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Dual optical sensor for oxygen and temperature based on the combination of time domain and frequency domain techniques

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ABSTRACT

In measuring specific conditions in the real world, there are many situations where both the oxygen concentration and the temperature have to be determined simultaneously. Here we describe a dual optical sensor for oxygen and temperature that can be adapted for different applications. The measurement principle of this sensor is based on the luminescence decay times of the oxygen-sensitive ruthenium complex tris-4,7-diphenyl-1,10-phenanthroline ruthenium(III) [Rudpp] and the temperature-sensitive europium complex tris(dibenzoylmethane) mono(5-amino-1,10-phenanthroline)europium(III) [Eudatp]. The excitation and emission spectra of the two luminophores overlap significantly and cannot be discriminated in the conventional way using band pass filters or other optical components. However, by applying both the frequency and time domain techniques, we can separate the signals from the individual decay time of the complexes. The europium complex is entrapped in a poly(methyl methacrylate) (PMMA) layer and the ruthenium complex is physically adsorbed on silica gel and incorporated in a silicone layer. The two layers are attached to each other by a double sided silicone based tape. The europium sensing film was found to be temperature-sensitive between 10 and 70 °C and the ruthenium oxygen-sensitive layer can reliably measure between 0 and 21% oxygen.

1. Introduction

Temperature and oxygen are two crucial parameters in numerous areas of science and engineering [1]. In highly controlled experiments, one can keep either the oxygen or temperature constant or both. But in real world situations, this is often not possible and the changing oxygen or temperature conditions will affect the oxygen or temperature readings and other parameters that are being measured. Thus, there is always a need for sensors that measure both oxygen and temperature.

Oxygen as the omnipresent oxidant on earth is essential for most life forms but detrimental at too high or too low a concentration. Hence, monitoring oxygen and maintaining it at an optimum level is essential to biotechnology, medicine, chemical and pharmaceutical industries and environmental science [2]. In fermentation processes, for instance, oxygen determines the growth and in some cases the quality and quantity of the desired cell product. In medicine, the measurement of oxygen in tissues is important in critical care (e.g. in intensive care units), for the optimization of cancer therapy and for aiding in the diagnosis and treatment of vascular disease [3,4]. In food, chemical and pharmaceutical industries, oxygen may be employed as a necessary ingredient for chemical synthesis but can also be an unwanted cause of degeneration and deterioration of food products and drugs. In environmental science, the amount of dissolved oxygen in bodies of water is a crucial indicator of the health of the water ecosystem [5].

The standard method to measure oxygen to date is the Clark electrode. However, this type of probe exhibits slow response times, small spatial resolution, interference with strong electromagnetic fields and is limited to short distance measurements [6]. Thus, many researchers requiring long distance measurements (e.g. maritime measurements in deep water) are motivated to look for alternatives. Among potential approaches, fluorescence based sensing technology has proven to overcome many of the mentioned shortcomings of the Clark electrode. Certain fluorescent organometallic complexes of ruthenium, platinum, palladium and iridium show strong oxygen sensitivity. Their fluorescence emission and decay time decrease when exposed to oxygen in a concentration dependent manner according to the Stern–Volmer equation. These fluorophores can be used in solutions or embedded in a polymer matrix. There is no spatial limitation and thus can be integrated in a wide range of systems from microfluidic devices up to building-size objects. In fact, oxygen sensitive films have been employed in the pressure analysis of planes [7]. More-

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over, fluorescence based oxygen sensors have been employed for deep sea applications [8]. The one drawback that needs to be addressed in fluorescence based oxygen sensors is their sensitivity to changes in temperature. Hence, when these oxygen sensors are used, it is crucial to either keep the temperature constant or to determine the temperature and compensate accordingly for temperature changes. In recent years, a variety of luminescence based temperature probes has been developed. In general, there are two types of luminophores being employed for temperature measurements; inorganic phosphors and organic luminophores. Alexandrite, an example of an inorganic phosphor, is found to be sensitive between 15 and 45 °C where its phosphorescence decay time decreases from 300 to 220 μs [9]. However, alexandrite crystals in powder form lose much of their luminescence, thus, cannot be incorporated in sensor films. Lanthanide phosphors such as La_2O_2S:Eu are also responsive to temperature over a wide temperature range. The decay time of this phosphor decreases over an order of magnitude as the temperature increases from 0 to 100 °C. Organic fluorescent dyes in general are temperature sensitive. Organic compounds and metal ligand complexes used specifically for temperature measurements include rhodamine B, ruthenium(II) tris-(1,10-phenanthroline) (ruphen), ruthenium(II) tris-bipyridyl (rubpy) and various β-diketonate-europium(III) complexes. Rhodamine B has been successfully used for temperature measurement in microfluidic systems as the bright fluorescence emission is strongly affected by temperature within the range of 0 and 100 °C. Rhodamine has a decay time of 4 ns at room temperature. On the other hand, rubpy and β-diketonate-europium(III) complexes have long decay times in the microsecond to millisecond range that are highly temperature dependent [10–12]. Since the decay time, τ, is independent of fluorescence intensity changes (e.g. caused by photobleaching or leaching), lifetime-based measurements are a more reliable method to determine the temperature. Nonetheless, for a short-lived dye like rhodamine B, the lifetime changes in the nanosecond range would require expensive, highly sophisticated instrumentation. Thus, the long lived dye complexes of ruthenium and europium are more amenable for the development of low-cost sensors using simpler, low-cost optoelectronics [13].

