absence and in presence of oxygen at a concentration $[O_2]$, respectively. K_{SV} is the quenching or Stern-Volmer constant and is a measure for the oxygen sensitivity of the dye. Both I_o or τ_0 and K_{sv} are dependent on temperature. The intensity ratio I_0/I or the decay time ratio τ_0/τ can directly be related to the oxygen concentration.

The measurement of luminescence intensity is usually performed with bulky instrumentation such as expensive fluorescence spectrometers or fibre-optic photometers, which are of limited applicability. Recently, oxygen measurement systems have been presented, which are based on low-cost semiconductor devices, such as light-emitting diodes (LEDs), photodiodes and low-cost analogue and digital components. Joanneum Research has developed such an oxygen sensor instrumentation, which is suitable for the determination of oxygen in gases or dissolved in liquids [2]. A ruthenium(II) complex, which is immobilized in a polystyrene layer, is used as the oxygen-sensitive dye.
A blue light-emitting diode is used for excitation of the luminescence, whereas a photodiode serves as the detector. The cell geometry has been optimized for signal yield, while minimizing straylight. The flow-through cell (the volume is approximately 50 µl) is compact, robust and

can be thermostatted by heating. It has been designed for sample flow rates of 0.1 to 2 ml/min. The measurement instrument is equipped with a built-in pressure sensor and an additional external temperature sensor, which are both needed for calibration of the device. Sensor calibration is performed with two solutions, an oxygen-free sulfite solution and air-saturated water. This two-point calibration proved to be sufficient for most of the water-monitoring applications.

The instrument has been applied to monitoring of oxygen in a lysimeter station, where ground water is drawn continuously from the soil at certain depths during its infiltration process. The oxygen concentration in the infiltration water can be considered as an indirect measure for the microbial and biochemical activity in the soil. The amount of water, which is collected, is small and may vary considerably with weather and season. This makes it difficult to use conventional polarographic oxygen sensors to determine the oxygen concentration. The optochemical oxygen sensor, which does not consume any oxgyen, is not affected by varying sample flow rate or drying out of the sensor membrane. Furthermore, this type of sensor is less affected by gases such as carbon dioxide or hydrogen sulphide and by membrane fouling. The measurement setup used in the lysimeter station is shown in Figure 1a. (see Figure 1a,b in annex)

Initially, the optical oxygen sensor was calibrated weekly. However, thermostatization by heating of the flow-through cell to a few degrees Celsius above ambient temperature was not convenient, since degassing of the sample solution lead to erroneous results. For this reason, the cell thermostatization was switched off and all calibrations were performed at ambient temperature. Since the temperature characteristics of the sensor were unknown, the temperature dependencies of the calibration parameters I_0 and K_{sv} were calculated after a measurement period of approximately half a year from the data, which were collected at the weekly calibrations. This was possible under the assumption that the sensor characteristics remained unchanged over this time period and that no photobleaching of the dye occurred. This was also veryfied for measurement periods of one to two months with calibrations at constant temperature. A comparison of the results obtained within the measurement period of March to August 1994 and of the oxygen concentrations of air-saturated soil infiltration water, which were calculated on the basis of the water temperature and of the barometric pressure, are shown in Figure 1b. The oxygen concentration was found to be always above 5 mg/l (i.e. more than 50 % air satuartion), although it decreased continuously within the observation period. This may be related to oxygen-consuming processes, which may be attributed to increasing biological and biochemical activity in the soil. Infiltration of rain water with higher oxygen content was identified as the main reason for short term fluctuations [3]. (see Figure 2 in annexe)

The measurement of luminescence intensity may be affected by e.g. changes in the light source intensity, by photobleaching or leaching of the dye, by intrinsic sample fluorescence or other optical properties of the sample solution. Many of these problems can be overcome by measuring the luminescence decay time of the oxygen-sensitive dye. Systems based on the measurement of the luminescence decay time offer the advantage that they are (in the first order) independent from the light intensity. This is of importance, since it reduces many requirements concerning the optical components, e.g. it becomes feasible to measure via

optical fibres, where the optical attenuation may be unknown or even variable. Furthermore, it becomes possible to measure in closed systems through the walls of a transparent vessel ("contactless sensing").

The decay time of the luminescence can be determined directly by employing a pulsed light source and high-speed detectors (with a sampling frequency of approximately 10 MHz). Such equipment, which is typically based on a laser, a photomultiplier and an oscilloscope, is large, expensive and cannot be considered practical for many purposes [4]. Another approach to the determination of luminescence lifetimes is based on phase modulation techniques [5-8]. In the meantime, several instruments of phase-modulated phosphorimeters and fluorimeters have been described. Oxygen measurement systems based on the use of an LED as the light source and a photomultiplier as the detector have been reported for oxygen-sensitive ruthenium-complexes, which have fluorescence lifetimes of up to approximately 5 µs [9 - 11]. In case that luminescent dyes with lifetimes in the range of tens and hundreds of microseconds (e.g. porphyrin dyes) are used, the required modulation frequencies can be essentially lowered. Thus, a substantial simplification of the system electronics becomes possible [12 - 14].

