Water Dissolved Oxygen Sensing By Means of RTP Measurements: A Comparison Between Different Immobilisation Procedures and Measurement Principles

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Chapter 1

Abstract

Chemical sensors are nowadays an established field of analytical chemistry with growing importance. Having its historic roots in electrochemical sensing devices, the repository of available techniques and implementations has grown steadily. Ongoing research efforts on this topic have yielded important advances concerning the miniaturisation, sensitivity, and response time of chemical sensors. Especially biosensors have attracted great interest in recent years. A huge variety of applications of chemical sensors can be found in industry and medicine. Moreover, they play an important role in environmental monitoring. Since the need for fast, reliable, and cheap ways of obtaining data on all kinds of processes and environments is ever increasing, it is believed that the interest in this research topic will continue and even augment.

In recent years optical chemical sensors have emerged as an important complement to other sensing principles. Such valuable advantages as for example their high bio-compatibility make them especially interesting for "in-vivo" or sterile sensing in medical applications, while the possibility of remote sensing facilitates their employment in hazardous environments or for environmental monitoring tasks. Among the optical chemical sensors, particularly luminescence sensors play an important role when high selectivity and sensitivity are of great concern. While there has so far been little work done on chemiluminescence, fluorescence and phosphorescence are well studied and frequently employed phenomena. Many publications can be found utilising the photoluminescence of various compounds for a multitude of analytes in all phases. A relatively novel approach in this context is the use of room temperature phosphorescence. However, as this sensing scheme permits measurements of high sensitivity with wide linear ranges and has the potential of outstanding selectivity, considerable efforts are underway for its exploitation.

The determination of dissolved oxygen in water is of high environmental relevance. The oxygen content is an important parameter determining the ability of natural waters to sustain diverse biota and to break down wastes. Therefore, it is one of the most immediate ways to judge

the water quality. Traditionally, volumetric or electrochemical methods are used for the determination of dissolved oxygen in water, but there are also several publications proofing the suitability of optical sensing approaches, especially RTP measurements, for this purpose. Hence, dissolved oxygen in water was believed to be an adequate analyte for the experiments at hand.

In sensor development, the measurement method is one of the crucial points to consider, but often appropriate data comparing different measurement techniques is not available. In the present work three different measurement approaches, namely intensity measurements, ratiometric measurements, and lifetime measurements, representing the most common techniques employed, were chosen for a comparison study. The aim was to establish the analytical figures of merit for each measurement method under equivalent conditions (i.e. leaving the sensor phase, experimental set-up, and the instrument all the same), changing nothing but the measurement technique. Doing so would yield suitable data for an inter-comparison of the three measurement approaches, providing a basis for the choice of an adequate sensing scheme for the future development of sensing devices.

From prior experiments in the working group, Al-ferron was known to exhibit fluorescence as well as phosphorescence. There have also been investigations that proofed a strong quenching of the phosphorescence by oxygen. In preliminary experiments, the independence of fluorescence from the oxygen level was established. Therefore, phosphorescence intensity measurements, lifetime measurements, and ratiometric measurements using the phosphorescence intensity to obtain the analytical information and the fluorescence as an internal standard, could be realised. Fulfilling these prerequisites, Al-ferron seemed ideal for the planned work. A flow system including a flow cell packed with sensing phase and placed inside of a luminescence spectrometer was chosen as the basic experimental set-up. After extensive optimisation of various experimental parameters, the first experiments were conducted with the Al-ferron immobilised in a sol-gel matrix. Although good results were reported in literature for this sensing phase due to the phosphorescence enhancing effect of the rigid environment, it proofed not to be suitable in this case. Hence, the solid support was changed for a resin, which showed considerably augmented luminescence intensities and enhanced spectra. The experimental set-up was also slightly modified, using in this case a mixture of two flows of buffer solution, one saturated with oxygen, the other deoxygenated by argon, as feed for the flow cell.

In total, five calibrations on five distinct days were made, where on two of these days samples were additionally measured after the corresponding calibrations. Using the Stern-Volmer equation, lifetime measurements yielded a linear calibration graph, while intensity measurements and ratiometric measurements showed a positive deviation. In order to explain this deviation from linearity several different quenching models were examined. Employing a model considering static and dynamic quenching, the data fitted the predicted function, thus this model was applied to obtain the calibrations for ratiometric and intensity measurements. For all measurement approaches calibrations up to saturation of water with dissolved oxygen were established.

Lifetime measurements showed the best performance in terms of repeatability and precision and have, together with intensity measurements, the lowest limit of detection. For sensor development, however, ratiometric measurements appear to be the most suitable. They offer short measurement times along with high robustness and supreme accuracy. Moreover, they can be realised using considerably less sophisticated instrumentation. All these advantages of the ratiometric measurement approach should permit the construction of robust, compact, and simple sensing devices not only for dissolved oxygen in water, but also for other analytes.

Chapter 2

Introduction

In recent years two main developments, leading into opposing directions in the spectrum of analytical methods, could be noted in analytical chemistry. One apparent trend leads towards highly sophisticated analytical instruments and hyphenated techniques. With this approach the goal is to acquire a maximum of analytical data from a given sample. Usually the samples are treated in some form of batch mode and require pre-treatment steps. Therefore, processing in a laboratory equipped with according instrumentation by an experienced operator almost always is required. On the other hand large efforts have been made in the development of portable, simple, in-field applicable devices for direct, reversible, continuous, and in-situ monitoring and screening tasks. The term coined for this instrumentation is “chemical sensors”.

2.1 Chemical sensors

In the past, chemical sensors often started of as by-products of developments in seemingly unrelated fields. Striking examples are timing circuits which evolved to quartz crystal micro-balances (QCMs), fibre optics once developed for data transmission and now just as well used for various types of optical sensors or chemically modified field effect transistors (CHEMFETs) which have their origin in ordinary insulated gate field effect transistor (FET). However, during the last 20 years chemical sensors gained more and more importance and are now acknowledged as a part of analytical chemistry. Benefiting from vastly augmented computing power available nowadays, novel materials and improved design and processing tools, they emerged to meet the industry’s ever growing need for means of fast, cheap and reliable data acquisition methods. The huge interest in this technology is illustrated by the fact that large companies now maintain their own sensor research groups, but above all by the growing number of publications on the topic. Figure 2.1 shows the results from a literature search on chemical sensors in the SciFinder database. Although the absolute values might not bear significance, the figure illustrates a steady

augmentation of publications related to the topic during the last twenty years.

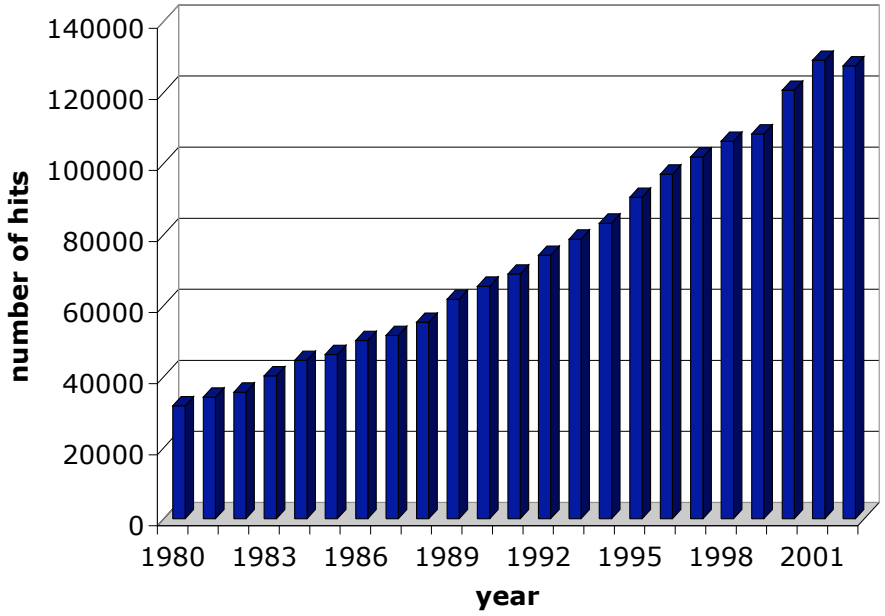


Figure 2.1: Results of a literature search on chemical sensors in the SciFinder database.

2.1.1 Definition of Chemical Sensors

With rising popularity of this field of investigation, the use of the term “chemical sensor” suffered from a certain blurring. This is not necessarily solemnly to be attributed to the willingly inaccurate usage of the term, but also to the inherent highly interdisciplinary characteristics of the field. Various aspects, like molecular recognition or chemical and physical transduction, from different sciences including materials and information science, inorganic, organic, physical, analytical, polymer, and biological chemistry are combined to yield a product with unique features.

In spite of the many attempts made, there was no universally accepted definition of a chemical sensor found until now. One approach, that provides a mean of discrimination due to fundamental properties, can be found in [1] and since it was used in virtually the same form in later years as well [2], it seems a save assumption, that this definition is applicable to a majority of cases:

> ...According to us a chemical sensor is a transducer which provides direct information about the chemical composition of its environment. It consists of a physical transducer and a chemically selective layer ...

This definition, although being rather general, allows to distinguish real sensors from batch-mode based techniques which might as well yield results in less time, but are, strictly speaking, not sensors.

A sensor as such consists basically of three zones [3, 4]:

Recognition Zone (Receptor) In this zone of recognition the interaction with the analyte takes place, resulting in a selective chemical or bio-chemical reaction. Thereby the physical properties of the system are changed, where the extend of the change is related to the concentration of the analyte. Semiconductor surfaces, ion-selective membranes, bio-molecules or reagent layers may, for example, be employed for this purpose.

Transducer The physical changes produced by the interaction with the species to be determined are converted into an electrical signal proportional to the analyte concentration. Among others, electrodes or photodiodes are common transducers.

Electronics Often a first treatment of the signal is performed close to the transducer to avoid interference. This may involve pre-amplification or AD-conversion, to name but the most frequently used techniques.

2.1.2 Classification

Although there are many possible ways of classifying, one of the most commonly adopted is by means of the transduction principle, as similarly presented in [5].

Electrochemical Sensors utilise changes in the electric properties of the system. Due to the high development level of modern electronics there is a number of phenomena (current, potential, resistance, etc.) that can be measured with high precision today. This group deserves not only special attention due its long history, but also because it contains some of the most popular and widespread implementations (e.g. ISFETs, the ion-selective electrode and the λ-probe). Further many trends that can just as well be spotted in other fields of sensor technology, have their corresponding implementations with electrochemical transduction principles. Illustrative examples of the latter might be found in recent advances in the employment of sol-gel matrices, the development of spatially resolved techniques (electrochemical microscopy) and the progress of electrochemical biosensors introducing enzyme-modified electrodes and immuno- and DNA-sensors [6]

Thermal Sensors As heat is one of the fundamental properties of every chemical reaction, it should be an ideal parameter for sensing. However, the flow of heat is generally difficult to control and not very specific. Commonly enzymatic reactions are utilised to achieve the needed specificity. Thermistors are still a popular realisation of a heat sensor due to

their low cost, high availability, stability and sensitivity. Alternative concepts being utilised employ the Seebeck effect or the change of heat conductivity, for example [1].

Mass Sensors rely on the changes of mass that interacts with the interface. They can therefore not only be used as balances, but also for the quantification of adsorbed gases or the measurement of viscosity. Two of the most prominent transduction principles with mass sensors are the use of surface acoustic waves (SAW), employing waves propagating parallel to the surface, and thickness shear mode resonators (TSMR), utilising waves perpendicular to the surface. The latter depends on longer wavelengths and therefore allows for thicker coatings. It is also less susceptible to temperature fluctuations [7]. Common implementations are quartz crystal micro-balances (QCM) and shear horizontal acoustic plate mode devices (SH-AMP) [8].

Optical Sensors are able to relate the concentration of an analyte with some change in the optical properties of the system (e.g. absorbance, reflectance, luminescence).

Various other proposals [3] for a classification of chemical sensors include:

- By the type of reaction used in the recognition step, i.e. whether the sensor is employing a chemical (chemical sensor) or a biochemical reaction (biochemical sensor).
- According to the nature of interaction: reversible or irreversible.
- Using the type of sensing element: plain, probe or flow cell.
- Employing the relation between the recognition element and the transducer: connected or integrated.
- Bearing in mind the operation mode: discrete measurements or continuous measurements.
- Considering the number of species detected: single or multiple.

Some of the cited suggestions might, however, interfere with a very strict interpretation of the term “chemical sensor” as described above. As this field is rapidly evolving and not yet anticipated developments might take place, it is likely that these classification change with time.

2.1.3 Important Development Aspects

Selectivity

A topic of great concern with (bio)chemical sensors is selectivity. Although there are many, and sometimes very distinct, ways in which selectivity is aimed to be achieved with sensors, two main routes, using fundamentally different approaches, can be distinguished [9].

The Conventional Approach aims at designing special recognition sites which, ideally, only generate an analytical signal on interaction with the desired analyte. For biochemical sensor these include mechanisms like enzymatic or immunochemical reactions as well as catalytic antibodies and even so called "genosensors" utilising DNA as recognition element. With classical chemical sensors popular methods of selectivity enhancement are e.g. molecular imprinting, self-assembled membranes and films, selective transport mechanisms or polymers (either used directly for signal generation or as matrix for immobilising a sensitive species like in sol-gels).

Sensing Arrays consist of multiple (cross-reactive) sensors or sensing layers using the same transduction principle, coupled to a signal processing system able to extract the analytical information. For this latter purpose chemometric methods for pattern recognition like artificial neural networks – so called "ChemNets" – are often employed. In this manner it is not necessary to prepare selective recognition elements for each analyte.

Fabrication and Miniaturisation

While in the 1970s, when the first CHEMFETs appeared, the feature size of electronics and chemical sensors was about the same, Moore's law just held true conventional electronics [2]. The chemical sensing fell behind in miniaturisation and integration due to the inherent limitations, where certain conditions for diffusion or dimensions for interphasial regions have to be met.

However, efforts on miniaturisation are ongoing as not only size itself is an important parameter, but also affect on other properties of a sensor like response time and above all the price. Hence an important research strain is the development of methods for the deposition of multiple selective layers in narrow defined areas of a support. Starting of from silicon employing integrated approaches, trends are ultimately heading towards non-silicon materials and more modular designs [9]. This development goes hand in hand with intentions to also integrate additional functions like sample preparation steps or calibration. Several methods (e.g. ion or electron beam, electrochemical and laser patterning) have already been devised in this concern and new materials like photo-curable polymers and novel approaches as for example immobilisation and patterning of bacterial surface layers promise further advances in the future [5]

2.1.4 Research Trends

In the attempt to solve some of the common problems of chemical sensors (e.g. lack of selectivity, long response times) some approaches have been investigated including the possibility to perform non-steady-state measurements or the use of higher-order systems.

Non-steady-state Measurements

Waiting for a sensing system to stabilise sometimes takes an considerable amount of time. Hence, efforts are made to use the dynamic response of a sensor to deduce information. This approach not only shortens the time until a reading can be taken, but sometimes also yields additional information about the interaction of the analyte with the system and hence providing more selectivity [10].

Higher-Order-Systems

Possibly one of the most important trends in chemical sensing is the use of more than one interrogation method on a sensing layer. These approaches can be compared to the use of hyphenated techniques in conventional chemical analyses. It is essential for the performance of these systems, that the responses of the single measurement methods are orthogonal. Advantages are not only a gain in information about an analyte, but also avoidance of problems like baseline instability, drifts or interference.

2.1.5 Biosensors

Throughout the years, the combination of a highly selective biological recognition mechanism with the sensitivity of a chemosensor all packed into a single sensor element gained in importance. Although commercial success is, for the time being, limited to medical applications, a lot of promising approaches have been presented [4].

Monoenzyme Sensors are one of the most studied biosensing devices and they also represent the first attempt that was made in this area. Commonly an enzyme is coupled to an optical or electrochemical sensor and the reaction partners of the substrate or the reaction product is detected.

Multienzyme Sensors circumvent the limits imposed by the need for the repeated use of expensive coenzymes, by in-situ generation with the help of a second enzyme.

Biosensors based on tissue sections or cell cultures use entire groups of intact cells and not just extracted enzymes as the recognition element. As the enzymes are allowed to work in their natural environment, they often show a prolonged activity and there is no necessity to add cofactors or coenzymes. The drawbacks are a sometimes reduced selectivity for the mixture of enzymes present. Carrying this concept a step further, the employed cell may be genetically modified, integrating signal amplification and transduction directly into the living cells. The result being self-replicating, highly sensitive sensors [9].

Microbial Biosensors employ micro-organisms to achieve selectivity. They are often combined with amperometric oxygen sensors.

Immunosensors rely on an antigen - antibody interaction, where one of the reaction partners is often labelled to facilitate the measurement. This principle is today in wide use with immunoassays.