Much effort has been applied to developing dual sensors able to measure both oxygen and temperature simultaneously [11,14–17]. Wolfbeis et al. developed a fiberoptical dual sensor for oxygen and temperature based on ruphen as the temperature sensitive probe and platinum(II)–5,10,15,20-tetra-kis(2,3,4,5,6-pentafluorphenyl)porphyrin (Pt–PFPP) as the oxygen sensing material [11]. Both dyes are incorporated in a PMMA film. The sensor measures the decay times of the two dyes using the frequency domain (phase modulation) method. In order to discriminate the fluorescence of the dyes the signal from the sensing layer is split into two beams which are then measured by two distinct photodetectors. The weaknesses of this system are the splitting of the beam which reduces sensitivity and the necessity for two photodetectors and two band pass filters which increase the complexity of the system. Baleizao, et al., on the other hand, utilized C_{70} fullerenes and ruphen embedded in two separate polymer layers and stacked on top of each other [18]. The detection method for this dual sensor is based on the time domain approach. The fluorescence of each dye is isolated by optical band pass filters. Since one photodetector (CCD camera) is employed, the measurement of temperature and oxygen has to be carried out sequentially and the band pass filter has to be changed accordingly.

In this paper, we discuss an approach for dual measurement of oxygen and temperature without the drawbacks of current methods mentioned above. We achieve this by combining both time and frequency domain methods and exploiting the advantages of each technique. This approach allows for the development of a low cost lifetime based dual sensor for oxygen and temperature.

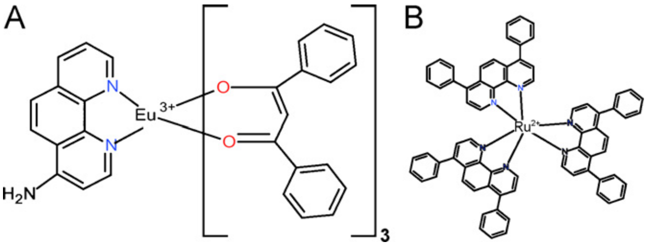

Fig. 1. Molecular structure of tris(dibenzoylmethane) mono(5-amino-1,10-phenanthroline) europium(III), (Eutdap) and tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II), (Rudpp).

2. Experimental

2.1. Materials

Tris(dibenzoylmethane) mono(5-amino-1,10-phenanthroline)europium(lll) [Eutdap] (molecular structure shown in Fig. 1), chloroform was purchased from Sigma–Aldrich (www.sigma.com), PMMA and microscope glass slides were bought from Thermo Scientific (www.thermo.com). The oxygen sensing membranes containing the oxygen sensitive tris-4,7-diphenyl-1,10-phenanthroline ruthenium(III) [Rudpp] (Fig. 1) were purchased from Fluorometrix Inc. (www.fluorometrix.com). The molecular structures of Eutdap and Rudpp are illustrated in Fig. 1.

Preparation of the dual sensing layer. 1 g of PMMA is dissolved in 4 mL chloroform at room temperature. 5 mg of Eutdap is added to the PMMA/chloroform solution and stirred until the europium compound is completely dissolved. 500 μL of this solution is spread quickly on a dry microscope glass slide which has been rinsed with acetone for several times. The slide is then spin-coated for 10 s at 2500 rpm. The prepared slide is allowed to dry for 1 h at 50 °C under vacuum. The PMMA film on the glass slide is estimated to be about 20 μm thick. The ready to use oxygen sensitive probe is then attached onto the temperature probe. It is important to note that the oxygen probe consists of a silicone layer incorporating the oxygen sensitive Rudpp, a 0.127 mm thick Mylar support and a double-sided adhesive layer on the bottom. As the oxygen probe is attached onto the temperature probe, the Mylar support and the adhesive film provide additional protective oxygen barriers for the temperature probe. The prepared dual sensing probe is shown in Fig. 2.