A cheap and simple oxygen measurement system has been presented, where the phase measurement is performed at a single frequency only and by measuring the delay time between a square-wave excitation and a square-wave in the detector, generated via suitable amplification of the emission signal coming from the sensor [15]. The instrument is based on low-cost components such as LEDs and photodiodes with the measurement module having an overall size of only 120x60x30 mm. Platinum(II) octaethylporphyrin-ketone is used as the oxygen-sensitive dye. It has an unquenched lifetime of approximately 60 µs and can be excited with a yellow/amber LED as the light source [4,13]. The instrument has been designed for either measurement of oxygen in gases or (in combination with a flow-through-cell) in liquids.

With this instrument, dissolved oxygen could be determined in concentrations of up to air saturation (i.e. approximately 0 to 200 hPa oxygen partial pressure) with an accuracy of better than ± 1 hPa for measurements over periods of typically 24 hours with the whole device being thermostatted (Figure 2). Due to the high oxygen sensitivity of the dye (when immobilized in a high-molecular weight polystyrene matrix), the resolution in oxygen reading was improved when compared to the previously used ruthenium(II) complex [2]. A resolution of 0.1 hPa was obtained at low oxygen concentrations. The small dimensions of the primary measurement unit (120x60x30 mm) are a substantial improvement, when compared to previously reported optical oxygen sensor units [2]. The new measurement technique, which is based on a luminescence decay time measurement, has proved to be clearly superior to intensity-based methods. (see Figure 2 in annexe)

Based on these results and new developments in measurement electronics, a new oxygen sensor device is now in development, which is designed for monitoring of oxygen in waste water, groundwater (e.g. in bore holes) and surface water. The optode has the same shape and dimensions as the commercially available oxygen electrodes CellOx 700 or TriOxmatic 700 from WTW (Weilheim, Germany) (Figure 3). The optical cell and a signal pre-amplification are integrated in the head of the probe, which is connected to a data processing unit by means of a cable. The final aim is to obtain an oxygen sensor, which is capable of measuring under the

conditions, which are specified in Table 1, and can be regarded as the ideal and the minimum requirements for environmental application of an oxygen sensor. (see Figure 3 and Table 1 in annexe)

From these specifications, it can be seen that the requirements are quite tough in order to be able to compete with already existing electrode systems. A high measurement stability is the pre-requisite for environmental application. Factory pre-calibration is desired and a single-point calibration (e.g. under air), as it is usually performed with oxygen electrodes, may be acceptable for the customer.

The main advantage of the optochemical oyxgen sensor (when compared to amperometric sensors) is its insensitivity to substances such as hydrogen sulfide, which have normally to be encountered in waste water monitoring. The absence of electrolyte solution will simplify the exchange of sensor membrane and the sensor handling. Furthermore, there is no long stabilization time necessary for the optical sensor, whereas oxygen electrodes may need up to hours to give a stable signal after switching on the instrument (the so-called "polarization time").

Despite the requirements on optical, electronical and mechanical design, it has to be pointed out that the major critical point is probably the oxygen sensor membrane, which has to be characterized very well. The oxygen response characteristics of this membrane, which have to be known in the whole temperature range of interest in order to allow for a temperature compensation, should not change over up to one year of continuous use under varying environmental conditions. Furthermore, they also should not change over a certain period of storage time before being used. This shelf lifetime should also be in the order of years.

Whereas the above-described oxygen sensor may be considered as a "macrooptode", oxygen microoptodes have been developed at the Max-Planck-Institute for Marine Microbiology in Bremen (Germany) and at the University of Regensburg (Germany) [16,17]. The microoptodes (Figure 4a) can be used for measuring oxygen gradients e.g. in marine and freshwater sediments with a high spatial resolution (better than 50 µm). The sensors are based on tapered silica/silica multimode fibers with tip diameters of 5 to 100 µm (depending on the type of application). Depending on the indicator and the polymer used, micropotodes with different measurement ranges can be obtained. A comparison of oxygen microelectrodes and oxygen microoptodes shows that the properties of the optical sensor are superior to that of the electrodes (Table 2). Whereas luminescence intensity measurement has been used at the beginning [16], the new generation of microoptodes is also based on a phase measurement technique [17]. A typical measurement of an oxygen profile in a sediment with both an oxygen microoptode and an oxygen microelectrode is shown in Figure 4b. (see Figure 4a,b and Table 2 in annexe)

4. Optochemical sensors for pH, ammonia and carbon dioxide

Most optochemical sensors for the parameters pH, ammonia and carbon dioxide have in common that a pH-sensitive dye is used as the indicator [1]. Typically, the colour or the luminescence properties of the dye are changed in presence of the analyte, which can be quantified e.g. by measuring the absorbance or luminescence intensity. In the case of optical pH sensors, the indicator dye is immobilized in a hydrophilic polymer or on the surface of a suitable substrate. A large variety of pH-sensitive dyes has been used to produce pH optodes [1]. In contrast to pH electrodes, no electrolyte solution is required with the optical sensor, no reference electrode is needed and the sensor is electrically isolated from the sample solution. Although pH electrodes are well-established and highly-developed, still new pH sensors for environmental application are of interest, which show a high measurement stability and which can be used at low sample conductivity (e.g. in rain water or most of the spring waters).
Optical pH sensors have been developed at Joanneum Research, where a pH-sensitive dye is immobilized onto a water-permeable celluloseacetate support [18,19]. The resulting sensor membranes change their colour from yellow to blue in a range of approximately pH 5 to 10 (Figure 5a) and show extremely good storage and measurement stability. A resolution of pH 0.001 could be obtained at the pK of the dye by measuring the transmittance of the membranes. Accuracies of pH $\pm$ 0.04 and of $\pm$ 0.08 were obtained immediately after and within one month after a four-point calibration, respectively.