Biosensors based on receptors Either single receptors or whole receptor structures are connected to a transducer. Although they have great advantages in terms of sensitivity and response times, there are still severe problems to be addressed with isolation and the stability of these structures when immobilised.

DNA-Senors An DNA hybridisation event is used for signal generation. Together with metal-chelates and various redox markers an appropriate electrochemical transduction system can be constructed, capable to respond to this changes [6].

2.1.6 Applications

As with many other research topics, market needs are an important guidance for development efforts related to chemical sensors. Industry, medicine, and municipal services, they all demand for new means of handling chemical analysis. Performance requirements are considerable and somewhat higher than in a laboratory environment. However, sensors have the potential to comply to the needs in terms of sensitivity, selectivity, accuracy, precision, long-term stability and ease of maintenance [11].

In industry, cost optimisation is one of the driving forces. Sensors used in process control help to maintain ideal reaction conditions and thereby minimise the amount of reagents needed. At the end of the production process, quality control gets increasingly more important. Here as well, sensors can contribute to optimise and check the result. In this concern, hydroquinone sensors have found broad application in different industries including coal-tar production, paper manufacturing or photographic development processes [12]. To protect the health of employers, the machinery and the environment, sensors can be used to monitor toxic by-products in the working environment as well as in effluents and wastes. Apart from that, some of the commercially most successful sensors, can of course be found in consumer products, as for example the λ-probe in vehicles [4].

Elevated public awareness and the resulting increasingly rigourous requirements of legislation have urged increased efforts in the field of environmental analysis. The large number of very distinct toxic substances, the different environments as well as the wide range of concentrations encountered, presents special challenges for the proposed sensors. Nonetheless vivid investigation activity can be noted that yielded a large repository of different devices from sensors for the detection of air pollutants [13] to approaches focusing on different gases and volatile

organic compounds [11]. Water quality control is another important area of research. This includes not only monitoring of effluents from industry, treatment plants or agricultural sites, but especially drinking water analysis where permissible levels of toxic substances are considerably lower. Besides devices for toxic organic substances [14], highly sensible sensors for trace level analysis of heavy metals [15] have been developed, as well as screening approaches for the same substance class [16].

In medicine other advantages of the sensor technology can be utilised. Sensors are generally cheap, so the often laborious task of disinfection can be omitted be simply replacing the sensor head [17]. Should that not be appropriate, it is often possible to construct sensing devices in way so that they withstand even the harsh conditions during thorough cleaning procedures. Also the often provided bio-compatibility combined with flexible designs presents new pathways to in-vitro and in-vivo measurements [18]. How powerful a good sensing principle can be is impressively illustrated by a whole set of sensors based on luminescence decay time measuring pH, oxygen, carbon dioxide, potassium, sodium, calcium, chloride, ammonia, urea and glucose [19]. Especially the latter together with lactose, lactate, cholesterol and ethanol are, due to their relation with various disorders and diseases, often chosen as analytes. Since some time a considerable interest is also dedicated to sensing nucleic acids [12]. Although they present a somewhat special case, sensors for chemical warfare agents, as for example nerve gas [20], might also be mentioned in this concern. The threat from these substances arises not only from their use in battle, but also from leakage during stockpiling.

As can be seen from the above examples, sensor development has gained great importance and numerous implementations can be found in the market. Having their roots in the advances achieved with using electrochemical sensors, sensing devices of all kinds are more and more frequently employed in industry have made the transition from somewhat “exotic” solutions to a special problem to routine applications.

2.2 Optical chemical sensors

For historical reasons, chemical sensor development was focused on electrochemical sensors for a long time. However, due to the rapid development of fiber-optical components for telecommunication and the power of established spectrometric methods, chemical sensors based on optical transduction principles are playing an important role today. The feasibility of miniaturisation, the possibility of remote sensing and applicability to even such delicate measurement tasks as “in-vivo” determinations are among the most often mentioned advantages of these devices [21, 22, 23] and account for the growing popularity of a wide range of applications.

With optical sensors the two basic detection schemes distinguished are direct and indirect determination. The former is based on the measurement of intrinsic properties of the analyte,

while the latter relies on the immobilisation of an indicator on the end of a fiber (or fiber bundle) and the detection of suitable properties of this mediator [23].

2.2.1 Advantages and Drawbacks

Due to earlier development and higher experience in the field of electrochemical sensors, comparisons of the properties are often made with respect to the latter [24]. Nonetheless, even compared to the growing variety of other types of sensors, optical chemical sensors possess some general advantages that make them especially appropriate and preferable for certain tasks [3, 21, 22].

- There is no reference signal needed, as for example a reference electrode with electrochemical sensors.
- Optical sensors are immune to electronic and magnetic interference.
- Reagent consuming reactions are negligible which in turn enhances the stability of the sample.
- Using an adequate discrimination element it is possible to employ the same transduction system for various analytes, thereby cutting the costs for instrumentation.
- Monitoring more than one wavelength or employing time-resolved techniques yields additional information about the investigated system.
- Advances made in miniaturisation and in other research fields (above all in telecommunication and electronics) are readily applied to optical sensing systems, thereby widening their range of application.
- Due to their bio-compatibility, size, and minor interference with the sample, optrodes often show a remarkable suitability for medical tasks without the risk of using electrodes or electrical contacts. Some optical sensors even sustain the harsh conditions of a sterilisation procedure.
- Since optical sensors do not need direct contact with the analyte, they open up new pathways to “sterile sensing”, limiting the risk of contamination in such delicate tasks as analysing biological samples, food or monitoring bio-reactors.
- The possibility of transmitting the obtained signal over long distances minimises the risk of working in dangerous environments and can be used to reduce costs, as signals from different sampling sites can be processed in one instrument.

Although they offer a lot of advantages, there are also some important drawbacks to optical sensors, which should not go unmentioned.

- It is necessary to eliminate interference from ambient light either by excluding disturbing light sources by means of a cover or by light modulation techniques.
- Many of the commonly used indicator reagents tend to show a limited stability. Hence a lot of sensors suffer from problems with photo-decomposition, which in turn reduces their lifetime.
- In some cases a mass transfer is needed to equilibrate the system, thus resulting in excessively long response times (a common problem also typically found in electrochemical sensors).
- Many optical sensing systems show a very small linear response range.
- Often reagents with appropriate selectivity and reversible interactions with the analyte can not be encountered.

The above mentioned problems might partly account for the ongoing research efforts and commercial success, that has yet to come.

2.2.2 Classification

As stressed before for chemical sensors in general, for the subclass of optical sensors just as well exist many classification schemes depending on which aspect the author intended to emphasise.

In a similar evolution as with other emerging techniques, the focus was first on the different approaches as a whole [24]:

Direct Sensors A simple equilibrium between the analyte and the reagent determines the response of the sensor.

Competitive-Binding Sensor The immobilisation phase contains not only a reagent able to specifically bind to the analyte, but also a ligand competing with the analyte for the binding sites of the reagent.

Absorbance-Based Sensors The analyte interacts with an immobilised indicator, the absorbance of which is measured.

Luminescence Sensors This type bears the advantage of the intrinsic selectivity of the luminescence phenomena and the separation of the incident and the detected light by wavelength.

Two-Wavelength Luminescence Measurements Either two excitation and one emission wavelengths are chosen or, just in reverse, one excitation and two emission wavelengths. Readings are taken for both of the cases, permitting to use one as a reference signal for the other.

Luminescence quenching Detection of the analyte by its ability to deactivate the excited state of an indicator and thereby to diminish the luminescence intensity.

Reflectance-Based Sensors When absorption measurements become unfeasible (often encountered in solids), another way of obtaining information is the measurement of reflected light.

The focus, however, changed, so that today a distinction by the nature of the interaction between the reagent and the analyte [23] is quite common.

Reversible Sensors where the interaction of the analyte with the recognition part of the sensor is fully reversible, which means that continuous measurements are possible and the there is no consumption of analyte.

Irreversible Sensors are divided into two subclasses, namely regenerable and not regenerable sensors. The former can be used several times, but require a regeneration step between the measurements, while the latter can only be used once and is then disposed. According to some of the definitions mentioned above, neither of the two may be considered a chemical sensor in a strict way of speaking.

Likewise, discrimination by the employment method are frequently encountered [3]

Probe-type Sensors (Optrodes) These devices consist of one or more optical fibers usually with some sort of sensing phase applied on one end and connected to an instrument on the other. They can either be employed in a flow system for continuous measurements or for distinct probing of samples.

Flow-cell Sensors (Optosensors) In this set-up the sensing phase is packed into a flow cell, which is either incorporated in or situated outside of an instrument. In the latter case wave-guides are commonly used to connect the flow cell with the instrument. In both cases the sample solution is applied by means of a flow system.

Furthermore, classifications based on the optical phenomenon employed [23] (absorption, reflection, raman dispersion, luminescence, etc.), on the type of analyte (gases, dissolved gases, vapours, humidity, ions, etc.) or the field of application (e.g. industry, environment, medicine) can be found in literature [22].

2.3 Luminescence sensors

The interest in using luminescence phenomena for sensors originates a great deal from the high sensitivity and selectivity typically encountered with such optical techniques. Therefore, much

effort has been spent to develop applications from remote sensing of environmental parameter to miniaturised clinical implementations. In the last years special interest could be noticed in the fields of sensing for nitro-aromatic compounds with respect to detection of explosives, nucleic acids, glucose sensing (where special emphasis was laid on miniaturised devices for the operation in human bodies), oxygen and pH [25].

2.3.1 Types of Luminescence

Classification of luminescence phenomena is most conveniently based on the two basic principles that yield the radiation, namely chemiluminescence and photoluminescence (i.e. fluorescence and phosphorescence) [26].

Chemiluminescence

The development of chemiluminescence methods in analytical chemistry commenced comparatively late. There is only a small number of compounds showing this type of luminescence, but some of these are of elevated environmental importance. The simplicity and high sensibility make chemiluminescence, in theory, a field of great interest for investigation. However, because of the low selectivity, caused by the susceptibility to various sources of interference, only modest research efforts have been made so far.

The basic process consists of the formation of an excited species as the result of a chemical reaction. If this species emits light on returning to the ground state or transfers its energy to another species which then emits radiation, we speak of chemiluminescence. However most reaction mechanisms exhibit a considerable complexity. Various examples of such reactions can be found in biological system (e.g. algae, bacteria), in which case the term of "bioluminescence" is also commonly used.

The intensity of the emitted light (I_{CL}) depends on the reaction speed (dC/dt) and the quantum efficiency of the chemiluminescence (ϕ_{CL}, usually about 0,01 to 0,2), which in turn depends on the quantum efficiency of the excitation (ϕ_{EX}) and the emission (ϕ_{EM}) as shown by equation 2.1.

$$I_{CL} = \phi_{CL}\frac{dC}{dt} = \phi_{EX}\phi_{EM}\frac{dC}{dt} \tag{2.1}$$

As the only source of radiation is the chemical reaction, the instrumentation for chemiluminescence experiments can be as simply as a recipient for the chemistry to take place and a detector for the emitted radiation. Usually a graph of time versus the emission intensity is recorded and the peak height or an integral of the peak is used for calibration.

Photoluminescence

Photoluminescence is the process of spontaneous emission of light from a molecule or atom previously excited by photons. The term refers in fact to two phenomena, namely phosphorescence and fluorescence, but due to their close relation they shall be treated here together. In Figure 2.2 a scheme of the energy levels and most important processes involved in the creation of fluorescence and phosphorescence is shown. With the horizontal lines, bold lines represent the vibrational ground state of the corresponding electronic states, while the fine lines stand for the vibrational excited states. Straight vertical lines are assigned to processes of absorption or emission of radiation.

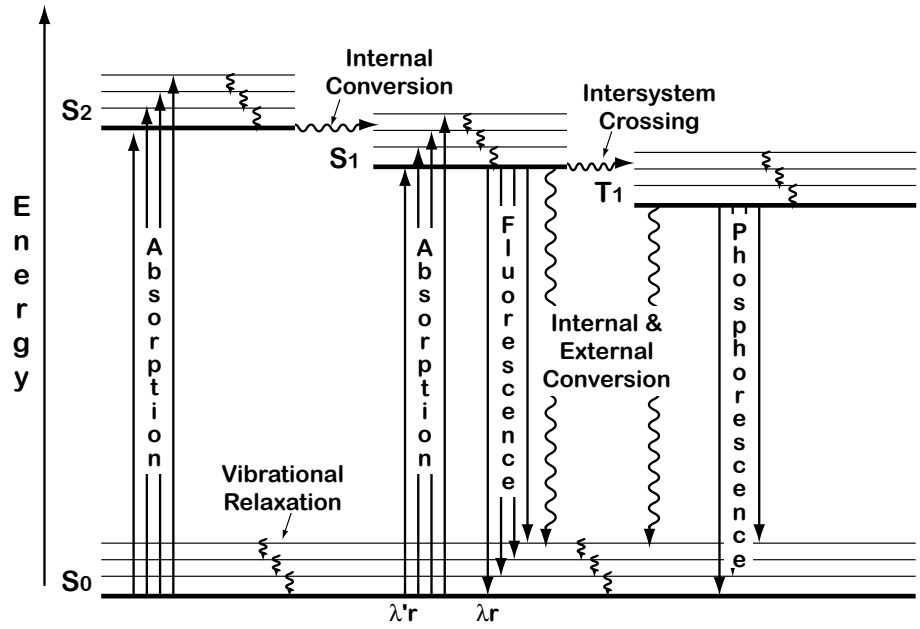


Figure 2.2: Illustration of the most important processes involved with fluorescence and phosphorescence in a Jablonsky diagram.

Photoluminescence is encountered in gases, fluids, and solids, with species ranging from single atoms to complex molecules. The simplest case is resonance fluorescence, where a systems excited by radiation (λ_r') returns to ground state emitting light of the same wavelength as the excitation source (λ_r). The time scale of this process corresponds to the lifetime of the involved electronically excited singlet state, i.e. 10^{-8} to 10^{-5} s.

More commonly the case of normal fluorescence is seen, with which the energy of the emitted radiation is lower than the one of the excitation source. After the very rapid (10^{-15} to 10^{-14} s) absorption of an electron, the species has various possible pathways to return to the ground state. Due to several radiation-less processes, fluorescence and phosphorescence phenomena

are limited to comparatively few species. Whether or not luminescence will be seen and to which extent, depends on the velocity constants of the different decay routes and is therefore determined by the specific structures and the environment. While photoluminescence processes are rather well understood, many of the radiation-less pathways are not completely elucidated.

One of the most important processes to consider is *vibrational relaxation*. Excitation may lead to any of the various vibrational excited states of the electronically excited states (S_1, S_2). However, provided the possibility, this vibrational energy will rapidly (10^{-12} s or less) be lost again by collision with the surrounding molecules. Since this process occurs in much shorter time than the lifetime of the electronically excited state, fluorescence will in these cases always originate from the vibrational ground state, but as some energy is lost to the environment the wavelengths of the emission are longer as compared to the excitation light (Stokes-shift). Upon decay to the electronical ground state (S_0), vibrational excited states of this state may just as well be yielded (which leads to peak broadening) and vibrational relaxation will occur once more, ensuring the fast return to the lowest vibrational state.

Internal conversion is a term referring generally to all intramolecular processes facilitating a transition of the molecule to a lower electronical state without emission of radiation. Therefore basically a rearrangement of electrons yielding another state with the same multiplicity is described [27]. Neither are these pathways well defined nor completely understood. For the relatively small number of compounds exhibiting fluorescence, it seems a safe assumption, that they are very efficient. Overlap of vibrational states favour these processes, because in these cases chances are that the transition may take place between energetically equal states. If internal conversion involves two excited states (as for example between in S_1 and S_2 in Figure 2.2), fluorescence is usually only observed from the lower excited state (S_1). A good example may be found in quinine (λ_{ex1} = 250 nm, λ_{ex2} = 350 nm, but λ_{em} always equals 450 nm).

Conversion may also take place between an electronically excited state and the electronical ground state. With some compounds (e.g. aliphates) a similar mechanism as described may apply as there is some overlap between the vibrational states of the electronical ground state and the first electronically excited state. However, different explanations must be considered if the new state involves the rupture of a bond (i.e. in the case of pre- or real dissociation).

Moreover, *external conversion* should be considered. It involves effects of deactivation of the electronically excited state by interactions with and energy transfer to the surrounding environment. Although these processes, sometimes also named *collisional deactivation*, are not very well understood, it is apparent that they take place, as strong influence of the solvent and other experimental conditions is frequently encountered with photoluminescence.

The last effect to be considered is the *intersystem crossing*, with which the spin of an excited electron changes and thereby forms an excited state with a different multiplicity (e.g. transition from the singlet state S_1 to the triplet state T_1). In case of the given example the lifetime of the

resulting triplet state is much longer (10^{-4} to various seconds or even more) than the lifetime of the singlet state. Light emission resulting from a deactivation of this triplet state is called phosphorescence. In theory, the transition leading to phosphorescence emission is spin-forbidden, but due to spin-orbit coupling there is a certain probability that is still happens. Since spin-orbit coupling is considerably stronger in heavy atoms, these favour phosphorescence at the cost of the fluorescence. A similar effect can be observed with paramagnetic species like molecular oxygen. The back-transition to the singlet state is even less probable and only yields very low intensities. This phenomenon is known as *delayed fluorescence* and depends strongly on temperature.