2.2. Instrumentation

For the decay time measurement of Eutdap the fluorimeter Eclipse (Varian, www.varianinc.com) with integrated temperature controlled cuvette holder is employed. The settings of the fluorimeter for decay time measurement are as follows: delay time = 6 μs, gate time = 20 ms, excitation = 400 nm, emission = 613 nm, collection time = 1 ms, number of measurements for 1 averaged decay time curve = 50, excitation slit = 5 nm, emission slit = 5 nm, PMT

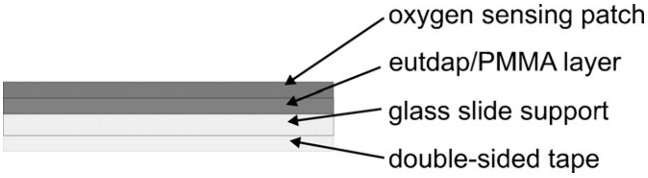

Fig. 2. The dual sensing probe consists of a polyethylene support, a temperature sensing PMMA layer with embedded Eutdap and on top the silicone layer with embedded oxygen sensing Rudpp. For attachment of this sensing patch a double-sided tape is used.

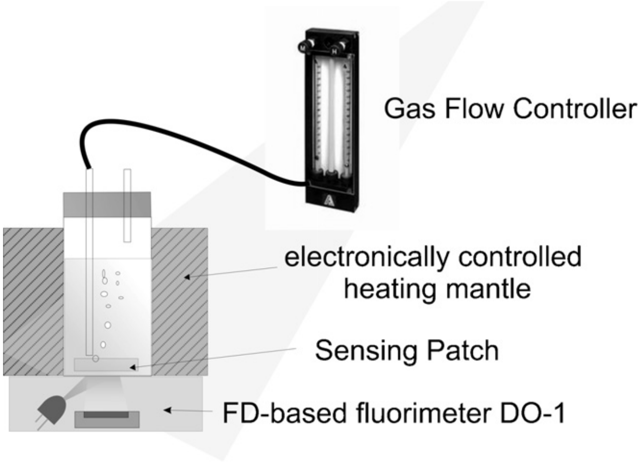

Fig. 3. Experimental setup for the measurement of oxygen. The temperature/oxygen sensing patch is attached on the bottom of the mini-bioreactor, which is incorporated in the HTBR system. The reactor is filled with deionized water. The oxygen concentration is adjusted by the gas flow meter. The luminescence is measured by the integrated fluorimeter.

control voltage = 700. For the measurement of the decay time of Eutdap at different temperatures the glass slide with the sensing layers is placed diagonally into a 4 mL quartz cuvette filled with distilled water.

For the measurement of oxygen the oxygen probe DO-1 from Fluorometrix is employed. The probe measures the decay time by using the frequency domain technique. The experimental setup is shown in Fig. 3. The dual sensing patch is adhered to the bottom of a mini-bioreactor, which is part of a high throughput bioreactor system (HTBR) developed in our laboratory [19]. The bioreactor is equipped with a gas inlet connected to a volumetric gas flow controller for adjusting the desired oxygen concentrations. The temperature and other parameters in the bioreactor are computer controlled through the HTBR system.

3. Results and discussions

The spectral properties of the oxygen-sensitive, Rudpp and the temperature sensitive, Eutdap are shown in Fig. 4. The excitation spectra (Fig. 4A) show a broad Eutdap excitation mainly in the UV area whereas the Rudpp has an excitation maximum in the blue region at around 480 nm. A strong overlap is apparent between 375 and 425 nm. The emission spectrum of Eutdap is completely eclipsed by that of Rudpp as shown in Fig. 4B. Hence, it is difficult, if not impossible to separate these signals using optical filters. Nonetheless, we show in this paper that the emission signals from the two luminophores can be successfully discriminated from their luminescence lifetimes measured by both time domain and frequency domain methods.