The response times, which are dependent on the buffer capacity of the sample solution, were less than one minute for 99 % of the signal change in 0.1 M buffer solutions. Despite these very promising sensor properties, it was found that the sensor response curve is strongly affected by the ionic strength of the sample solution (Figure 5b). For application of the sensor, this would mean that the sample ionic strength or its range of variation should be known, and that a constant or a (more or less constant) high ionic strength would be required for pH measurement. This is the case, for example, in sea water. Since the pK of any pH-sensitive dye (whether dissolved or immobilized on a solid matrix) is influenced by changes in the ionic strength of the sample solution, the development of optochemical pH sensors for environmental application with varying or unknown composition of the sample solution (e.g. waste water) turns out to be quite difficult. The problem may be minimized by choosing a dye with low sensitivity to ionic strength in combination with a matrix, which additionally reduces these ionic strength effects. Furthermore, it has to be considered that, for practical purposes, the optical pH measurement has to be optically isolated from the sample solution, since it may be influenced by sample colour, turbidity or intrinsic sample luminescence. (see Figure 5a,b in annexe)

In the more "classical" approach for ammonia and carbon dioxide measurement, the pH indicator is separated from the sample solution by means of a gas-permeable membrane [1]. Ammonia and carbon dioxide diffuse through this membrane into an internal buffer solution, where the pH is changed. This again causes a change in the spectral properties of the indicator. The principle can be compared to the potentiometric ammonia and carbon dioxide electrodes,

where a pH electrode is inserted into an internal buffer solution. For this reason, optical sensors based on this measurement principle are also prone to similar influences than the corresponding electrodes and show similar disadvantages. A change in sample ionic strength leads to a difference in osmotic pressure between sample solution and internal buffer solution. This causes diffusion of water into or out of this internal buffer solution, which again results in drifts in the sensor signal and changes in the sensitivity for the analyte. However, optical sensors of this type have shown to be suitable for applications, where the ionic strength of the sample solution remains rather constant [20]. This is the case especially in sea water monitoring, where a high sensitivity to CO_2 is required, which cannot be obtained with conventional CO_2 electrodes. Optical carbon dioxide sensors have been developed by various institutions especially for this application and tested successfully [21-24].

In the past years, direct immobilization of the pH-sensitive dyes in a gas-permeable polymer matrix has shown to be a suitable technology for the preparation of carbon dioxide [25-27] and ammonia sensors [28,29]. This is achieved in most cases by the formation of ion pairs between the dye and lipophilic counter ions (e.g. quaternized alkyl ammonium salts). The sensor membranes obtained in this way do not require an internal buffer solution and are not influenced by varying sample osmolarity or ionic strength. This simplifies the sensor production substantially.
The sensors are based on the measurement of absorbance, luminescence intensity and even luminescence decay time, although the decay times utilized are still too short (i.e. in the range of a few nanoseconds) for realization of a simple and cheap sensor instrumentation [30,31].

Joanneum Research has been following this new approach with the aim of the application of the carbon dioxide and ammonia sensors to water monitoring [32-34]. Figure 6 shows a typical carbon dioxide measurement performed with a sensor, which was based on the fluorescent dye 8-hydroxypyrene-1,3,6-trisulfonate (HPTS) immobilized in ethylcellulose. The sensor membrane was protected from the sample solution by an additional chemically and mechanically resistant gas-permeable membrane. The fluorescence measurement was performed with a fibre-optic setup in combination with a flow-through cell, which was used for testing purposes.

The sensor provided a linear calibration graph and measurement stability over short periods of up to a few days. At present, this type of CO_2 sensor is in investigation with respect to key features such as the measurement and storage stabilities and their improvement. The ammonia sensor is based on the dye bromothymol blue, which is immobilized (again as an ion pair) in a thin layer of silicone, and the change in colour from yellow to blue in presence of ammonia is monitored. Again the sensor was basically characterized in the laboratory and very promising results were obtained. However, for practical application in environmental sensing (especially for long-term monitoring), the sensor still needs improvement with respect to its measurement stability, which is limited to a few days only [34]. (see Figure 6a,b in annexe)

Both, the carbon dioxide and ammonia sensors may provide several advantages over the commercially available electrodes, which are laboratory devices only, and which are not intended to be used in field applications. The optical sensor membranes can be prepared very easily and on a cheap cost basis. Corresponding low cost instrumentation for this type of

sensors is also in development. The instrument prototype will be based on solid state components in combination with fibre optics. Such instrumentation can also be easily used with sensor membranes for other analytes (e.g. ionic species such as heavy metal ions) by adapting the optical components (LEDs, photodiodes, filters) to the spectral characteristics of the respective sensor membrane. Again, the availability of sensor membranes with long luminescence decay times (in the range of µs to ms) as the measurement parameter would allow to simplify the optical setup considerably.