As mentioned above, internal and external conversion are very efficient processes. Therefore, phosphorescence is usually only observed at low temperatures, in very viscous solutions, bound to a solid support or in an otherwise protected environment (e.g. micellar solution). Ultimately much attention was paid to systems that show phosphorescence at ambient temperatures, also known as *room temperature phosphorescence* (RTP). This special case will be treated a little bit more in detail in Chapter 2.3.2.

Considering the above mentioned processes certain parameters can be identified influencing the luminescence of a species. Each species in a system has a so called *quantum efficiency* to it, defined as the number of molecules emitting luminescence over the number of molecules excited in total. This quotient can in theory take values from 0 to 1 and in fact also in practice the two extremes are almost reached. For the formulation of an equation for the decay from the singlet excited state to the ground state, as with equation 2.2, the velocity constants of all of the above mentioned processes, namely fluorescence (k_f), intersystem crossing (k_{isc}), internal conversion (k_{ic}), external conversion (k_{ec}), pre-dissociation (k_{pd}) and dissociation (k_d), have to be considered.

$$\phi = \frac{k_f}{k_f + k_{isc} + k_{ec} + k_{ic} + k_{pd} + k_d} \tag{2.2}$$

Equation 2.2 permits a qualitative interpretation of the parameters influencing the fluorescence, where k_f, k_{pd} and k_d depend mainly on the chemical structure, while the other constants are stronger influenced by the environment.

Fluorescence is rarely provoked by radiation with wavelengths smaller than 250 nm, as, due to the high energies evolved, the deactivation by pre-dissociation and dissociation is the predominant pathway in these cases. Hence, not $\sigma^* \rightarrow \sigma$ transitions, but rather less energetic $\pi^* \rightarrow \pi$ and $\pi^* \rightarrow n$ transitions are accountable for fluorescence emissions. Empirically it can be found, that compounds having a $\pi \rightarrow \pi^*$ transition as the least energetic transition are more likely to show strong fluorescence. This is due to the high molar absorption factor (i.e. short lifetime of the transition) as well as to the low rate of intersystem crossing.

Considering the above mentioned explication, it only seems rational that the molecules with the strongest fluorescence possess aromatic functional groups with low energy $\pi \rightarrow \pi^*$ transitions. While one aromatic ring usually does not suffice to yield fluorescence, adding of further conjugated aromatic elements leads to the desired luminescence effect. Introduction of halogens tends to diminish fluorescence. This is believed to be due to the heavy atom effect, which permits easier intersystem crossing, and also the augmentation of deactivation by predissociation may contribute in this respect. Carboxylic acids and carbonyl groups inhibit fluorescence, as with this groups an $n \rightarrow \pi^*$ transitions, which is considerably less efficient, has the lowest energy.

From experiment it is known that rigid molecules exhibit significantly stronger fluorescence. This finding is also believed to explain the augmentation of luminescence with chelates complexes of a metal ions and certain organic ligands. Although the process is not fully understood, it seems that rigidity limits the velocity of internal conversion and thereby the probability of a radiation-less decay.

Higher temperatures or lower viscosity of the solvent commonly reduce fluorescence. Under these conditions more collisions occur and external conversion is more likely to take place. Heavy atoms in the solvent as well as in dissolved species tend to show the same effects as if incorporated in the fluorescent molecule, i.e. intersystem crossing is favoured and phosphorescence is augmented at the costs of fluorescence.

The luminescence of an aromatic structure with acidic or basic substituents usually depends on the pH. Changes may not only involve the intensity, but also the wavelength of the emission. These variations depend on the number of available resonance structures for the protonised and deprotonised form, respectively. The more resonance structures are available, the more stable is the first excited state and the more fluorescence is yielded. It should be noted that the pKa value of the excited species frequently differs from the one of the species in its ground state, sometimes as much as 5 orders of magnitude.

Dissolved oxygen usually reduces the fluorescence. This quenching is sometimes due to photochemical oxidation, but more frequently to the paramagnetic properties of molecular oxygen, which favour intersystem crossing and energy transfer to the oxygen. Other paramagnetic species show the same effects.

From theory a linear relationship would be expected between the concentration of the fluorescent species and the intensity of the luminescence. However, in reality this only holds true for low concentrations. With higher concentrations the simplifications made to obtain a linear graph do not apply anymore and intensities fall behind the expectations. Other effects that also contribute to this deviation is on one hand self-quenching, i.e. on collision, two excited molecules return to their ground state in an radiation-less process similar to an external conversion. Such occur with higher probability if the concentration of the luminophor is higher. On the

other hand auto-absorption can take place if the wavelength of the emission overlaps with the absorption peak. In this case the light emitted by one species is reabsorbed by another molecule before it leaves the solutions and can be measured.

2.3.2 Room Temperature Phosphorescence

Although fluorescence is the much more extensively used principle with luminescence measurements, phosphorescence and especially room temperature phosphorescence has significant advantages for some applications [23].

- The wavelengths of phosphorescence emission are longer, which yields a better separation and discrimination between excitation and emission light.
- Long lifetimes of the excited triplet state facilitate the construction of simple instrumentation for lifetime measurement.
- Analytical signals with low baselines, yield good signal/noise ratios and therefore improve detection limits.
- Since there are much less phosphorescent than fluorescent species, the former method should be more selective.

In the last years RTP on solid support has grown to a well established technique permitting measurements of high sensitivity, wide linear ranges of concentration and outstanding selectivity. The latter being an inherent property of RTP, as the emission occurs in a spectral region where few interference from other processes is situated and the number of molecules exhibiting phosphorescence is limited compared to that of fluorescence. Moreover, due to the long lifetime of the excited triplet state emission may be discriminated in time as well. All these properties make RTP especially interesting for sensor development.

Like with fluorescence, there are some important points to consider, which influence the phosphorescence of a given species in a certain environment. As for the origins of the phosphorescence, all the mechanisms that affect the fluorescence will likewise have an influence on the phosphorescence emission. Hence, in the following list, special attentions was paid to somewhat more specific interactions.

Rigidity of the system To protect the excited triplet state from radiation-less deactivation, it is desirable to limit the degrees of freedom in the system as well as movement in order to avoid external conversion. This may be achieved by the use of ordered media (e.g. cyclodextrine cavities, micelle stabilised luminescence) [28], immobilisation on a solid support or by reduction of the temperature.

Heavy atom effect While intersystem crossing is not desired with fluorescence, quite the reverse holds true for phosphorescence. Inter- or intra-molecular heavy atoms will augment the population of the excited triplet state and thereby enhance the phosphorescence intensity [29, 30]. Although not all heavy atoms have the same effect on different phosphorescent species [31, 32], which can on the other hand be used to obtain analytical information [33].

Molecular oxygen In its ground state molecular oxygen represents a system in a triplet state, therefore it interacts very efficiently with the excited triplet state of phosphorescent species and reduces the RTP emission [34]. Hence, it is desirable in most cases to remove oxygen from the system completely. Convenient ways of doing so are for example bubbling Ar or N_2 through the solution, or chemical desoxygenation with Na_2SO_3 [35].

Humidity Depending on the immobilisation of the phosphorescent species and measurement set-up, humidity sometimes has an important influence on the emission intensity. This may be explained by the common use of hydrogen bridge bonds for immobilisation (the strength of which is of course strongly affected by the water content of the surrounding environment), or water may otherwise compete with the luminescent molecules for the available binding sites on the matrix. The result of either of this mechanisms is a less rigid environment and higher accessibility of the phosphorescent species to quenchers, leading to the effects outlined above [29].

Temperature A rise of temperature diminish fluorescence due to a higher rate of external conversion, it therefore comes as no surprise that phosphorescence should be reduced as well.

2.4 Instrumentation

The parts of a luminescence spectrophotometer and their functions are very similar to standard UV/vis spectrophotometers. Light from the excitation source is passed through an excitation monochromator to select a single excitation wavelength. Subsequently the light is directed on the sample to excite the luminescent species. The radiation emitted by the luminescent agent is collected and passed through the emission monochromator before it finally gets to the detector. It should be noted, that the excitation source and the excitation monochromator may be omitted in the case of chemiluminescence measurements. For phosphorescence experiments additional devices or techniques are required to avoid light other than originating from the phosphorescence emission to reach the detector. In the simplest case this is achieved by a so called "phosphoroscope", although pulsed lamps for time-resolved measurements are more frequently found in modern equipment. Commonly the sample is mounted in such a way that it is possible to collect the emitted radiation in a 90 degree angle to the incident beam, as to minimise the interference from dispersed radiation. Many instruments used for measuring fluorescence or

phosphorescence rely on a dual beam referencing, where one beam passes through the sample while the other gets straight to a photo-multiplier.

2.4.1 Important parts of a luminescence spectrophotometer

As the luminescence effects usually yield rather low intensities, strong light sources are required. High pressure arc lamps are in widespread use for this purpose. They consist of two tungsten electrodes in a quartz housing filled with xenon, mercury or a mixture of the both at high pressure. These lamps are commercially available providing an intense quasicontinuum radiation from 300 to about 1300 nm. With mercury various distinct emission lines are yielded, which usually suit the purpose as photo-luminescence excitation can most often be achieved with a number of different wavelengths. For their smoother continuum type spectrum, xenon lamps are usually preferred for research tasks.

If simplicity and economic considerations are of special concern, low pressure mercury vapour lamps are sometimes employed, which yield a few intense lines in the UV only. Another option in this concern are tungsten filament incandescent lamps, possessing a very smooth continuous spectrum but little intensity in the UV.

The use of pulsed flash lamps as excitation sources not only provides the advantage of potentially higher intensities, but also permits instrumentation employing an electronic approach for achieving time-resolved phosphorescence measurements. In this case the sample is excited by a short pulse (typically about 10 μs) from a flash lamp and the emission is determined by a photomultiplier operated in a synchronised manner, able to take readings delayed with respect to the excitation pulse. Figure 2.3 illustrates this gated-detection system. It works turning the detector on only after a convenient delay time (t_d) has passed, allowing the excitation light pulse and eventual fluorescence emission to cease to a negligible value. As the phosphorescence has a relatively long mean lifetime of the excited state, its emission continues until after delay time and can subsequently be recorded during a given gate time (t_g). This cycle can be repeated with each pulse of the lamp and the integrated intensities (indicated in grey in Figure 2.3) can be recorded. The delay between the excitation and the observation of luminescence ensures the discrimination between phosphorescence and other shorter-lived emission phenomena. Therefore with this technique no mechanical chopper device is needed.

In more sophisticated equipment a laser (especially N_2 laser or dye laser pumped by Nd:YAG laser) can also be found as excitation source. They offer some important advantages as for example their high intensity, the limitation of interference for their highly monochromatic output. Additionally they can be used in pulsed mode providing another alternative for pulsed light sources, where mechanical phosphoroscopes can be omitted. In remote sensing the coherence of the beam is an essential advantage just as well as for the work with very small sample amounts. For some measurement modes (e.g. lifetime determinations by phase shift) also the

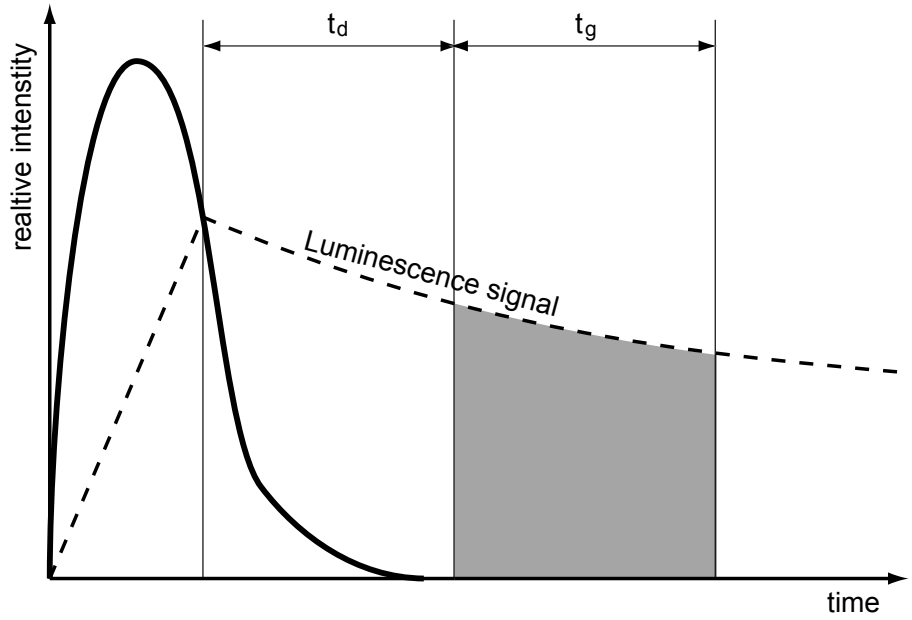


Figure 2.3: Illustration of the decay processes involved with fluorescence and phosphorescence.

phase coherence of the lasers is of special interest.

Depending on the desired use of the instrument simple filters or more expensive prism or grating monochromators are possible alternatives for the dispersive device.

A crucial part in the phosphorescence spectrophotometer is the phosphoroscope. The main purpose of this device is to shield the detector from scattered excitation light and fluorescence emission from the sample or the support. This is especially important because the phosphorescence emission is usually very weak compared to the excitation beam or even the fluorescence output of the support or the sample itself. The simplest way of realising such a device is a mechanical chopper. Excitation radiation is periodically blocked and after a short time delay (usually in the order of a few tens of μs) the cover is removed from the detector to permit the radiation to be recorded. In this way the fast ceasing fluorescence and the scattered light from the incident beam are mostly removed and only the phosphorescence emission containing the analytical information is collected. A linear arrangement where the sample is placed in the middle of two disc with apertures, permitting the described out-of-phase excitation and detection principle, is known as the Becquerel Discs. Other implementations of the same system can be found in rotating cylinders or mirrors or chopper disc attachments. With all of the latter, the emission radiation is usually collected at an angle of 90 degrees, to further enhance the removal of light from the excitation source [36].

Both cuvettes made of glass as well as such made of quartz may be used. Special attention has to be paid to the remove of all stains from the surface, as for example fat is fluorescent.

The low signal level also challenges the detectors. Commonly photo-multiplier tubes are employed, which are sometimes cooled to obtain a better signal/noise ratio. But just as well diode arrays [37] and charge coupled devices [38] have been proposed as detectors, permitting rapid acquisition of spectra, which is of special interest in chromatography and electrophoresis.

Luminescence spectrophotometer are generally easy to use and inexpensive equipment, as they can be build with simple constituents commonly available. Therefore a wide range of compact and robust equipment is commercially available.

2.4.2 Instrumental configurations

Two important configurations can be distinguished with luminescence sensors, namely flow cell set-ups and probe type sensors. With the former, the sensing phase is packed in a flow cell and the analyte is pumped through the cell. The flow cell can either be installed in a luminometer or connected to one of those instruments by optical fibres. Probe type sensors always depend on optical fibres to guide the excitation light to the sensing phase and later collect and transmit the luminescence emission back to the detector.

2.4.3 Immobilisation

A key step in developing a luminescence sensor is the adequate immobilisation of the phosphorescent reagent in the solid phase ("active" phase synthesis). For the measurement of luminescence phenomena different environments were tested in the past to enhance the output. While fluorescence emissions are sometimes quite strong, these techniques are of special interest for (room temperature) phosphorescence, where typical intensities are considerably lower. A good immobilisation method should be simple and fast, producing stably immobilised reagents (which do not show any leaching from the substrate) and it should maintain the chemical and biochemical activities of the reagent after immobilisation. Three widely used methods for immobilisation can be considered: Adsorption, covalent binding and encapsulation (or entrapment) of the sensing reagents within a solid matrix [21, 39]. Adsorption often suffers from considerable leaching and due to the resulting loss of signal, the lifetime of the sensing phases are limited. Covalent binding overcomes most of the problems with insufficient sensing agent binding and therefore results in longer lifetimes of the sensor. However, this comes at the price of sophisticated preparation procedure involving very specific reaction [40, 41].

Concerning the solid supports, a variety of substrate materials, including cellulose supports, polymeric resins, silicones, different hydrophobic polymers and silica materials have been widely used as RTP sample supports for a variety of compounds. Both, the particular solid support, used to entrap the RTP active reagent, and the immobilisation technique used play an active role in the performance of the developed RTP sensing "active" phases. An adequate choice of

the solid support and immobilisation technique is not a simple task and depends on a number of factors, particularly on the nature of the analyte and the physicochemical properties of the substrate.