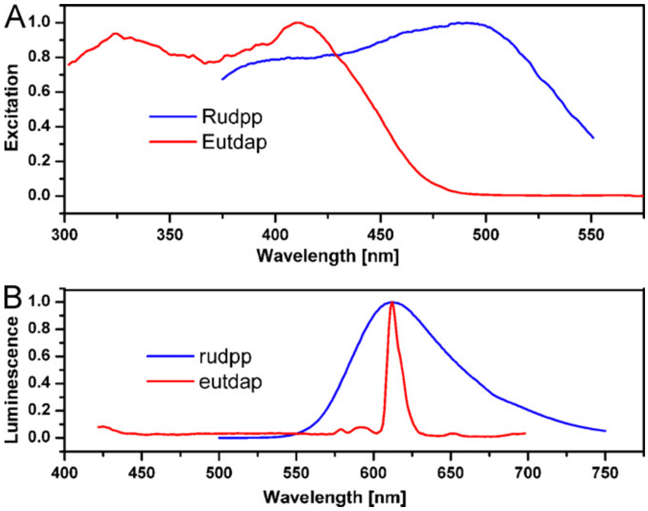

Fig. 4. (A) The excitation spectra of Rudpp and Eutdap. (B) The emission spectra of the two dyes. The excitation spectra of the two dyes partially overlap. However, the emission spectrum of Eutdap is completely eclipsed by the emission of Rudpp.

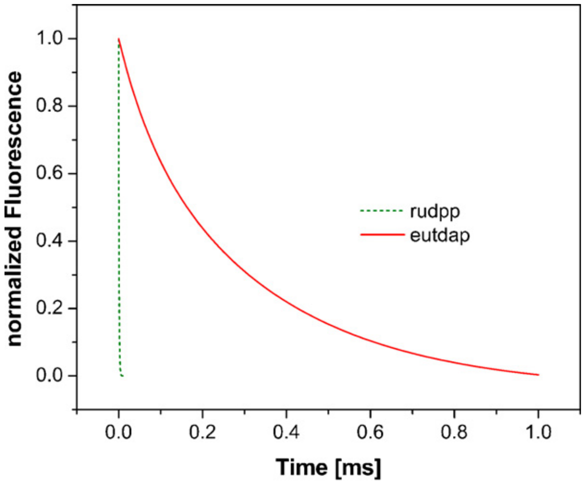

Fig. 5. The theoretical decay curves of Eutdap and Rudpp are shown. The luminescence of Rudpp decays much faster than that of Eutdap. Hence by delaying the measurement until Rudpp completely decays, the decay time of the longer lived Eutdap can be determined without any interference from the ruthenium dye.

In the time domain method, the luminophore is excited with a relatively short pulse of light and the resulting luminescence decay is measured directly. For a single emitting species, this process can be mathematically described by a single exponential function as in Eq. (1) [20].

$$I = I_0 \times e^{-t/\tau} \tag{1}$$

The time domain decay time measurements require a light source with a switch-off time that is much faster than the decay time of the luminophore and a photo detector that is fast enough to measure the decay at sufficient resolution. The main advantage of this method is that the actual measurement is carried out after the excitation light has been switched off. In principle no optical filter is necessary to block excitation light from entering the detector. While measurement of short lifetimes of a few microseconds or less requires very fast and relatively expensive optics, long luminescence lifetimes (hundreds of microseconds or more) can be easily measured by low cost optoelectronics. The luminescence lifetimes of Eutdap and Rudpp are about 280 μs and 1 μs, respectively, at standard temperature and in air. Thus, the decay time of the europium complex can be determined without any interference from the ruthenium complex by applying the gated detection technique. The measurement of the decay process of Eutdap is commenced after a delay period long enough for the Rudpp luminescence to completely decay (Fig. 5). Because the lifetime of Eutdap is almost 300 hundred times longer than the lifetime of Rudpp, this process of discrimination is particularly straightforward. Consequently, temperature measurements from the Eutdap emission are achievable with negligible interference from Rudpp.

To determine oxygen concentrations, the frequency domain method was utilized to discriminate one emission signal from the

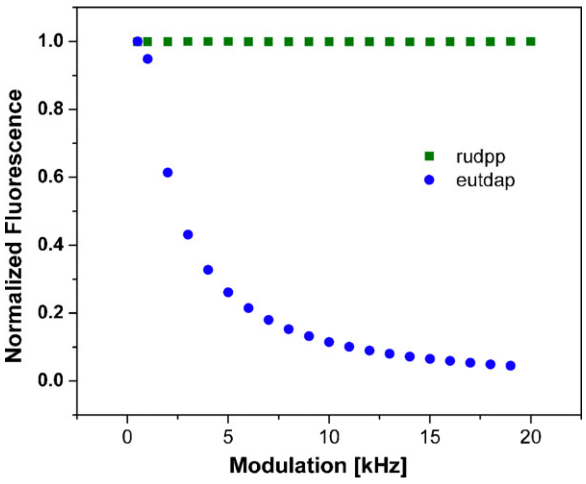

Fig. 6. Modulation frequency sweep. The modulation frequency of excitation light is steadily increased from 500 Hz to 20 KHz. The modulated luminescence of Rudpp and Eutdap is measured at each modulation modulation frequency step.