5. Conclusions and outlook

Optochemical sensors have shown to be a suitable tool in monitoring of analytical parameters relevant for the determination of water quality. For specific applications, they may offer advantages over already existing measurement methods. Besides the sensors presented in this review, optochemical sensors have also been reported for numerous other parameters, for example, for gases (e.g. SO_2 and Cl_2), ions (e.g. NH_4^+, Na^+, K^+, Ca^{2+} and Cl^-), heavy metal ions (e.g. Ag^+, Pb^{2+} and Hg^{2+}) and organic substances, such as hydrocarbons, glucose, lactate, amino acids, biodegradable material in general etc..
New technologies and materials are becoming available every year: new dyes and polymers, light sources (e.g. LEDs and laser diodes), integrated optics and microsystems. This is also leading to continuous improvements in already existing sensor systems, and new sensors are becoming available for new parameters and for new applications.

What is required now, is a clear identification of the needs in water monitoring. It has to be identified, which parameters are of analytical interest and for which parameters already existing methods may be regarded as insufficient. It should be clearly stated, what the requirements (depending on the type of application) on these new sensors are, what sensor characteristics they should have in order to be useful for practical purposes, and which sensor characteristics of already existing sensors should be improved. These needs, which should be identified by scientists, the companies, which are already producing sensors, national and international institutions and the end users, should be clearly summarized in a kind of catalog or "hot list". This catalog would enable researchers immediately to focus on sensors for the right parameters, and to avoid developments, which may be considered as a "cul-de-sac", because they are of no practical use.

In a second step, the various research groups (i.e. at the universities, at private and (inter)national research institutions and in the companies) dealing with sensor systems and working on methods for the determination of specific parameters should be identified and also listed in a second part of the catalog. Furthermore, companies, which are able to produce and market sensors as well as the potential end users should also be included. This could lead to the formation of networks and clusters covering basic research, production facilities and the marketing of the final product. Of course, it would be necessary to have annual revision of the needs, the active groups in the field, the companies and the state-of-art. This would, finally, also allow to revise the system of financement of research and sensor development and to help to identify the critical steps from the point of view of the costs. For this purpose, committees

could be formed within the networks or clusters, which are responsible for coordinating or reviewing specific tasks or sections in sensor development.
The universities and institutions, which do the basic research, are mainly interested in developing and demonstrating new sensor principles, and very often stop their work with the publication of some results. The work is usually stopped at a point, where further investigations become tiresome and sometimes, from a "scientists point of view", also boring. To identify sensor membranes with properties, which make them suitable for practical application, to characterize these membranes and to improve these characteristics step by step means just a hell lot of work. The costs for this work are very often too high to be carried by small companies, and the large companies have the tendency to wait until a sensor system is really functioning. Once a working prototype system is available, it is quite easy to find some interest from the industrial side in commercializing a system. However, there is a big gap between the basic results and a working prototype measurement system, which very often can only be closed by contributions from national or international fundings, although contributions from industrial side would be highly desirable. For this reason, it is essential to avoid that several groups are working on the same subject (if there is no real need for it), or that identical work is done in parallel.

Cost effectiveness is maybe one of the prerequisites for future sensor development, especially with respect to the steadily increasing costs, the decreasing amounts of money available for research activities and to the large future potential, which is available, for example, in the field of optochemical sensing.

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ANNEXES

Table 1. Main requirements on the performance of an optical oxygen sensor for environmental monitoring.

Dimension	ideal	minimum requirement
dissolved oxygen measuring range	0 .. 1000 hPa pO_2 or 0 .. 50 mg/l O_2	0 .. 500 hPa pO_2 or 0 .. 25 mg/l O_2
measuring accuracy	0.2 hPa pO_2	2 hPa pO_2
calibration	factory pre-calibration	factory pre-calibration + 1-point calibration by user
drift with air saturation	< 1 %/month	< 2 %/month
service life		1 year
response time (system)	t_{90} < 10 s (20 °C) t_{99} < 60 s (20 °C)	t_{90} < 30 s (20 °C) t_{99} < 180 s (20 °C)
sample flow rate		0 ... 5 m/s

Dimension	ideal	minimum requirement
measuring temperature	0 .. 80°C	5 .. 40°C
ambient pressure	10 bar	5 bar
no sensitivity to:	CO_2 (5 g/l) H_2S (500 mg/l) NH_3 (100 mg/l)	CO_2 (2 g/l) H_2S (100 mg/l) NH_3 (50 mg/l)

Table 2. Comparison of different types of oxygen microsensors [17].