In RTP sensor development organic polymeric matrices have been widely used for immobilisation of the RTP reagents. Inorganic matrices received little attention until a few years ago when the sol-gel technology was introduced as an efficient tool for simple reagent immobilisation in sensing applications. The sol-gel process offers a very convenient method to incorporate indicator molecules in porous inorganic matrices: The relative simplicity of sol-gel technology and the important physicochemical advantages offered by these inorganic supports (including physical rigidity, negligible swelling in both aqueous and organic solutions, chemical inertness, high photochemical and thermal stability, along with excellent optical transparency and low intrinsic fluorescence) are, at least partially, responsible for its rapid proliferation in the field of optical chemical sensors. For its importance and the usage in with the experiments at hand, this method shall be looked at in section 2.4.3 in a little more detail.

Micellar solutions have also proven to be useful [42]. They seem to augment the proximity of heavy atoms to the phosphorescent molecule and thereby enhance phosphorescence intensity [43, 44]. Additional information about the environment and the interactions of the analyte can be obtained determining the lifetime of the triplet state with micelle stabilised RTP (MS-RTP) [45, 46].

For a long time, some kind of "protection" for the excited triplet state, whether a solid support or ordered media, seemed to be a prerequisite for RTP measurements. In recent years this believe changed as work was presented where the occurrence of RTP did not depend on a protecting medium. The terms of non-protected fluid room temperature phosphorescence (NP-RTP) [47] and heavy atom induced room temperature phosphorescence (HAI-RTP) [48, 49, 50], respectively, were suggested for this new kind of RTP emission.

Sol-Gel

Particularly in the field of the RTP sensors, sol-gel glasses offer several additional advantages mainly derived from the high physical rigidity achieved by entrapping the active agent in these solid supports. As a result of this rigid environment, enhanced relative RTP intensities and triplet lifetimes of the immobilised agent are observed, making these materials very attractive as solid supports for the development of RTP optical sensing phases. Different supports can provide different rigidity patterns, which are a crucial parameter for observing analytically useful RTP emissions.

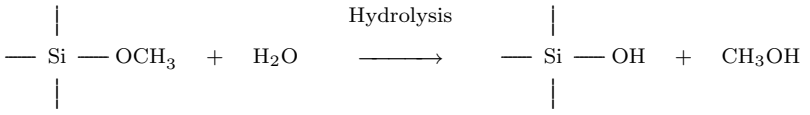
Sol-gel materials are generally optically transparent, glass-like matrices produced by hydrolysis and subsequent polycondensation of organometallic compounds at low temperatures. Com-

monly silicon alkoxides in aqueous solution are used. First a colloidal suspension, the sol, is produced. Gelation by agglomeration leads to a wet 3D SiO_2 network. Finally, drying of the wet-gel, by evaporation of water and the organic solvent from the glass cavities, leads to the final product: A dry-gel (xerogel). These polymers possess interconnected pores (sometimes also called cages or cavities) of sizes of a few nanometers. Agents of relatively large size are securely trapped within the pores, while the small are still able to diffuse throughout the material [39]. The pore network allows external molecules, including the desired analytes, to diffuse into the matrix and react with the trapped molecules. A sol-gel of inorganic oxide particles is prepared likewise by polymerisation of metal alkoxides in the presence of the desired dopant molecules.

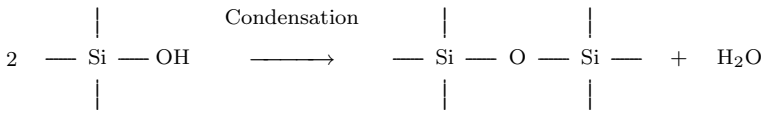
The Sol-Gel Process involves, as already briefly outlined above, usually four steps:

Mixing In this step the reagents are mixed and the reaction is initiated.

Gelation In the gelation step the monomers are first hydrolysed (equation 2.3) so that they can than condense (equation 2.4) to a network.



(2.3)



(2.4)

Ageing Some time is allowed for the network to strengthen.

Drying Water and solvent are removed.

Sensing species are frequently added right at start, but can just as well be added at a later point in the process. The obtained polymer can be cast as a monolith or coated on a solid support such as glass or silica slides or fibers.

During the synthesis reagents are entrapped within the matrix but can move freely inside the pores. Thus they can also interact with the silanol groups on the inner surface of the pores or

with each-other. Analytes, which are usually considerably smaller, can diffuse into the pores and react with the sensing molecule. Sol-gels normally possess a high porosity (around 30%) and also a very large specific surface area (> 300 cm^2/g).

Advantages

- Sol-gels are compatible with many organic and inorganic reagents.
- They are chemically, photochemically, thermally and mechanically stable compared to organic polymers and therefore also usable in harsh environments.
- Their optical transparency makes them preferably to most organic matrices, which absorb in the UV region.
- Due to the formation in mild conditions, also indicators with poor stability can be used.
- In some cases the lifetime of the reagents is prolonged by the entrapment.
- Sol-gel matrices can be used for various sensing configurations (casting, coating, etc.).

Disadvantages

- As response in aqueous media is governed by diffusion, response times tend to be poor (various minutes).
- Leaching is greatly reduced in comparison to resins, but keeps being a topic of concern. Important parameters are the preparation procedure, the relative sizes of the reagent and the pores and the conditions of the measurement. Approaches for tackling this problem include the following.
  - drying the sol-gel for a long time (1-2 weeks) before use
  - polymerisation at high temperature and with low water content
  - covalent bonds between reagent and the inner surface of the pores
- Sol-gels from tetrafunctional precursors will continue to condense even after the gelation step. This leads to shrinking of the polymer upon drying and hence to smaller pore sizes. Analyte diffusion will therefore be reduced and this will negatively affect response time and selectivity.
- The spectroscopic properties, chemical behaviour and biological activity might change due to the reduced degree of freedom in the pores and/or the interaction with the inner surface if the pores.

Applications of sol-gel materials yielded an exciting new repository of sensors, including for example pH sensors [51], sensors for ionic species [52], gas sensors [53, 54, 55, 56], fiber-optic evanescent wave sensors [51, 57] or glucose sensors [58].

2.4.4 Measurement types

The possibility to directly measure the luminescence of an analyte is, as mentioned before, limited to a relatively small number of compounds. An example where this approach is employed successfully are polycyclic aromatic hydrocarbons (PAH) [32], which are, due to their toxicity, of high environmental relevance.

Another group of compounds accessible to photoluminescence analysis are quenching agents. In this case the loss of intensity with higher concentration of the quencher represents the analytical signal. The term "quenching" as used up to this point, only referred to one type of quenching, which is dynamic quenching. As described earlier, this mechanism is based in the deactivation of an excited state. Oxygen sensors are very popular examples for this principle [40, 59], but also less common implementations can be found [60]. Static quenching is the second type of quenching. It is as such not a deactivation process as it refers to the formation of non-luminescent complex of the quencher and the luminescent species [61].

A similar process, but with the reverse effect can be applied with some metal ions. Some of which form luminescent complexes that can be used to obtain analytical information [16].

Nonradiative energy transfer between a luminescent species and an indicator is another alternative. In this case there is no need for the luminescent molecule to interact specifically with the analyte. The only prerequisite is a spectral overlap of the luminescence emission with the absorption of the indicator. The interaction of the latter with the molecule, desired to determine, will decide the extent of the energy transfer and thereby the luminescence intensity [62].

Possibly the most simple and therefore frequently employed method with photoluminescence are *intensity* measurements. After the delay-time the emission peak is integrated for a certain time called gate-time, which is usually in the order of a few nano-seconds. Although these readings are readily obtained, the method suffers from several drawbacks, as not only the parameters that affect the luminescence directly, but also variations in the experimental set-up (like instability of the excitation source or photo-bleaching of the luminescent dye) will interfere [63, 64].

In order to overcome most of the problems encountered with intensity measurements, *lifetime* measurements can be used instead. As lifetimes of the excited states are constant for a given system and concentration of quencher, it can serve as an internal standard [65]. Methods based on lifetime measurements are therefore usually very reliable. However, general purpose instrumentation is rather sophisticated and hence harder to handle and more costly.

For obtaining lifetimes either of two approach can be used [66]. In time-resolved measurements samples are excited by a pulsed source and the decay profile is measured. Methods employing phase-resolved techniques rely on the modulation of the light intensity of the excitation source (usually a continuous wave laser). When the light, modulated at a frequency near the lifetime of the excited state, then interacts with the sample, its phase characteristics change. From the relation of the phase of the incident light and the emitted light, the lifetime can be obtained [67].

Taking the described techniques one step further, many derived measurement methods were developed. Frequently a ratio between two signals is used, whereby one acts as an internal standard for the other. As for example a ratiometric flourometer for oxygen was developed, where a dual emission (phosphorescence and fluorescence) dye was employed. While the phosphorescence emission of the indicator changed strongly with the oxygen concentration, the fluorescence was not affected at all. Therefore the phosphorescence signal could be used to extract the analytical information, while the fluorescence served as a standard [68]. Lifetime measurements of distinct excited states can just as well be employed in a similar way [65], where it should be mentioned that it is not mandatory to use dual-emission dyes, for two different dyes will suit the purpose as well [69].

2.5 Determination of Dissolved Oxygen

The oxygen concentration is of great importance in a number of processes. Not only is it necessary to measure and control oxygen in industrial reactions, even more important is its role in biological systems. A lot of effort is therefore spent on the development of oxygen sensing devices in environmental control and for medical applications.

Water is one of the fundamental resources nature provides us with. One factor that very much decides over the quality of sea as well as fresh water is its content on dissolved oxygen. Warm water ecosystems with oxygen concentrations below 5 mg/L cannot adequately support diversified biota. The ability of micro-organisms in the water to deal with pollution is also heavily dependant on the availability of oxygen, as the waste (i.e. mainly carbon) is removed by forming carbon dioxide. Waste discharges are therefore required to have a high level of dissolved oxygen (5-8 mg/L). Oxygen levels below 5 mg/L will impede fish growth and if the oxygen content drops to about 1 mg/L fish will even die within a few hours. For domestic and industrial water supply lower oxygen levels are preferred, as to protect the piping. However, in most countries there are no upper limits [70].

The importance of oxygen determination in medicine and pharmacy extends to a point that urged authors to call the investigations in this field the “holy grail” of (fiber-optic) sensor development [71]. Development in this field has reach a level that provides doctors today with such

advanced tools like fluorescence imaging of the spatial distribution of the oxygen supply of the skin [72] to base their diagnoses on.

2.5.1 Volumetric Determination of Oxygen(Winkler)

Although one of the oldest ways to determine dissolved oxygen in waters, the Winkler method is still a very common and recommended reference method [70, 73]. The reaction scheme is based in the reduction of Manganese hydroxide forming a brown precipitate and the following determination by iodometry, as illustrated by equations 2.5 to 2.8.

$$4Mn(OH)_2 + O_2 + 2H_2O \longrightarrow 4Mn(OH)_3 \downarrow \text{brown} \tag{2.5}$$

$$Mn(OH)_3 + I^- + 3H^+ \longrightarrow Mn^{2+} + 0,5I_2 + 3H_2O \tag{2.6}$$

$$2S_2O_3^{2-} + I_2 \longrightarrow S_4O_6^{2-} + 2I^- \tag{2.7}$$

$$1\ \text{ml}\ 0,025M\ Na_2S_2O_3 = 1\ \text{mg dissolved Oxygen L}^{-1} \tag{2.8}$$

Considerable drawbacks of this method are that most organic matter will cause negative interference with this and sample conservation is needed [73]. Nitrites, as frequently encountered in treated sewage, will just as well interfere [70]. To deal with the latter problem, several approaches have been developed, e.g. the addition of sodium azide.

$$HNO_2 + HN_3 \longrightarrow N_2 + N_2O + H_2O \tag{2.9}$$

2.5.2 Electrochemical Oxygen Sensors (Clark Electrode)

The Clark electrode is nowadays the standard method in industry for oxygen determination. This amperometric bipolar electrode consists of a small platinum cathode and a silver chloride plated platinum anode immersed in calcium chloride. The whole system is protected and separated from the environment by an oxygen permeable Teflon membrane. On application of a polarisation potential the oxygen, diffusing in through the membrane, is reduced at the cathode (equations 2.10 and 2.11).

$$O_2 + 2H_2O + 2e^- \longrightarrow H_2O_2 + 2OH^- \tag{2.10}$$

$$H_2O_2 + 2e^- \longrightarrow 2OH^- \tag{2.11}$$

The current produced by these reactions is proportional to the amount of oxygen reduced. By this methods measurements of the oxygen partial pressure from ppms upto percent levels may be realised.

However, the Clark electrode presents a number of drawbacks:

- Poor long-term stability due to the necessity of changing the electrolyte from time to time. Continuous use of the electrode bares the risk of drifts due to electrolyte consumption.
- The reaction used for the determination of oxygen consumes itself oxygen and therefore influences the system intended to measure.
- As with all electrochemical sensors, the Clark electrode is as well prone to interference from external electromagnetic fields.

Many of the mentioned shortcomings can be avoided or their effects at least reduced by modifications of the original design, so that the Clark electrode has found applications in many different fields [3].

2.5.3 Optical Sensors for Dissolved Oxygen

The topic of optical sensors for oxygen in aqueous solution or biological fluids has received considerable attention for some years now. Especially the use of modern optical fibre technology is a very attractive approach [74].

Chemiluminescence has been used successfully in direct measurements [75] as well as indirectly by determination of hydrogen peroxide produced in an enzymatic reaction [76]. Colour change reactions are another alternative. Investigation has been reported using the shift of the Sorbet band of haemoglobin as a measurement principle for construction of fibre-optical sensors [77]. Also a wide range of other reagents have been used for absorption sensors [74, 78, 79].

The majority of optical oxygen sensors is still represented by devices based on dynamic quenching of luminescence [80]. This technique offers the advantage that rapid and reversible reactions are used and, which is of special importance, the sample is not altered.

One important group of luminescent species are polycyclic aromatic hydrocarbons (PAH). Especially Pyrene and its derivatives were used in several studies [81, 82], but also other molecules of the same group were investigated [83, 84, 85]. Serious problems are presented by the short wavelengths necessary associated with the excitation and emission of this compounds, as UV lamps and quartz fiber optics have to be used to achieve optimal conditions.

An alternative is presented by luminescent metal complexes. A selection of different metals has been studied [86], among which Ruthenium enjoys great popularity. Various of its complexes

present long lifetimes, great Stokes shifts and wavelengths accessible to cheap and small LEDs. Also the possibility to use plastic fibers is an important advantage. Hence a multitude of studies of these complexes on different supports was conducted [64, 87, 88, 89].

Among the different optical sensors for oxygen based in luminescence, there is also a significant group of phosphorescence sensors. These devices are almost exclusively based in the quenching of the room temperature phosphorescence of different molecules immobilised on a solid support. This was just as well the principle of the first sensor of this kind constructed in 1935 [90]. Since then, a large collection of sensors was developed distinguished by the different phosphorescent molecules used as well as by the solid supports. Although an attempt to summarise all of the work done on this field renders, due to the limited availability of sources and time, seemingly impossible, the three apparently most important approaches will be introduced.

The working group of Prof. Sanz Medel examined various different metal chelates [86]. However, most of the work was published using quinolinesulfonic acid derivatives as ligands bound to lead [91], but especially to aluminium [40] yielding strongly phosphorescent chelate complexes. Studies of the properties of these compounds were conducted using anionic resins and sol-gel glasses as solid supports. In particular, Al-ferron encapsulated in a sol-gel matrix showed promising characteristics for oxygen sensing, thus this sensing phase was then chosen for the construction of oxygen sensing devices [92, 93].

Another approach adopted by different groups involves the use of Erythrosine B as an indicator. For the importance of the role of the solid support a multitude of studies can be found, assessing different possibilities such as anionic and non-ionic resins [94], encapsulation in silicon rubber films [95] or covalent binding to a silica-based amino-functionalised exchanger [96]. Embedded in sol-gel matrices, this phosphorescent species also suits for oxygen sensing in gas phase [97].

Just as well many studies have been dedicated to Porphyrin and its derivatives. Commonly either platinum or palladium complexes of these compounds are used. Among those platinum octaethylporphyrin has found great attention such in a polystyrene matrix [98, 99, 100], as immobilised in sol-gel glasses [101, 102].

Chapter 3

Objectives

For different applications, distinct aspects of a measurement method are dedicated special attention to. In sensor development, these would be simplicity, robustness, and the possibility of cheap and small implementations, to name but the most important. Being able to employ less sophisticated measurement approaches can cut costs and augment the ease of use considerably. It is therefore desirable to have data comparing the performance of different measurement methods. On the bases of such experiments further research decisions can be made to optimise the development process.