Property	sensor type		
	O_2-microelectrode (needle-type)	*O_2-microelectrode (Clark-type)*	*O_2-microoptode*
measuring range (% air saturation)	1 - 500	1 - 500	0.05 - 500 (depending on the type of O_2-sensitive layer)
response time (t_{95})	< 1 second	< 1 second	< 1 second
calibration curve	linear	linear	hyberbolic
	O_2-microelectrode (needle-type)	*O_2-microelectrode (Clark-type)*	*O_2-microoptode*
cross sensitivity	H_2S, pH, ionic strength (especially Ca^{2+}, Mg^{2+})	H_2S	SO_2
longterm stability	poor	moderate	excellent
storage stability	moderate	moderate	excellent (> 1 year with stable calibration curves)
stirring sensitivity	high	moderate	no effect
disturbance of the micromilieu	mechanically, polarisation potential	mechanically	mechanically, excitation light
price	low cost sensor	expensive	low cost sensor

Figure 1 :

a) Schematic of the setup for the measurement of oxygen in soil infiltration water with an optochemical oxygen sensor at a lysimeter station;

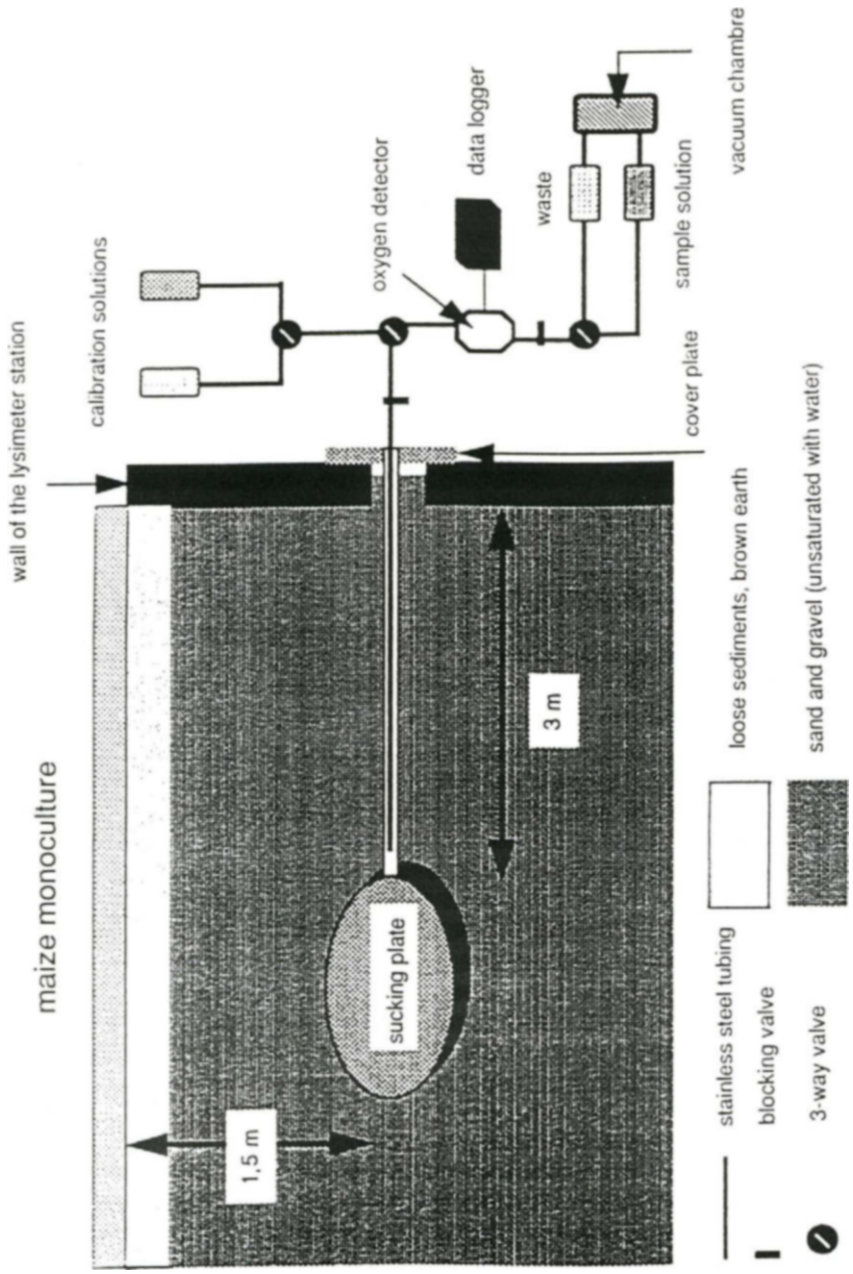


Figure 1 :

b) Comparison of oxygen concentrations in soil infiltration water, which were measured with the optical sensor in the period of March to August 1994, with oxygen concentrations, which were calculated for air-saturated water.