Aim of the work at hand is a comparison of three different measurement methods for the development of optical sensors based on luminescence. The selected approaches cover, to a certain extent, the available spectrum of distinct approaches. Intensity measurements were chosen for their common application and their simplicity. From the analytical point of view, they are expected to be very susceptible to a wide range of different sources of interference. Any other method would have to perform better than intensity measurements to present an advantage at all. Lifetime measurements were selected for this study because they overcome most of the problems presented with the intensity measurement approach. For the reasons outlined in the introduction, analysis based on lifetimes usually yield very good results, but come at the cost of augmented complexity. A compromise between the simplicity of intensity measurements and the robustness of lifetime measurements is often presented by ratiometric analysis based on intensity measurements. Hence, this was approach was chosen as the third alternative.

In order to obtain truly comparable values, it will be necessary to encounter a suitable sensing system, with which all three measurement approaches can be realised without changing anything but the measurement method. Hence, an appropriate sensing phase has to be found, exhibiting adequate photo-luminescence properties. Moreover, an experimental set-up has to be devised, which provides stable conditions and allows the application of standard solutions of different levels of water dissolved oxygen and various samples to the sensing phase. All exper-

imental parameters have then to be optimised and ways have to be found to exclude possible interference as far as possible.

From previous work realised by the working group of Dr. Alfredo Sanz Medel, Al-ferron, either immobilised on a resin or encapsulated in a sol-gel matrix, is thought to be a promising candidate as an indicator molecule. Likewise, various different designs of flow systems, employing gases or streams of liquids, are potentially useful for the application of the standards and samples to the sensing phase.

From what was outlined above the following objectives can be deduced:

- A suitable sensing has to be found constituted by a adequate solid support and a appropriate indicator molecule. The latter should exhibit fluorescence and phosphorescence, where the intensity of the latter should be correlated to the concentration of dissolved oxygen, while the former should remain uninfluenced to be used as an internal standard.
- An experimental set-up has to be devised, providing stable conditions for the measurements, allowing the application of different concentrations of dissolved oxygen to the sensing phase, and the determination of real world samples.
- Calibration graphs for each measurement method have to be established and samples have to be measured in order to obtain the analytical figures of merit for every measurement approach.
- All the investigated measurement schemes have to be evaluated and compared as to their suitability for the development of chemical sensing devices.

Chapter 4

Experimental

4.1 Instrumentation and Materials

4.1.1 Reagents, Solutions, and Instruments

For synthesis 8-hydroxy-7-iodo-5-quinolinesulphonic acid ($\geq$99% Fluka), methyl-tri-methoxy-silane ($>$98%, Fluka), tetramethoxy-silane ($\geq$99%, Fluka), aluminium standard solution (aluminium nitrate in nitric acid 0,5 M, 1000 mg/L Al, Merck) were used as delivered from the respective suppliers, as were ammonium acetate (p.a., Merck), acetic acid (glacial, 100%, Merck) and 1,10 phenanthroline 1-hydrate (99%, Fluka) for buffer solutions.

Dowex 1x2-200 was purchased from Aldrich and washed thoroughly with 2 M HCl solution and water before use. All the solutions were prepared with de-ionised ultrapure MilliQ water from a Millipore Milli-Q plus 185 water purification machine.

For pH measurements a Crison micropH 2000 digital pH-meter was employed. For validation purposes and and comparison dissolved oxygen determinations in water and buffer solution, as described with some experiments, were realised using a commercial Crison Oximeter (OXI 330i) with a galvanic sensor (cellOx 325).

Apart from the preliminary experiments, which were conducted in a Shimadzu spectrofluorophotometer RF-5000, all the measurements were taken using a Perkin Elmer luminescence spectrometer (LS 50 B) equipped with a flash Xe arc lamp.

4.1.2 Preparation of the Sensing Phases

The preparation of the resin was carried out almost exactly as described in literature [86]. 35 ml of ferron solution (3x10^{-3} M), 3 ml of Al standard solution (1 g/L) and 10 ml of NH_4OAc/AcOH

buffer solution (2 M, pH 5,5 with acetic acid, containing 50 mg/L of 1,10-phenanthroline 1-hydrate) were mixed in a 100 ml flask and made up to the mark with MilliQ water. The obtained Al-ferron solution was pumped through a mini-column packed with Dowex 1x2-200 during 30 minutes at a flow rate of about 2 ml/min. Later the column was rinsed with a 1 M NaCl solution for 20 min and finally with MilliQ water for about 10 min. Until use the resin with the Al-ferron immobilised on it, was stored in MilliQ water in the dark.

For the preparation of the sol-gel material, a procedure from literature [59] was followed to the letter. To the moment of usage, the sol-gel was stored dry under exclusion of light, which could potentially induce photochemical processes deteriorating the luminescent complex.

4.2 Experimental Set-Up

4.2.1 Parameters and Sample Preparation for the Experiments with Sol-Gel

For the basic set-up a flow system was chosen (Figure 4.1). The heart of the system was a flow cell filled with Al-ferron encapsulated in the sol-gel matrix. An Erlenmeyer flask was used as the source for the solutions. The pump was installed in between the flask and the instrument. Changes of the oxygen concentration were realised by exchanging the flasks as a whole. As there are no "certified" standard solutions available for the analytical problem of dissolved oxygen in water, deoxygenation by argon was used. With this method, described as well in other work [59, 86], argon is bubbled through a solution for varying lengths of time, resulting in different concentrations of dissolved oxygen. To determine the oxygen level in the solution a commercial oximeter, validated with the Winkler-method, was used. As this deoxygenation method also remove other dissolved gases, as for example carbon dioxide, the pH of the solution was influenced and hence a buffer solution (0,5 M NH_4OAc/AcOH; pH = 5,5) had to be used instead of pure MilliQ water. All samples, whether used for calibration or referred to as "real" or "natural" samples, were prepared alike.

The spectral characteristics of the sol-gel sensing phase and the experimental settings are summarised in Table 4.1. Appropriate excitation and emission wavelengths were optimised for each day anew, but they showed hardly any variation. Therefore typical excitation and emission wavelengths could be given. The delay was, as outlined above, varied for the different measurement methods. For intensity measurements a delay time of 0,04 ms proofed to be the ideal setting as at that time virtually all emission originating from the excitation source and the fluorescence has ceased. Ratiometric measurements are preferably conducted with a delay time of 0,03 ms. This setting provides the most suitable ratio of the fluorescence and phosphorescence peak intensities. To obtain the needed decay curves for determining the phosphorescence lifetimes, intensities were recorded at 0,03 to 0,17 ms at steps of 0,01 ms. In addition a final reading

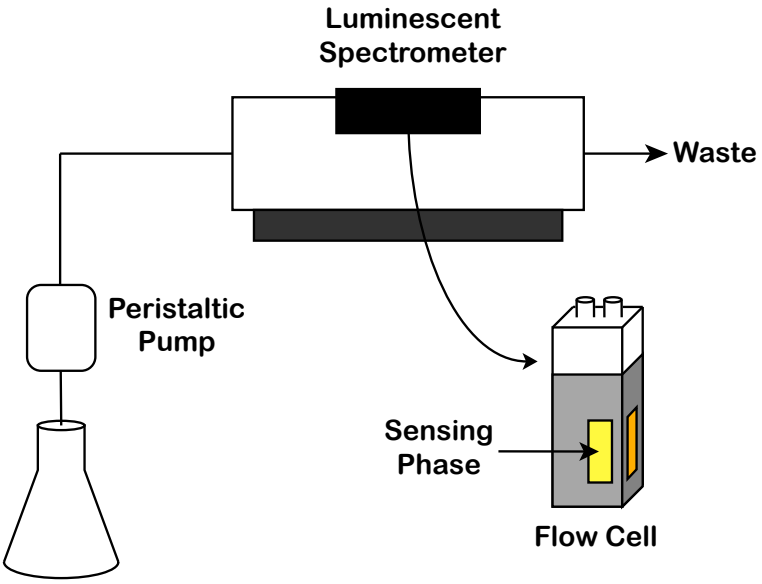


Figure 4.1: Experimental set-up as used with the experiments with sol-gel.

was taken at 0,20 ms.

λ_{ex} : 360 nm	$\lambda_{em,fluo}$: 465 nm
$\lambda_{em,phos}$: 575 nm	t_{gate} : 2 ms
t_{delay} : $0,03 - 0,20$ ms	flash count : 1
$slit_{ex}$: 10 nm	$slit_{em}$: 20 nm

Table 4.1: Spectral characteristics of the sol-gel sensing phase and the experimental settings.

4.2.2 Parameters for the Experiments with Resin

A major challenge with the experiments in sol-gel was the preparation of the standard solutions and their stability. To overcome this problem a flow system was designed, using the different mixtures of a deoxygenated and an oxygen saturated solution. In addition a water-bath was used to maintain a constant temperature of 20 °C, as depicted in Figure 4.2. A small but noticeable influence of the fill level of the flask containing the deoxygenated solution was noted in the first experiments. Therefore an automatic refill system was devised to ensure a constant amount of solution in the flask. Strong interference from iron was noted with the resin, while this was not a problem with the sol-gel. To overcome this problem 100 mg/L of 1,10 phenanthroline were added to the buffer solution.

Using this experimental set-up, all the oxygen concentrations were obtained as percentage

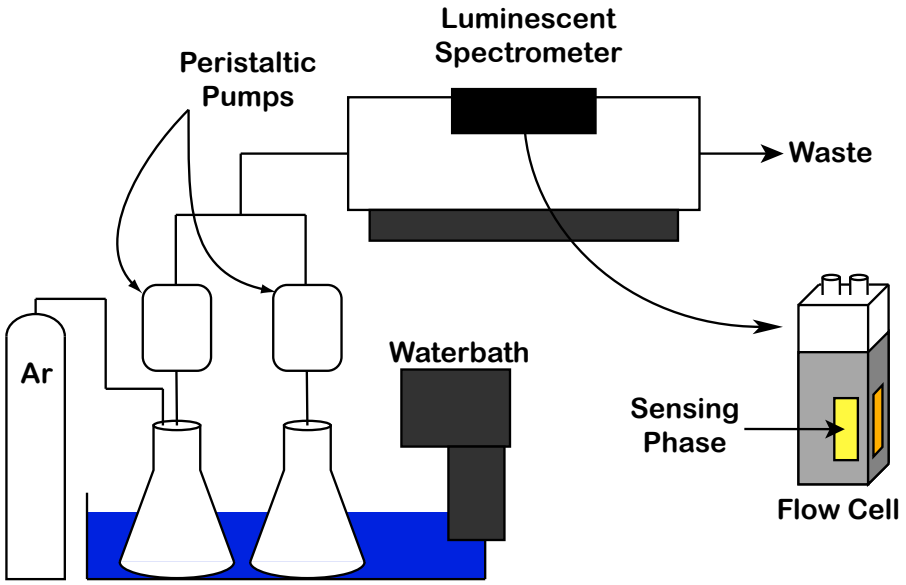


Figure 4.2: Experimental set-up as used with the experiments with resin.

of saturation of the buffer solution with oxygen. Therefore they had to be converted to ppm of dissolved oxygen before use for the calibration graphs. This was done employing the equation devised by Benson and Krause for the solubility of oxygen in saline waters (equation 4.1 [103]).

$$lnCs = I + J/T + K/T^2 + L/T^3 + M/T^4 - S(N + P/T + Q/T^2) \tag{4.1}$$

With:
- Cs ... dissolved oxygen (O_2) solubility concentration [μmol/L]
- T ... temperature [K]
- S ... salinity [g/kg]
- I ... -135,90205
- J ... +1,575701 x 10^5
- K ... -6,642308 x 10^7
- L ... +1,243800 x 10^{10}
- M ... -8,621949 x 10^{11}
- N ... +0,017674
- P ... -10,754
- Q ... +2140,7

The μmol had finally to be converted to mg, as to obtain ppm of dissolved oxygen.

Apart from the wavelengths of excitation and emission, only slight adoptions were made to the instrument settings from the experiments with sol-gel. Intensities for lifetime measurements

were only recorded with delay times ranging from 0,03 ms to 0,10 ms using steps of 0,01 ms. Measurements with longer delay times proofed not to be reliable due to the high contribution of noise. The settings used for the experiments with the resin as a solid support are summarised in Table 4.2.

λ_{ex} : 395 nm	$\lambda_{em,fluo}$: 500 nm
$\lambda_{em,phos}$: 595 nm	t_{gate} : 2 ms
t_{delay} : $0,03 - 0,10$ ms	flash count : 1
$slit_{ex}$: 10 nm	$slit_{em}$: 20 nm
temperature : 20 °C	total flow : 2 ml/min

Table 4.2: Spectral characteristics of the resin sensing phase and the experimental settings.

4.3 Preliminary Experiments

First of all the basis of the proposed measurement principles, the dependence of the phosphorescence and fluorescence emission on oxygen, had to be investigated and clarified. Therefore preliminary tests were conducted in a spectrofluorometer. With these experiments resin and sol-gel sensing phases were employed in gas streams as well as in liquid. It was found that the fluorescence emission is not significantly influenced by the oxygen concentration applied and seemed therefore suitable as an internal standard. The strong influence of oxygen on the room temperature phosphorescence of the used luminescence complex could be shown in experiments in the luminescence spectrometer and had also been established in already published work [59]. The sensing phase seemed therefore suitable for the planned investigations.

4.4 Measurement Methods

For this work three different measurement methods were employed: Intensity measurements, ratiometric measurements using the ration of the phosphorescence emission and the fluorescence intensity and lifetime measurements. As all the experiments used the quenching phenomenon to obtain the analytical information, the Stern-Volmer equation [61] was used for the calibration graphs. Excitation and emission wavelengths were adjusted in each case to coincide with the maximum of the respective peaks.

4.4.1 Intensity and Ratiometric measurements

As the used instrument was designed for intensity measurements neither special precautions had to be taken nor had any adoptions to be made. For ratiometric analysis, total luminescence

spectra of the sensing phase were recorded and data extracted from these spectra was used for calculation.

4.4.2 Lifetime measurements

With this measurement method a workaround had to be found, because the instrument did offer the option to deduce the lifetime directly from the decay curves. Instead intensities at various delay times were recorded, from which the lifetime was later determined by adjusting the data to an exponential decay. Figure 4.3 illustrates the method in an example.

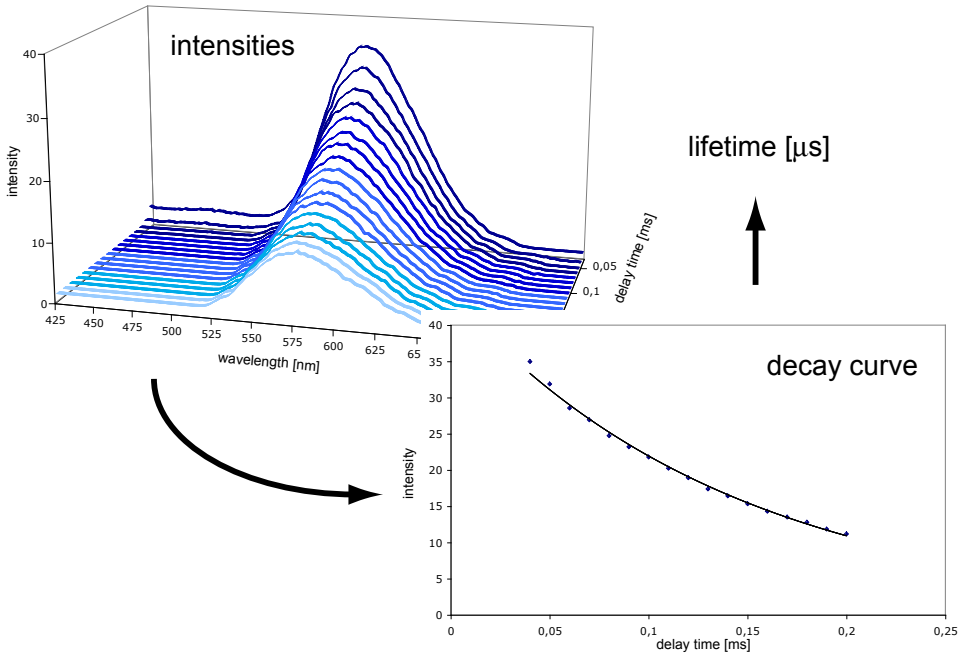


Figure 4.3: Illustration of the lifetime measurement method.

4.5 Optimisation

To obtain the best possible results, several experiment series were conducted, varying instrumental parameters and environmental conditions. It was found that the optimum of most of the settings coincided for sol-gel and resin experiments. An important difference occurred, as was to be expected, with the wavelengths of excitation and emission. These were, in any case, adjusted from day to day anew, so that ideal conditions were assured. Although these preliminary experiments do not bear analytical information as such, they are of certain interest as they show some of the above mentioned principles at work.