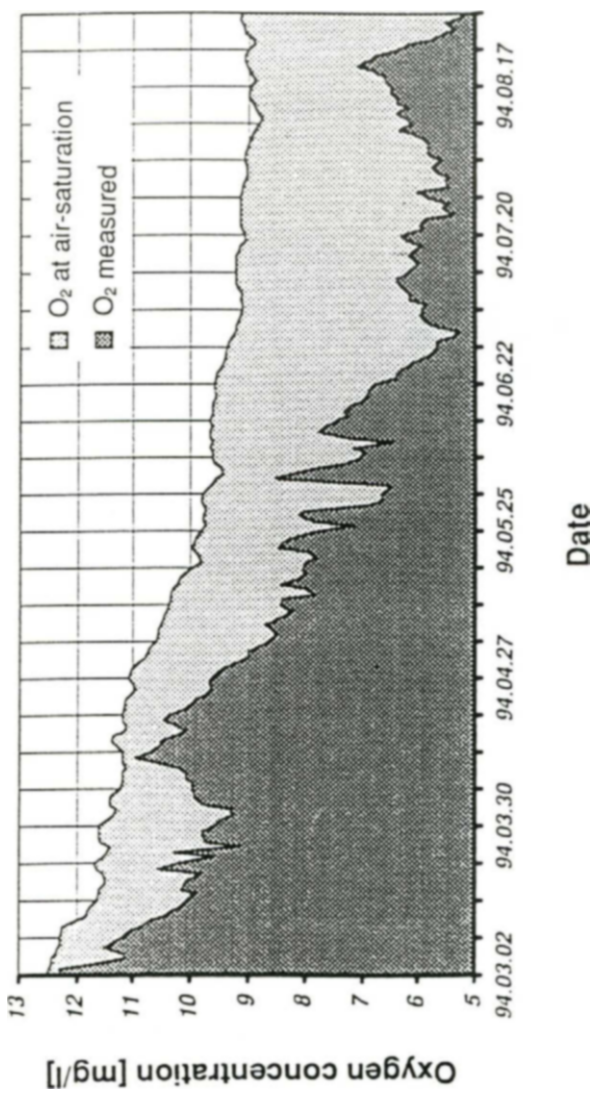


Figure 2 : Oxygen measurement in water with an optochemical oxygen sensor based on a phase measurement technique [15]. Sample solutions were tonometered with calibration gases of varying O_2 concentrations (0, 0.1, 1, 5, 10 and 20.6 % O_2) at 30 °C. The measurement was performed at a flow rate of 0.8 ml/min and at 25 °C.

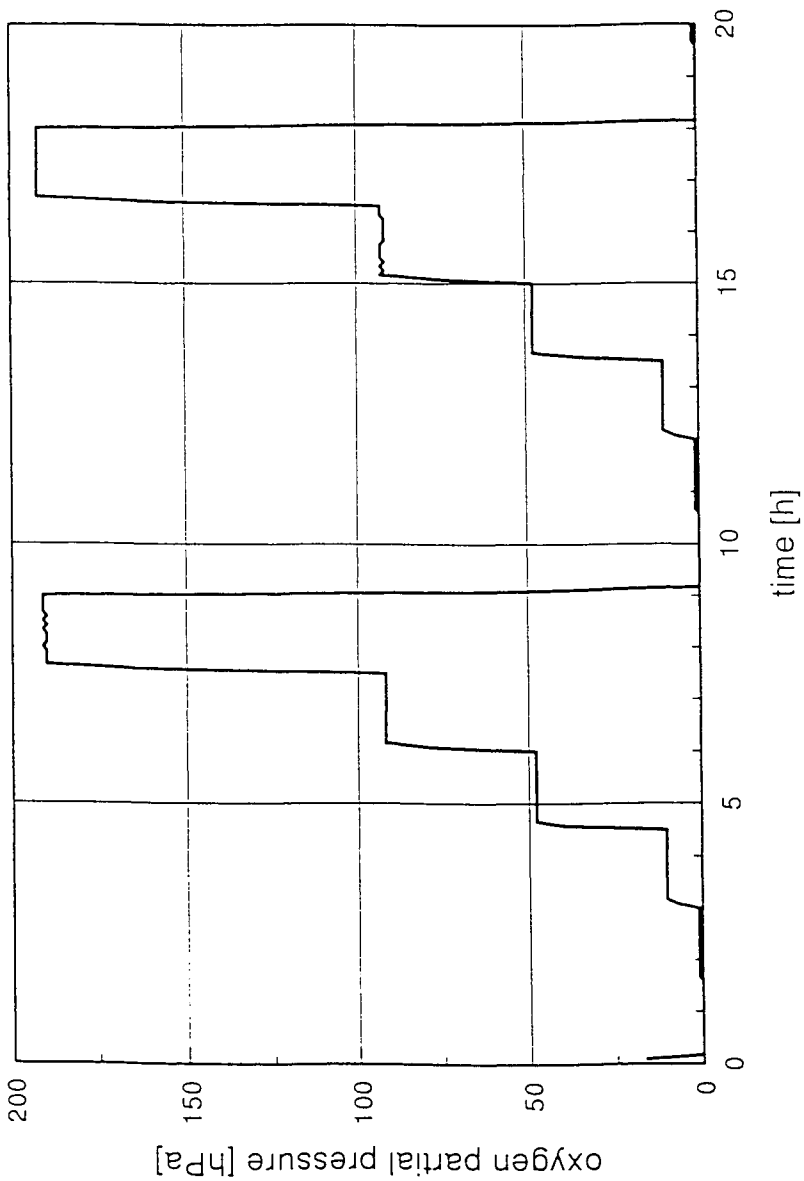


Figure 3 : Schematic of an optode specially designed for oxygen measurements in waste water, groundwater and surface water (outer diameter: 40 mm; length: ca. 190 mm).

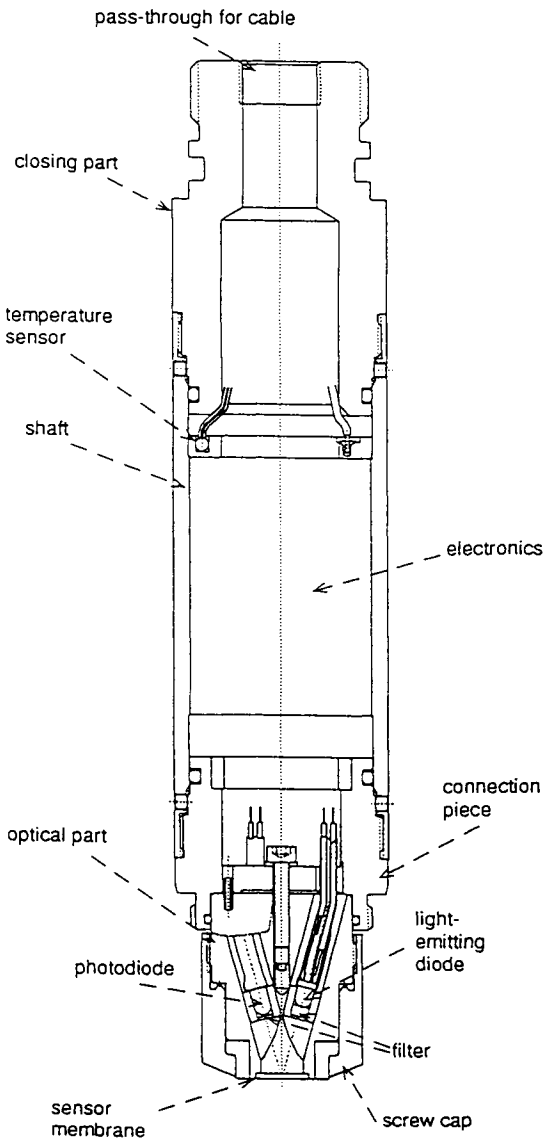
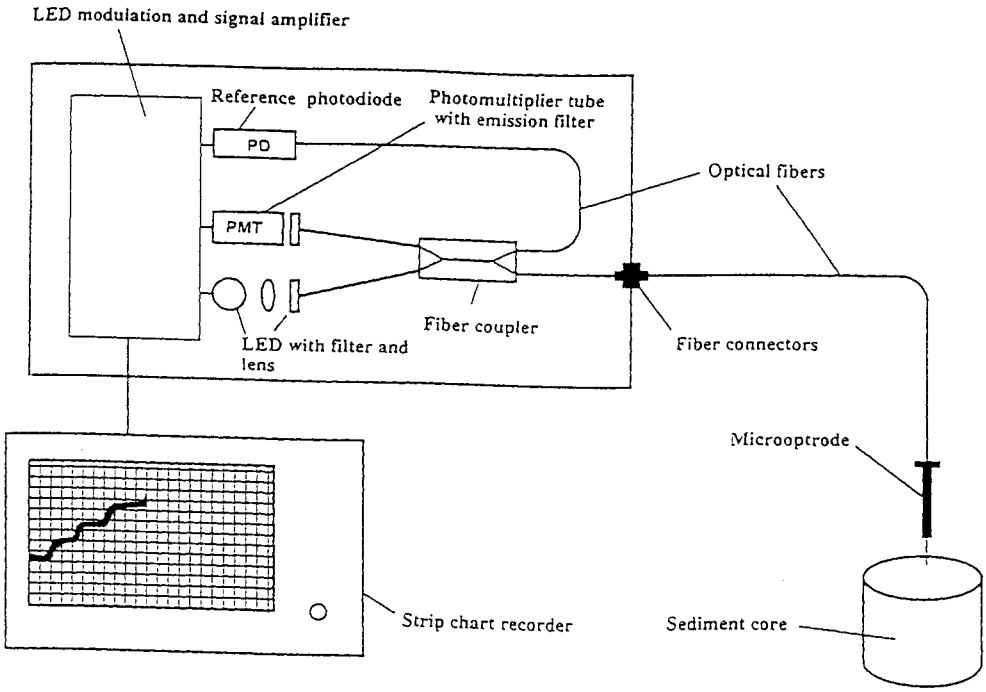


Figure 4 : Schematic of an oxygen microoptode measuring system oxygen profiles measured in a coastal North Sea sediment with an O_2 microoptode and an O_2 microelectrode [16].



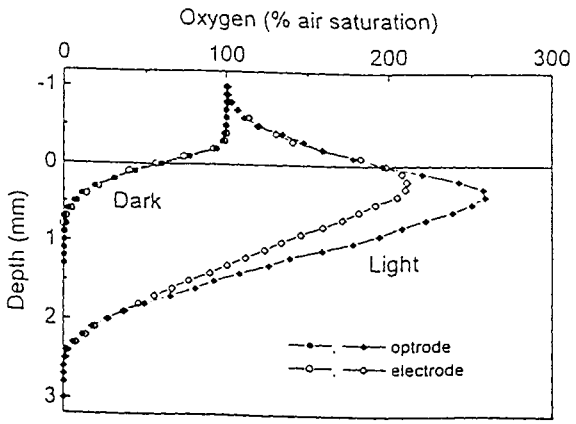
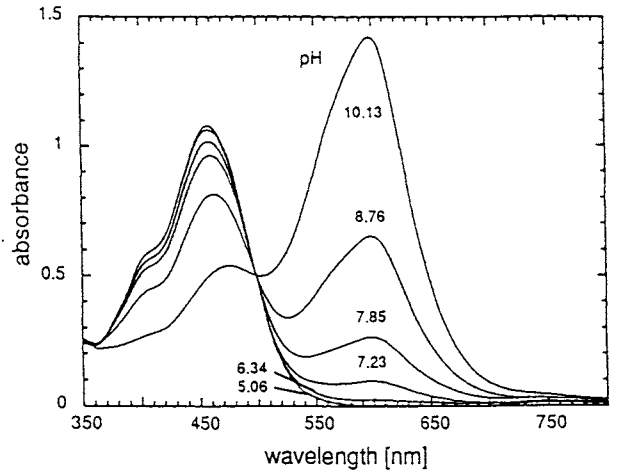