4.5.1 Delay-time

First an appropriate delay had to be found for intensity and ratiometric measurements, respectively. In Figure 4.4 the total luminescence spectra for a gate-time of 2 ms and different-delay times in sol-gel are shown. For the case of a delay time of 0,02 ms the spectrum had to be scaled dividing it by a factor of 5 to be able to fit it in the figure in total. As can be seen, the fluorescence is far stronger than the phosphorescence for delay times smaller than 0,03 ms, and overlaps the latter completely. With a delay time of 0,03 ms there was only a small fluorescence peak which allowed to distinguish the phosphorescence peak as well. However, there was a strong overlap between the two peaks which in turn influenced the results considerably. Using resin as a solid support, the findings were basically the same, although less overlap of the peaks was encountered. With a delay-time of 0,04 ms (and above) there was no significant fluorescence, it was therefore thought, that a delay time of 0,03 ms and 0,04 ms for the ratiometric and intensity measurements, respectively, represented the optimal settings.

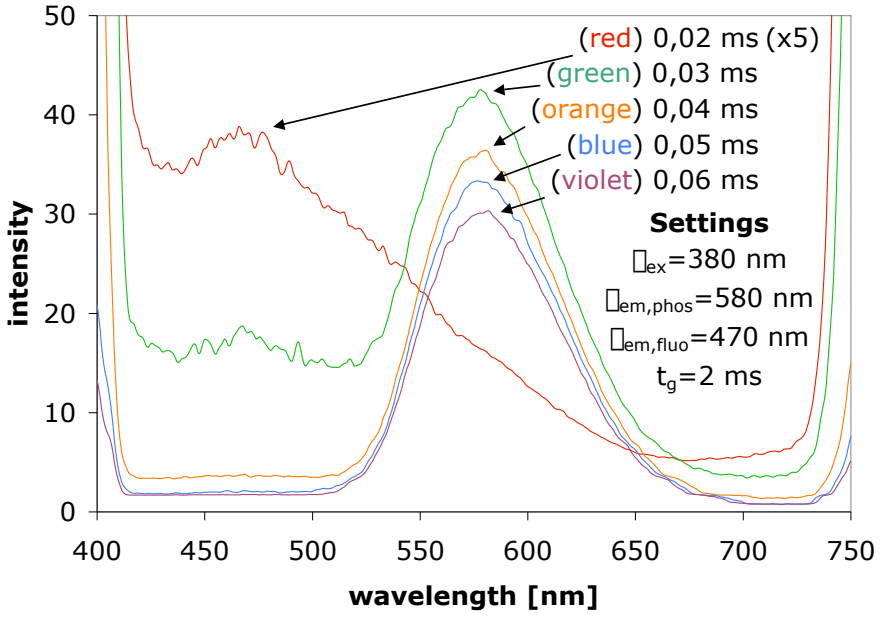


Figure 4.4: Optimisation of the delay-time in a sol-gel matrix. The spectrum for a delay time of 0,02 ms is scaled by dividing it by a factor of 5.

4.5.2 Gate-time

In order to optimise the gate time, spectra at gate times from 1 ms to 5 ms were recorded for delay times of 0,03 ms and 0,04 ms, respectively. As can be seen in Figures 4.5 for a delay-time of 0,04 ms, there was little change in intensities for different gate times. However, in prior experiments

the intensities with a gate time of 1 ms appeared to be considerably lower and in literature [59] longer gate-times were just as well found to be the more appropriate setting. Hence, it was decided to use a gate time of 2 ms.

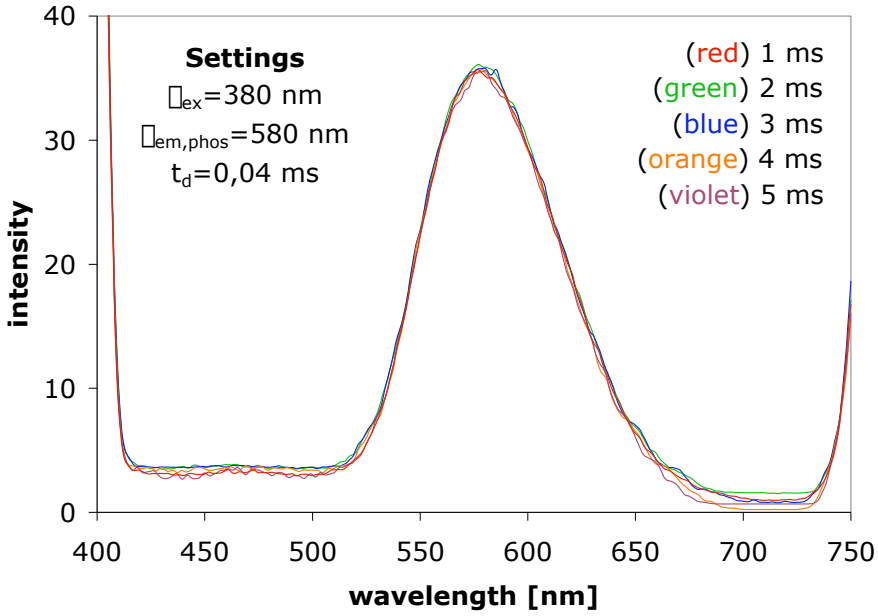


Figure 4.5: Optimisation of the gate-time in a sol-gel matrix.

4.5.3 Averaging

In order to reduce the noise especially in the spectra used for ratiometric analysis, the mean of several runs was calculated. For optimisation mean spectra of different numbers of repetitions were investigated. The procedure is illustrated in Figure 4.6 for ratiometric measurement. As can be seen, there is no significant improvement above a certain number of spectra used for averaging. Therefore it was decided to use 7 and 3 spectra for the mean for ratiometric and intensity measurements, respectively, in sol-gel. With the measurements on resin three spectra were recorded in each case.

4.5.4 Scan Speed

For both, ratiometric and intensity measurements, experiments at different scan speeds were recorded to investigate the increase of noise with augmentation of the velocity of the scan. As shown in Figure 4.7 for the experiments with sol-gel, the spectra at a scan speed of 400 nm/min showed slightly lower noise levels, while at higher scan speed the distortion of the peaks hardly

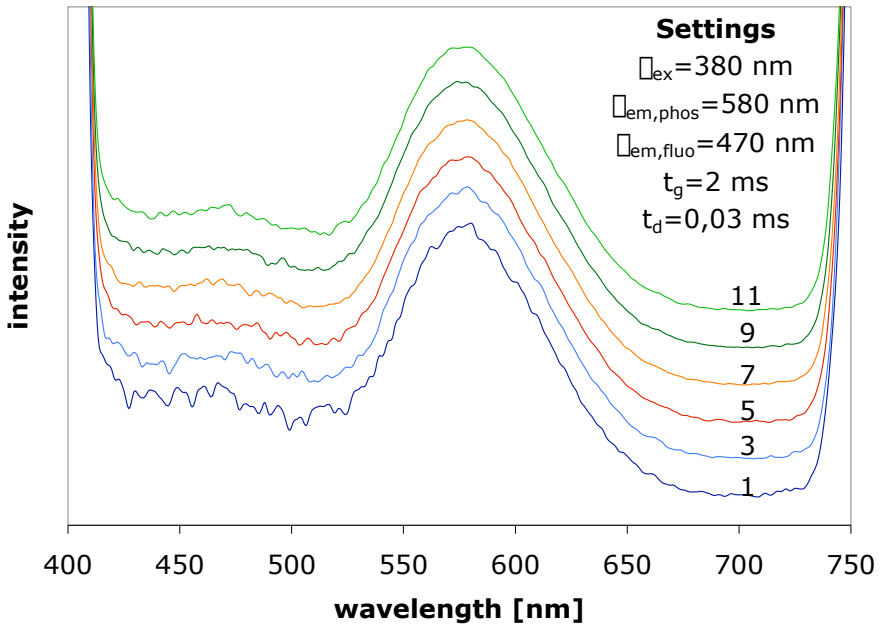


Figure 4.6: Optimisation of the number of spectra used for averaging in a sol-gel matrix. Spectra are staggered for clarity.

varied. 800 nm/min were therefore chosen as scan speed for the experiments in sol-gel, representing a compromise between velocity and quality of the spectra. As before with the number of spectra used for averaging, the settings were slightly changed for the experiments with the resin and 600 nm/min were used.

4.5.5 Smoothing

With the spectra of the ratiometric measurements a lot of noise distorting the fluorescence peak was encountered. This was expected, since due to the delay time of 0,03 ms, only the last part of the decay curve of the fluorescence emission was recorded. Hence, it was necessary to apply a smoothing to the spectra. The program used offered four different smoothing algorithms, namely moving average, triangular, quadratic Golay-Savitzky and cubic Golay-Savitzky. In all cases, it was possible to select the number of points used. The triangular algorithm yielded the best results and was therefore applied to the recorded data (Figure 4.8). In case of the sol-gel experiments 20 points were used, while for data obtained with the resin 15 points seemed to suffice, due to better overall quality of the spectra.

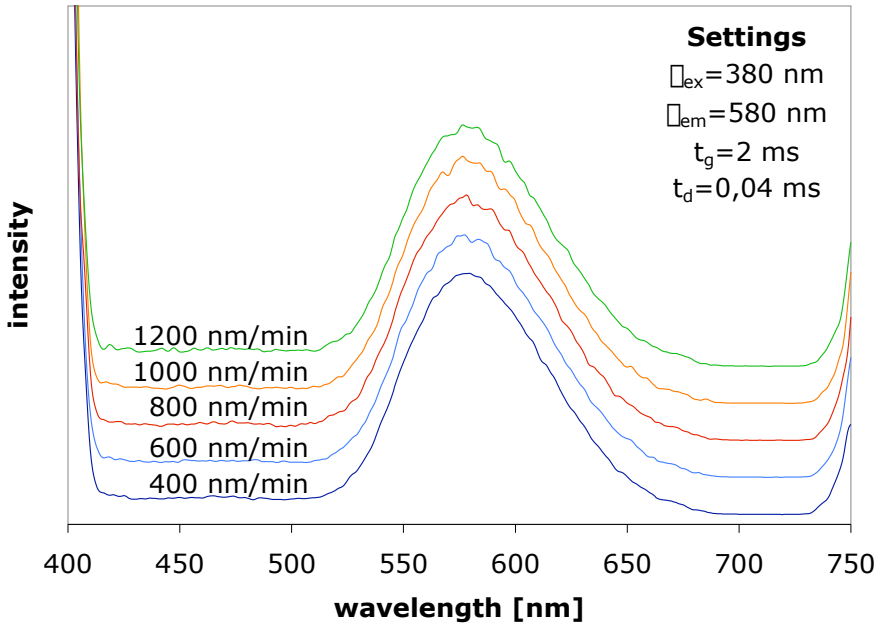


Figure 4.7: Optimisation of the scan speed in a sol-gel matrix. Spectra are staggered for clarity.

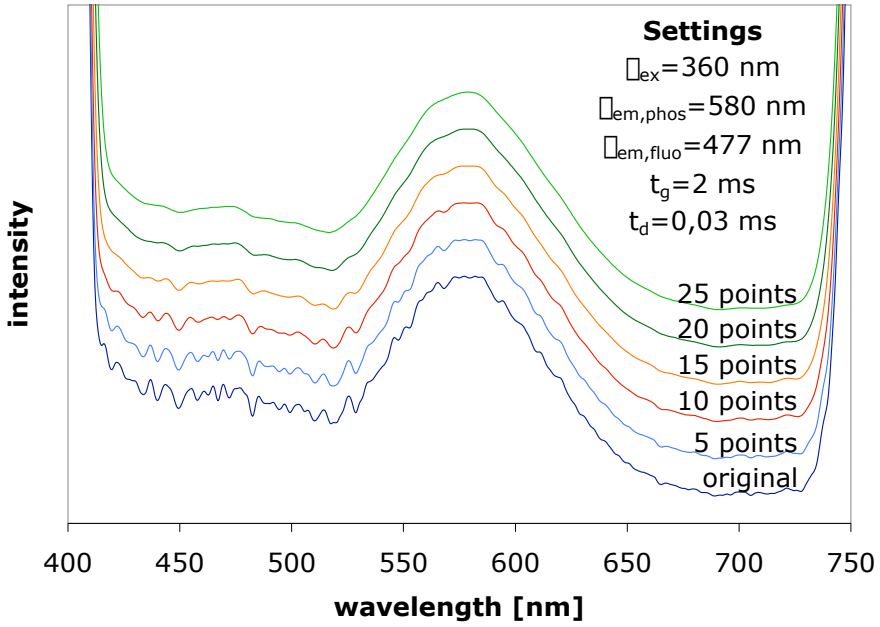


Figure 4.8: Optimisation of the smoothing using spectra obtained with a sol-gel matrix. Spectra are staggered for clarity.

4.5.6 Temperature

Partly due to the experimental set-up, there was no temperature control used in the experiments with sol-gel. The samples and all solutions were prepared and used at room temperature. With the resin, however, a thermostated water-bath was used for maintaining at a constant temperature and hence a series of experiments at different temperatures could be conducted to evaluate the influence of the temperature and provide a foundation to choose a suitable setting for the experiments.

In Table 4.3 the results for the ratiometric measurement method are summarised. With higher temperature the phosphorescence intensity diminished. The fluorescence was influenced as well, but not proportionally, so that the ratio changed. Also the relative standard deviation of the signal changed somewhat with temperature. Based on this results, 20° C were chosen as a compromise between signal intensity and noise.

	15° C	20° C	30° C	40° C	50° C
I_{RTP}/I_F	2,22	2,04	1,91	1,73	1,57
RSD [%]	2,14	1,87	2,04	3,35	0,70

Table 4.3: Data obtained for ratiometric measurement at different temperatures using resin.

4.5.7 Flow rate

Due to the experimental set-up, optimisation of the flow was only applicable to the experiments using the resin. Again various experiments were made using different flow rates. Figure 4.9 shows the spectra recorded for ratiometric analyses. As only changes in the phosphorescence peak can be seen, it is supposed that up to a flow rate of 2 ml/min a considerable re-oxygenation of the solution occurred.

4.5.8 Slits

Variation of the setting for the slit width exhibited a maximum of obtained intensity using 5 nm and 20 nm as excitation and emission slit width, respectively. Figure 4.10 shows the spectra obtained for ratiometric measurements using the resin. The reason why 10 nm and 20 nm were chosen as the respective excitation and emission slit width for the experiments can be found in the augmentation of noise with the setting that produced the highest signal. This is especially apparent in the spectra for ratiometric measurements.

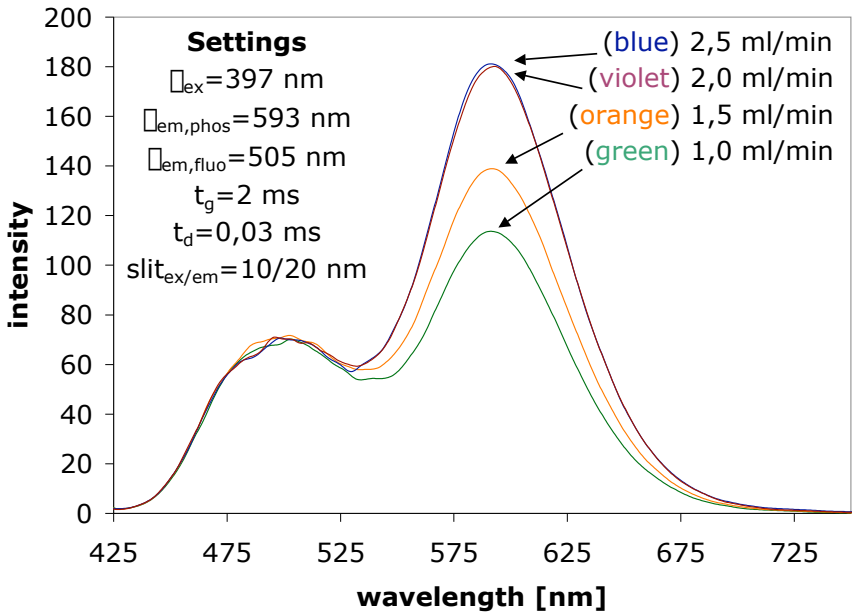


Figure 4.9: Optimisation of the flow rate using a resin as solid support.

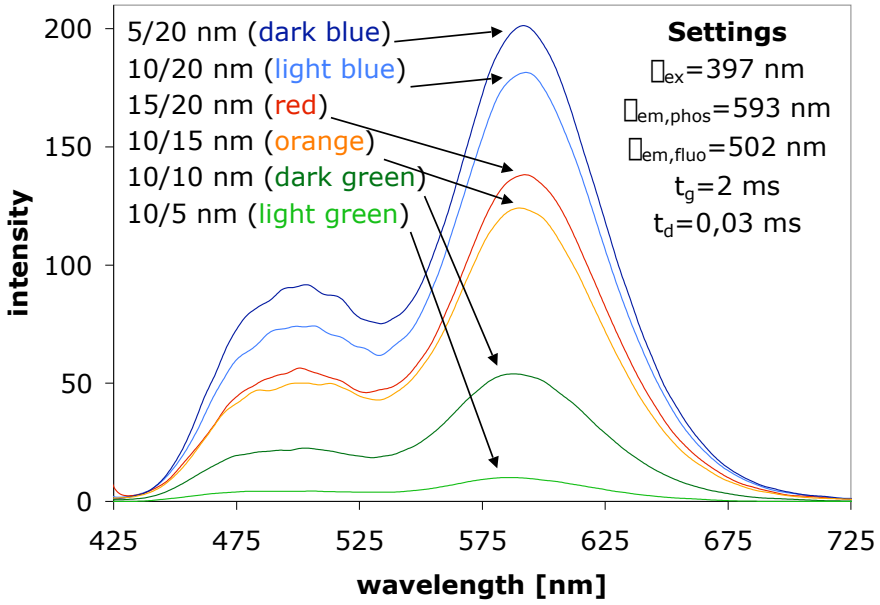


Figure 4.10: Optimisation of the slit width using a resin as solid support. The numbers refer to the excitation and emission slit width, respectively.

4.5.9 Other parameters

Some of the settings possible to vary with the instrument, like the flash count or the cycle time, showed little or no effect on the signal and were therefore left with their default values.

4.6 Results of the Experiments with Sol-Gel

From publications [59] of prior work with the sol-gel in question, the latter seemed highly appropriate for the task at hand and was therefore chosen as the first attempts to establish a calibration for the three different measurement methods.

Although the system appeared stable before measurements were begun, after recording all the data and spectra needed for a calibration of all three measurement methods, i.e. after one day working with the sol-gel, it was, in the majority of the cases, not possible to recover the same signal intensity as in the beginning. Leaving the sensing phase for days purged with buffer solution, yielded higher stability, but the luminescence intensity dropped to a level where only data for a very limited range of concentration of dissolved oxygen could be recorded or even no sensible measurements were possible at all.

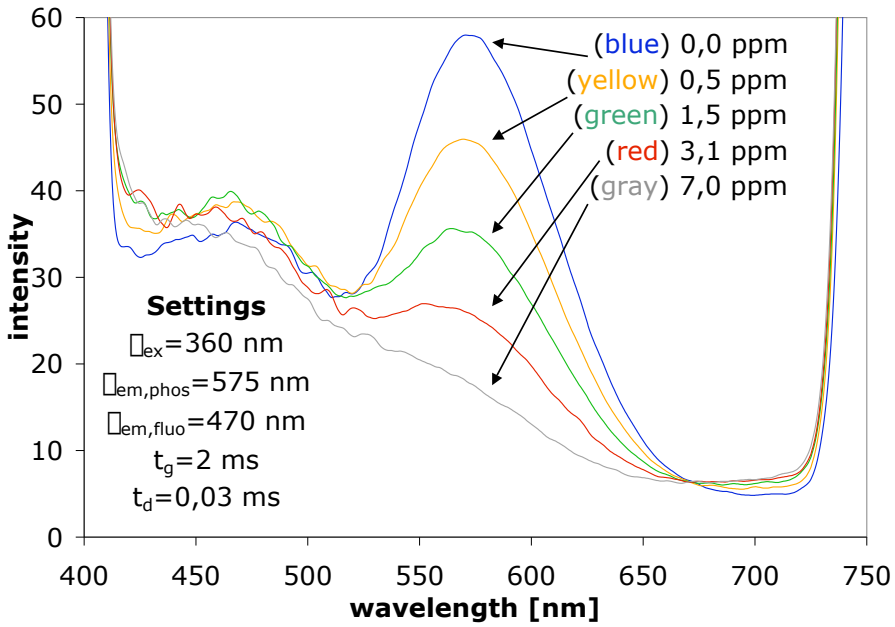


Figure 4.11: Spectra obtained with different concentrations of dissolved oxygen using the sol-gel sensing phase.

Exemplary spectra obtained with different concentrations of dissolved oxygen using the de-

scribed system are depicted in Figure 4.11. Immediately apparent are the slight changes in the fluorescence, which appear not to be correlated to the oxygen concentration. The fluorescence peak was, in general, not very clear to see, due to overlap from what was thought to be a peak originating from scattered light from the excitation source at shorter wavelengths and the phosphorescence peak at longer wavelengths. Apart from the interference in signal intensity, these contributions from overlaps lead to a distortion of the peak shape that rendered it difficult to clearly pinpoint the maximum. In fact it seemed that the maximum even shifted slightly towards shorter wavelengths with continuing use of the sensing phase. Moreover, a relative high base line was observed that, combined with the low signal intensity, limited the range of concentration for which useful data could be obtained. Data from these experiments could therefore not be used for calibrations or sample measurements.

Reasons for the untypical characteristics of this well-studied sensing phase were thought to be encountered in problems with the preparation (e.g. old reagents or bad temperature regulation during drying). As the main focus of these experiments were not the further studies of the indication molecule or its immobilisation, but rather the comparison of different measurement techniques, the sensing phase was exchanged for Al-ferron immobilised on a resin.

4.7 Results of the Experiments with Resin

As an alternative to sol-gel as a solid support, a resin was tested as well. The first immediately apparent advantage of the latter is a considerably reduced preparation time (a few hours compared to two weeks with sol-gel). Signal intensities were also augmented considerable with this sensing phase. Using the experiences from the first part of this work, the experimental set-up was modified to provide better stability of the samples during measurements.

As can be seen in the exemplary spectra for the ratiometric measurements with the resin (Figure 4.13), the base line was considerably lower for these experiments than with the sol-gel. At the same time the signal intensities were higher and the overlap of the peaks was negligible. Just as well, the very intense emissions attributed to scattered light with the prior experiments, were shifted so far away from the peaks of interest that the fluorescence and the phosphorescence peaks could be obtained decently separated and with little distortion.

As mentioned above, the resin yielded a considerably augmented signal level as compared to the sol-gel. Even with the bare eye the much more intense yellow colour of the sensing phase, originating from the Al-ferron complex, could be noted. These findings suggested a larger quantity of the luminescent complex being immobilised on the resin than in the sol-gel, yielding higher phosphorescence and fluorescence intensities. The lifetimes in turn were much shorter. These findings might find an explanation in the different immobilisation methods. The sol-gel presents a much more rigid environment, which enhances the phosphorescence lifetime. At

the same time the wavelengths of excitation and emission were shifted as can be seen in Table 4.4. Although this also relates to the different solid supports, the reasons for that phenomenon are rather related to the different chemical situation the indicator molecule encounters itself in. While in sol-gel the indicator molecule is thought to be merely entrapped, in the resin a bond is formed between the Al-ferron and the solid support.

	Sol-Gel	Resin
λ_{ex}	360 nm	395 nm
$\lambda_{em,fluo}$	465 nm	500 nm
$\lambda_{em,phos}$	575 nm	595 nm
lifetime$_{phos}$	300 μs	70 μs

Table 4.4: Comparison of the spectral characteristics of the sol-gel and the resin sensing phase.

As the emission intensities were high and since lifetime measurements relied on phosphorescence intensities with the experiments at hand, calibrations could be obtained for all measurement methods up to saturation level of buffer solution with oxygen. With the resin sensing phase five calibrations on different days were made. In addition, on two of these days samples were prepared as described above for the experiments with sol-gel and measured after the corresponding calibration. Exemplarily the spectra of the third day (Figures 4.12 and 4.13) are depicted here. Just as well, decay curves for selected oxygen concentrations are shown in Figure 4.14

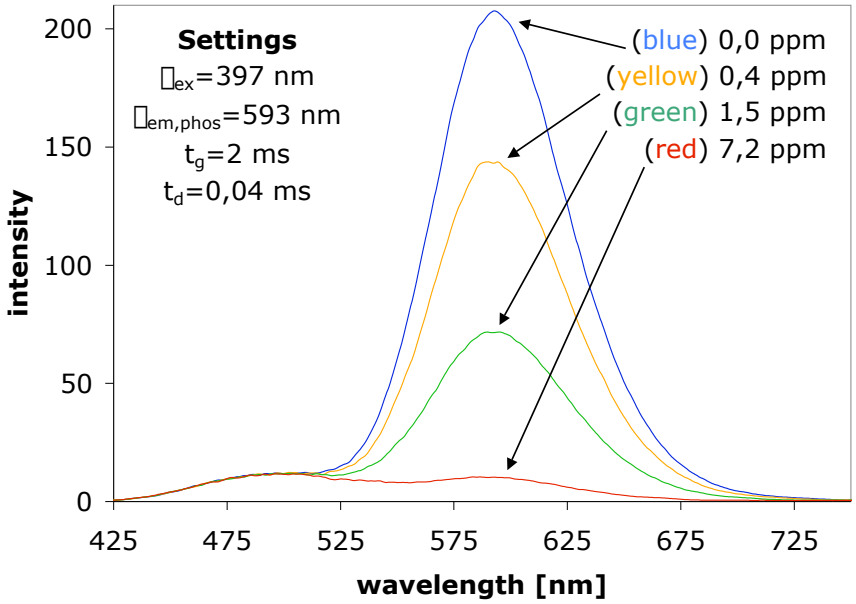


Figure 4.12: Spectra used for the calibration with intensity measurements.

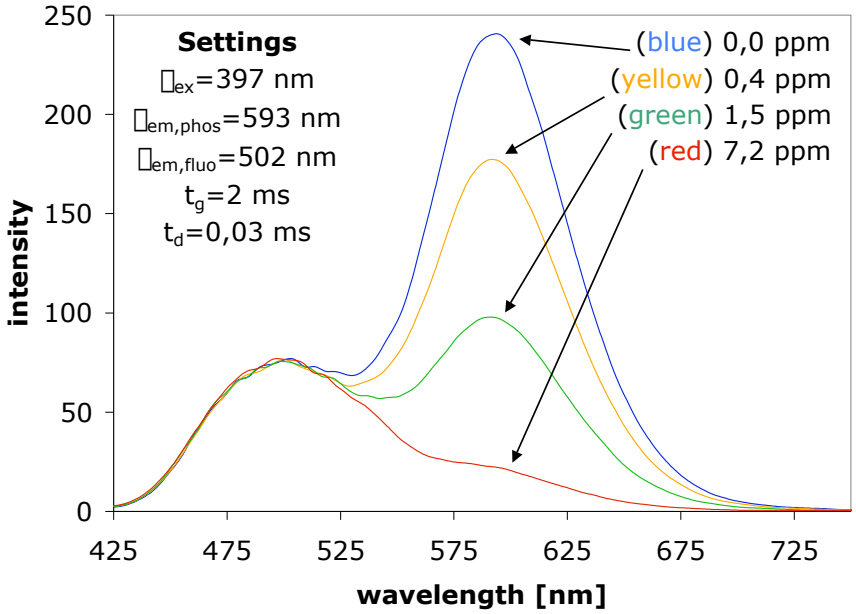


Figure 4.13: Spectra used for the calibration with ratiometric measurements.

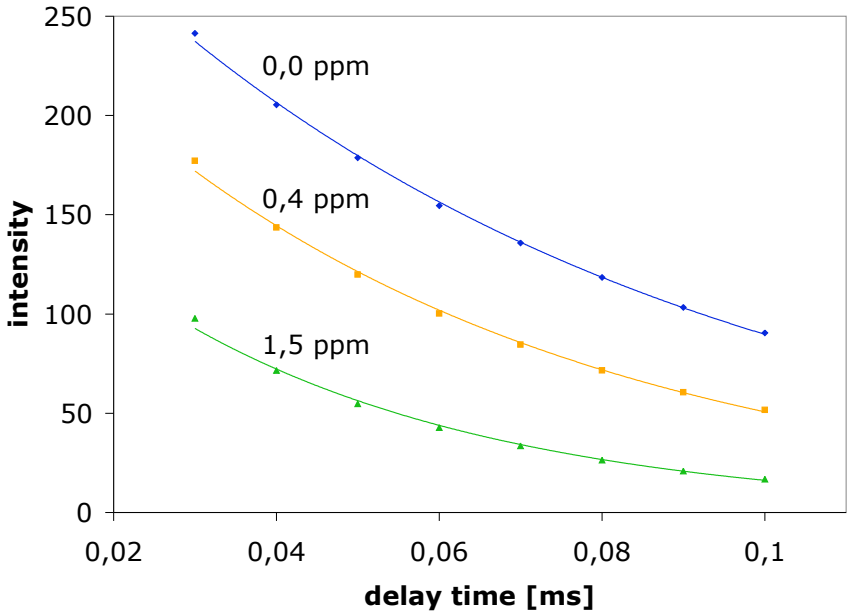


Figure 4.14: Decay curves used for the determination of the lifetimes.

Drawing a graph of τ_0/τ versus the concentration of oxygen yielded again a straight line. For intensity and ratiometric measurements, however, a positive deviation from linearity was found. In order to still be able to work with the data, another model for explaining the findings had to be found. For this reason several modifications of the Stern-Volmer equation described in literature were reviewed.

One of the most common approaches is the consideration of an in-homogeneous environment. Many materials do not possess only one uniform kind of binding sites, but various distinct options, which result in slightly different interactions with the luminescent species or distinct accessibility of the analyte to the immobilised agent [104]. To allow for this fact, the so called "multi-site" models were developed, which are generally based on a superposition of independent Stern-Volmer plots (as described by equation 4.2), corresponding to the different sites [105].

$$\frac{I_0}{I} = \left(\sum_i \frac{f_{i0}}{1 + K_{SVi}[Q]} \right)^{-1} \tag{4.2}$$

With:
I_0 ... intensity in absence of the quencher
I ... intensity in presence of the quencher
f_{i0} ... fraction of contribution of the i-th component
K_{SVi} ... Stern-Volmer constant for the i-th component
$[Q]$ ... concentration of the quencher

From this equation it is clear that for positive Stern-Volmer constants always negative deviations from linearity will occur. Usually considering two different sites suffices to fit the experimental data to the model, but also multitudes of sites have been investigated using for example Guassian distributions for description [106].

Even if the solid support is homogenous, the luminescence decay curves are often not simple exponential decays, but again a combination of the various components yielding a multi-exponential decay [107]. This, however, is ascribed to other phenomena and summarised in the model of micro-in-homogenous environments. Bases of the latter is the inclusion of temporal fluctuations of the system into the approach. If these variations occur on a time-scale slower than the excited state decay, a superposition of various exponential decays will be obtained [105]. As phosphorescence lifetimes are comparatively long, this should be less a concern with the experiments at hand.

Another approach to fit positive deviation involves the consideration of static and dynamic quenching occurring at the same time. As described elsewhere [108], dynamic and static quenching, respectively, can be described mathematically as shown in equations 4.3. Although these equations were developed for fluorescence, they just as well hold true for phosphorescence and other photophysical or photochemical processes [109].

$$\text{Dynamic Quenching:} \qquad \frac{\Phi_f^0}{\Phi_f} \approx \frac{P_f^0}{P_f} = 1 + k_d \tau_f^0 C_q = 1 + K_d C_q \tag{4.3a}$$

$$\text{Static Quenching:} \qquad \frac{\Phi_f^0}{\Phi_f} \approx \frac{P_f^0}{P_f} = 1 + K_s C_q \tag{4.3b}$$

With:
- Φ_f ... quantum efficiency
- P_f ... fluorescence power
- τ_f ... fluorescence lifetime
- C_q ... concentration of the quencher
- k_d ... bimolecular rate constant for dynamic quenching
- K_d ... Stern-Volmer constant
- K_s ... static quenching constant

The subscript f denotes to fluorescence while the superscript 0 refers to cases in absence of a quenching agent.