Figure 5 : Absorption spectra of a cellulose-immobilized pH indicator in 0.1 M phosphate buffers of varying pH [18], dependence of the pH calibration curve on the concentration of the buffer employed [19].



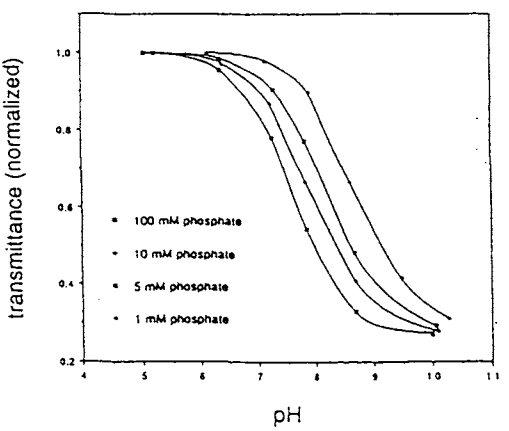


Figure 6 :

a) Carbon dioxide measurement with a fluorescence-based CO_2 sensor in 0.9 % NaCl solution, which was tonometered with gases of varying CO_2 concentration (0, 1, 3, 6, 10 and 15 % CO_2) (sample flow rate: 0.5 ml/min; 37 °C)

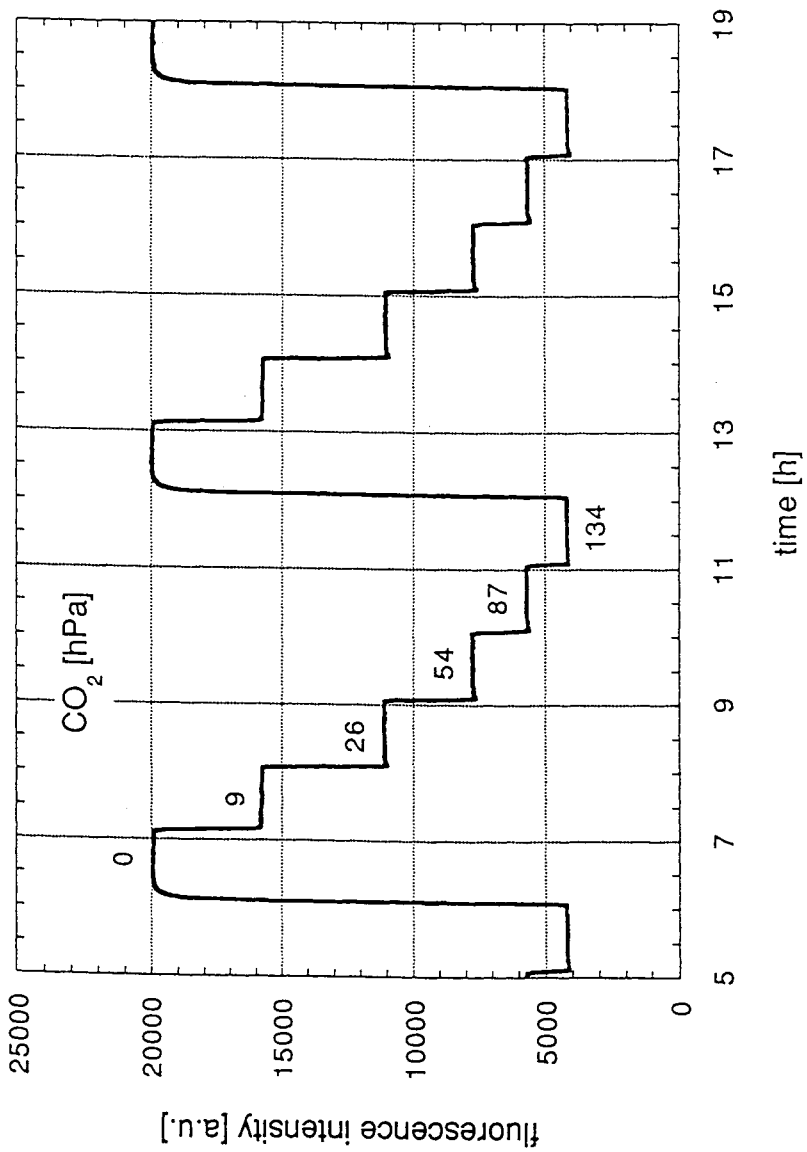


Figure 6 :

b) calibration graph obtained with the same measurement.