The two processes can subsequently be combined, yielding a model that considers both dynamic and static quenching occurring at the same time [110].

$$\frac{I_0}{I} = (1 + K_{SV}[Q])(1 + K_{eq}[Q]) \tag{4.4}$$

With:
- I_0 ... intensity in absence of the quencher
- I ... intensity
- K_{SV} ... Stern-Volmer constant
- K_{eq} ... complex formation constant (equilibrium constant of static quenching)
- $[Q]$ ... concentration of the quencher

As lifetimes are not influenced by static quenching, the approach developed so far will yield the same set of equations 4.5 as previously described elsewhere [111, 112].

$$\frac{\tau_0}{\tau} = 1 + K_{SV}[Q] \tag{4.5a}$$

$$K_{SV} = k_2 \tau_0 \tag{4.5b}$$

$$\frac{I_0}{I} = 1 + (K_{SV} + K_{eq})[Q] + K_{SV} K_{eq} [Q]^2 \tag{4.5c}$$

With:
τ_0 ... lifetime in absence of the quencher
τ ... lifetime of the excited state
I_0 ... intensity in absence of the quencher
I ... photoluminescence intensity
K_{SV} ... Stern-Volmer constant
k_2 ... bimolecular quenching constant
K_{eq} ... complex formation constant (equilibrium constant of static quenching)
$[Q]$ ... concentration of the quencher

Hence, purely dynamic quenching (i.e. $K_{eq} = 0$) would be indicated by linear and coinciding graphs of I_0/I and τ_0/τ. Ratios of I_0/I larger than τ_0/τ and the resulting upward curved plots are, on the other hand, indicators that static quenching might also occur [111, 112]. Even so, a positive deviation from the Stern-Volmer relation on its own is no proof for static quenching to take place as for example with the sphere of action model the same qualitative finding is predicted. In the latter model, no non-luminescent complex is formed by the quencher and the indicator, but, due to high concentrations of the quencher, the two reaction partners encounter themselves in such a proximity that deactivation occurs almost instantaneously. A possibility to distinguish between the two processes is provided by the mathematical description for the active sphere model for one part (equation 4.6 [113]) and static quenching for the other (equation 4.3b).

$$\frac{\Phi_{F0}}{\Phi_F} = \exp\left(\frac{[Q]N_A V_m}{1000}\right) \tag{4.6}$$

With:
Φ_{F0} ... fluorescence quantum yield in absence of the quencher
Φ_F ... fluorescence quantum yield at a quencher concentration of [Q]
$[Q]$ ... concentration of the quencher
N_A ... Avogadro constant
V_m ... molecular sphere of action volume

Hence, dividing the ratio of luminescent quantum yield with and without quencher by the corresponding ratio of the lifetimes and thus eliminating the effect of dynamic quenching, should yield a straight line in case of static quenching being the second process accountable for quenching (equation 4.7a) and an exponential curve for active sphere effects contributing (equation 4.7b) [113].

$$\frac{\Phi_{F0}/\Phi_F}{\tau_{F0}/\tau_F} = 1 + K_A[Q] \tag{4.7a}$$

$$\frac{\Phi_{F0}/\Phi_F}{\tau_{F0}/\tau_F} = \exp(N_Q V_m) \tag{4.7b}$$

With: Φ_{F0} ... fluorescence quantum yield in absence of the quencher
Φ_F ... fluorescence quantum yield at a quencher concentration of [Q]
τ_{F0} ... lifetime of the excited state in absence of the quencher
τ_F ... lifetime of the triplet state at a quencher concentration of [Q]
K_A ... equilibrium constant for static quenching
$[Q]$ ... concentration of the quencher
N_Q ... number density of quenching centres
V_m ... molecular sphere of action volume

For all data obtained, a graph of $(\Phi_{F0}/\Phi_F)/(\tau_{F0}/\tau_F)-1$ was drawn, one of which is exemplarily shown in Figure 4.15 for the data of the forth day. The linearity of these plots indicates the contribution of static quenching rather than the applicability of the active sphere model.

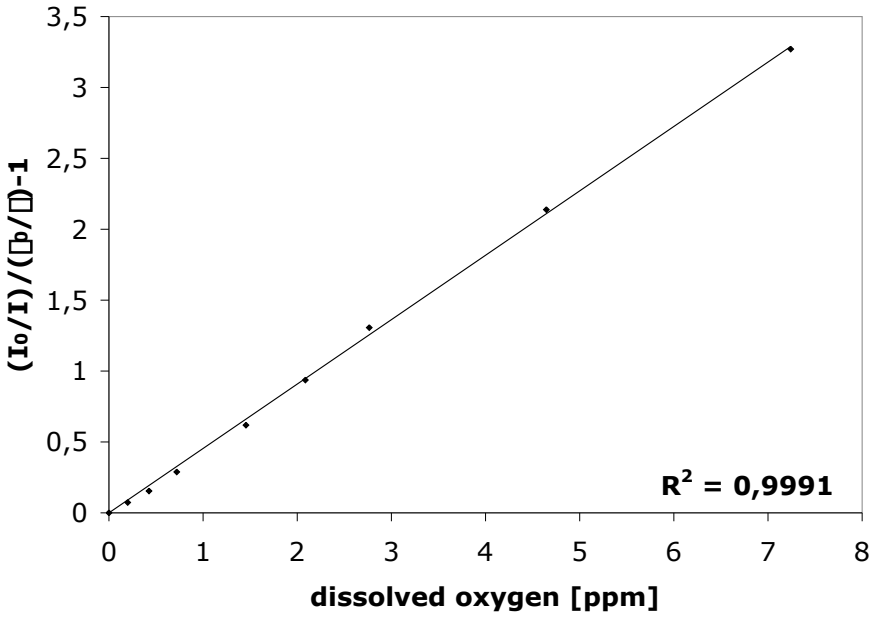


Figure 4.15: Intensity ratio over lifetime ratio versus quencher concentration using the data of the forth day.

The good accordance of the experimentally obtained data with the theoretically derived functions as well as the attribution of differences in the ratios of lifetimes and intensity measurements to static quenching in previous work using virtually the same sensing phase [86] are indicators that a model introducing dynamic and static quenching at the same time might be appropriate to describe the processes with the sensing phase at hand.

Examples of the calibration graphs obtained from the fourth day are shown in Figures 4.16, 4.17 and 4.18. The resulting calibration functions are summarised in Table 4.5

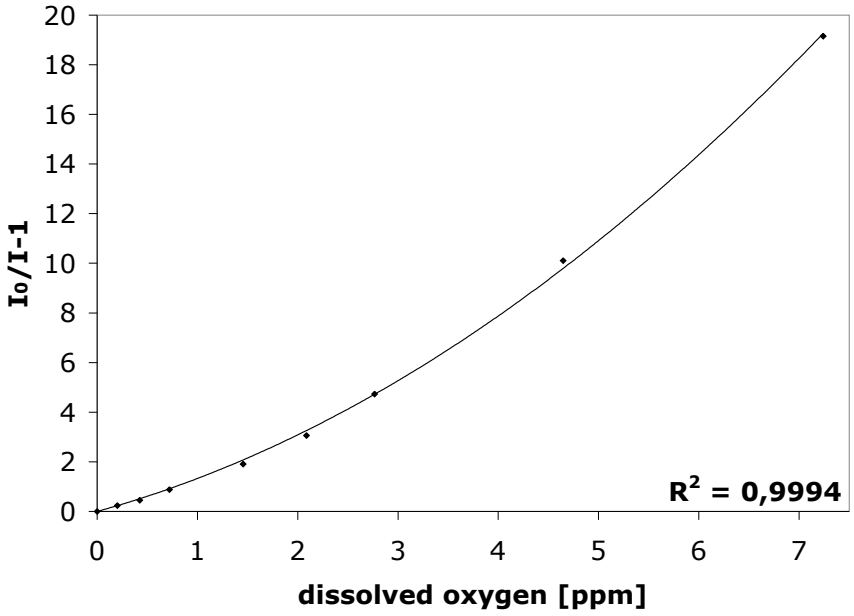


Figure 4.16: Calibration obtained for intensity measurements on day 4.

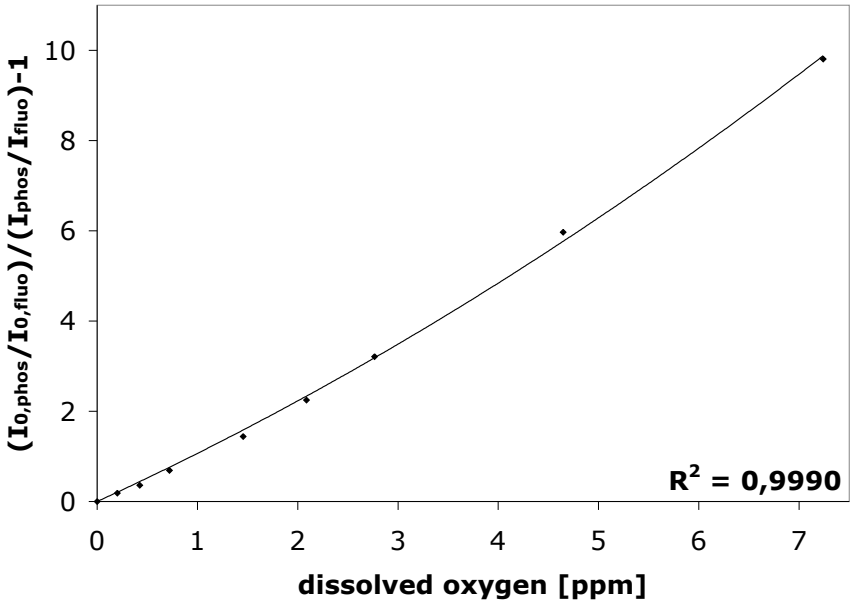


Figure 4.17: Calibration obtained for ratiometric measurements on day 4.

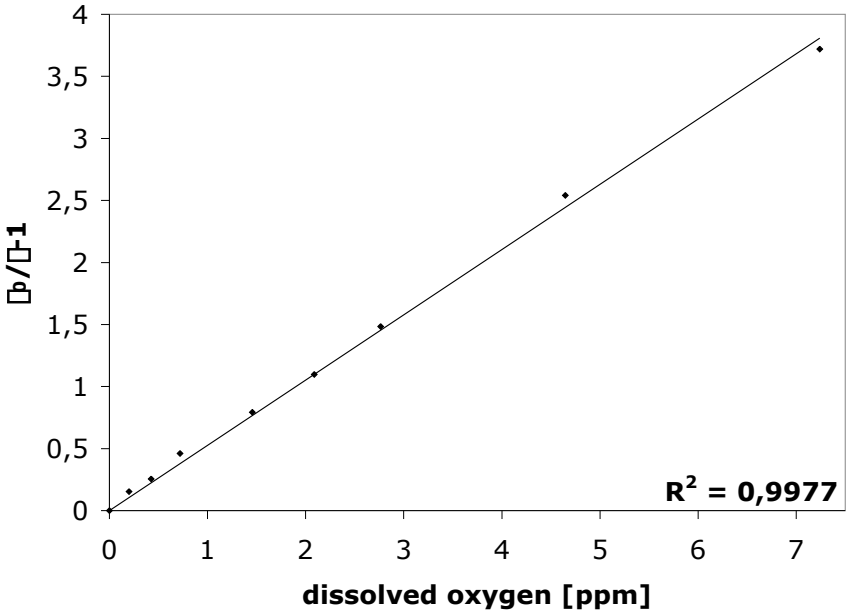


Figure 4.18: Calibration obtained for lifetime measurements on day 4.

	Intensity Measurements	Ratiometric Measurements	Lifetime Measurements
Day 1	$y = 0,154x^2 + 0,729x$	$y = 0,0457x^2 + 0,677x$	$y = 0,405x$
Day 2	$y = 0,178x^2 + 0,594x$	$y = 0,0594x^2 + 0,698x$	$y = 0,429x$
Day 3	$y = 0,155x^2 + 0,828x$	$y = 0,0495x^2 + 0,784x$	$y = 0,423x$
Day 4	$y = 0,213x^2 + 1,117x$	$y = 0,0476x^2 + 1,020x$	$y = 0,526x$
Day 5	$y = 0,177x^2 + 0,962x$	$y = 0,0506x^2 + 0,824x$	$y = 0,435x$

Table 4.5: Obtained calibration functions for experiments in resin.

Having established the calibration functions for the distinct measurement approaches, the limit of detection, using five replicas, could be calculated. Furthermore, interest was taken in the repeatability, wherefore eight non-consecutive measurements for a certain concentration level were taken. The results are, together with the other figures of merit, shown in Table 4.9.

Finalising the inter-comparison of the different measurement methods, various samples were run on different days. The obtained readings for the different samples were applied to all the calibrations, as if they had been measured on that day (i.e. the values of I_0, $I_{phos,0}/I_{fluo,0}$ and τ_0 together with the corresponding calibration functions were used on all the samples). The results are summarised in Table 4.6 for intensity measurements and in Table 4.7 and 4.8 for ratiometric and lifetime measurements, respectively. The oxygen levels are given ppm of dissolved oxygen in buffer solution.

Intensity Measurements	Sample 1 Value [ppm] (Error [%])	Sample 2 Value [ppm] (Error [%])	Sample 3 Value [ppm] (Error [%])
True Value	2,01 ± 0,01	1,21 ± 0,01	1,83 ± 0,01
Day 1	2,22 ± 0,04 (9)	1,55 ± 0,03 (22)	2,15 ± 0,10 (15)
Day 2	3,18 ± 0,05 (37)	2,41 ± 0,03 (50)	3,10 ± 0,11 (41)
Day 3	*1,94 ± 0,04 (4)*	1,32 ± 0,03 (8)	1,88 ± 0,09 (3)
Day 4	1,93 ± 0,04 (4)	1,37 ± 0,02 (12)	1,88 ± 0,08 (3)
Day 5	1,79 ± 0,04 (12)	*1,22 ± 0,02 (1)*	*1,73 ± 0,08 (5)*

Table 4.6: Results obtained for the samples with intensity measurements. Values in italic refer to measurements conducted on the same day as the corresponding calibration.

Ratiometric Measurements	Sample 1 Value [ppm] (Error [%])	Sample 2 Value [ppm] (Error [%])	Sample 3 Value [ppm] (Error [%])
True Value	1,40 ± 0,01	0,73 ± 0,01	1,83 ± 0,01
Day 1	1,48 ± 0,07 (5)	0,69 ± 0,12 (5)	1,86 ± 0,30 (2)
Day 2	1,58 ± 0,07 (11)	0,80 ± 0,12 (9)	1,95 ± 0,29 (6)
Day 3	*1,34 ± 0,06 (5)*	0,63 ± 0,10 (15)	1,68 ± 0,27 (8)
Day 4	1,46 ± 0,06 (4)	0,79 ± 0,10 (8)	1,79 ± 0,26 (2)
Day 5	1,45 ± 0,07 (3)	*0,74 ± 0,11 (1)*	*1,81 ± 0,28 (1)*

Table 4.7: Results obtained for the samples with ratiometric measurements. Values in italic refer to measurements conducted on the same day as the corresponding calibration.

In Table 4.9 all the analytical figures of merit for the three different measurement methods, using a resin as solid support, are compiled.

Lifetime Measurements	Sample 1 Value [ppm] (Error [%])	Sample 2 Value [ppm] (Error [%])	Sample 3 Value [ppm] (Error [%])
True Value	1,05 ± 0,01	1,41 ± 0,01	1,21 ± 0,01
Day 1	0,96 ± 0,01 (9)	1,36 ± 0,03 (4)	1,30 ± 0,03 (7)
Day 2	1,15 ± 0,01 (9)	1,55 ± 0,03 (9)	1,49 ± 0,03 (19)
Day 3	*0,99 ± 0,01 (6)*	*1,38 ± 0,03 (2)*	1,32 ± 0,03 (9)
Day 4	1,24 ± 0,01 (16)	1,61 ± 0,03 (12)	1,55 ± 0,03 (22)
Day 5	0,86 ± 0,01 (21)	1,23 ± 0,03 (15)	*1,18 ± 0,03 (3)*

Table 4.8: Results obtained for the samples with lifetime measurements. Values in italic refer to measurements conducted on the same day as the corresponding calibration.

	Intensity Measurements	Ratiometric Measurements	Lifetime Measurements
Calibration Range [ppm]	0,06-7,2	0,30-7,2	0,06-7,2
Limit of Detection [ppm]	0,02	0,10	0,02
Repeatability (RSD) [%]	3	4	1
Measurement time [min]	3	3	7

Table 4.9: Summary of the analytical figures of merit for the different measurement methods using a resin as solid support.

Chapter 5

Conclusions

In Al-ferron immobilised on a strongly anionic exchanger resin a suitable sensing phase was encountered to determine the concentration of dissolved oxygen. Given that the RTP emission is strongly affected by oxygen, intensity and lifetime measurements could be conducted in the same instrument without any changes, but the measurement approach. As the fluorescence emission did not show any affection by oxygen and could therefore be used as an internal standard, ratiometric measurements could additionally be made using the same sensing phase. Hence, the obtained data could be used for a comparison of the different measurement approaches to investigate their suitability for the development of robust luminescence sensors.

Out of the three measurement principles investigated, the most simple equipment is available for intensity measurements. In any case, intensity measurements are known to suffer from many different sources of interference. Also with the experiments at hand it was seen, that accuracy is rather poor and especially when applying the sample values of one days to the calibration of another day large deviations arise. Intensity measurements are generally quick and simple, but for good data to be obtained it seems inevitable to perform a new calibration before each measurement series. This important drawback not only prolongs the time needed for analysis, but may also present major problems as standard solutions might not be readily available.

In terms of precision and repeatability lifetime measurements show supreme analytical properties. This proofs that lifetime measurements are a very powerful technique, but this potential comes at the cost of a more complicated handling and the demand for more sophisticated instrumentation. From the results it can also be seen, that in terms of the overall performance ratiometric measurements present some important advantages. The latter exhibit better accuracy and more robustness, i.e. they are less susceptible to instrumental variations or fluctuations of the experimental conditions with time. In addition the data acquisition is simpler and considerably less time consuming.

For many applications lifetime measurement might, due to its good performance, still be the

method of choice. However, if simplicity of instrumentation and robustness are of major concern, ratiometric analysis based on intensity measurements clearly present advantages. Therefore the latter approach should be especially useful for the construction of simple and compact instrumentation as desired and employed in sensor development.

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