other. In the frequency domain method, a luminophore with a decay time τ exposed to intensity-modulated light with frequency ω ($\omega = 2\pi \times$ frequency in Hz) will emit modulated light with the same circular frequency ω. However, depending on the lifetime of the luminophore, the modulated luminescence is shifted or delayed in phase by an angle ϕ. In our device, the intensity-modulated light is generated from an LED excitation source. The phase shift ϕ of the resulting emission is related to the decay time τ by the following equation:

$$\tau = \frac{\tan(\phi)}{\omega} \qquad (2)$$

Additionally, the modulated luminescence $f(\omega)$ is inversely proportional to the modulation frequency ω and the lifetime τ of the dye as described in Eq. (3) [20].

$$f(\omega) = \frac{1}{\sqrt{1+\omega^2\tau^2}} \qquad (3)$$

This relationship can be exploited as follows. The oxygen sensitive ruthenium complex (Rudpp) has a luminescence lifetime of less than 1 μs in air and at room temperature. In contrast the temperature sensitive europium complex (Eutdap) has a lifetime of 300 μs under the same conditions. According to Eq. (3), when the modulation frequency ω is increased, the modulated luminescence $f(\omega)$ decreases until it approaches zero. However, this decrease in modulated luminescence $f(\omega)$ for Eutdap will be more drastic than for the Rudpp because it has a much longer lifetime (>300×). Thus, a frequency ω can be reached where for a mixture of Eutdap and Rudpp, only the modulated luminescence of Rudpp is observed. This is the basis for separating the luminescence signal of the short lived dye from the long lived dye. The modulation frequency sweep shown in Fig. 6 confirms this. The experiment reveals that the modulated luminescence of Eutdap decreases drastically and approaches zero (demodulated) asymptotically as the frequency increases. Since the electronic system was very basic, that consists of only a photomultiplier, transimpedance amplifier, a first order passive low pass filter and an external lockin amplifier, the measurement has a high electronic offset. Taking this offset into consideration, one can say that at ≤10 kHz the luminescence of Eutdap is virtually demodulated. In contrast, the modulated luminescence of the ruthenium complex remains practically unchanged within the frequency range of the sweep. Hence, the modulated luminescence of Rudpp can be discriminated with no interference from the Eutdap at the higher frequencies.

To further demonstrate the frequency domain and time domain approaches for discrimination of Rudpp (oxygen) and Eutdap (temperature) signals, we prepared two sensor probes. One probe had a bilayer consisting of the oxygen sensitive Rudpp/silicone layer and the temperature sensitive layer of Eutdap/PMMA as shown in Fig. 1. The other probe has one layer of Rudpp in silicone, that is known to be very permeable to oxygen (permeability 6.95×10^{-15} cm^3 cm cm^{-2} s^{-1} Pa) [21], hence allowing a fast response of the probe.

First, the lifetimes of the dual layer and the Rudpp monolayer sensor probes were measured with the standalone Fluorimeter DO-1 (Fluorometrix, Inc.). This system was manufactured specifically to measure dissolved oxygen (DO) with the Rudpp/silicone oxygen 'patch'. The excited state of Rudpp is strongly quenched when it comes in contact with the oxygen molecule. This dynamic quenching of Rudpp is observed as an increase in the phase angleϕ, a decrease in modulation m, and a decrease in lifetime τ, consistent with Eqs. (2) and (3). The modulation frequency of fluorimeter DO-1 is optimally set at 75 kHz to measure the Rudpp phase shifts (reported as lifetime) associated with changes in oxygen concentrations. Based on Fig. 5, at this frequency Eudtap luminescence should be totally demodulated. In other words, the observed modulated fluorescence should be from Rudpp alone. The results in Fig. 7 for the lifetime data of the single and dual probes at varying oxygen concentrations and constant temperature strongly support this contention. As can be seen, the two curves are virtually identical which proves that only the Rudpp signal was detected while the luminescence signal of Eudtap was virtually filtered out. Hence, the Eudtap has no impact on the oxygen dependent luminescence of Rudpp when using the frequency domain technique under these conditions.

For the second experiment, we demonstrate the signal discrimination of Eutdap from the Rudpp signal in the dual probe using the time domain approach. At constant DO and varying temperatures, the detection is initiated at a delay time of 6 μs after the excitation. Fig. 5 shows that this delay should allow the luminescence of Rudpp to completely decay prior to the start of the actual measurement. As such, the observed decay should be from Eutdap alone with no interference from Rudpp. In Fig. 8, the lifetime data for the dual sensing probe and the Eutdap monolayer probe show that this is indeed the case when plotted against different temperatures. The measured lifetimes range between 150 and 340 μs

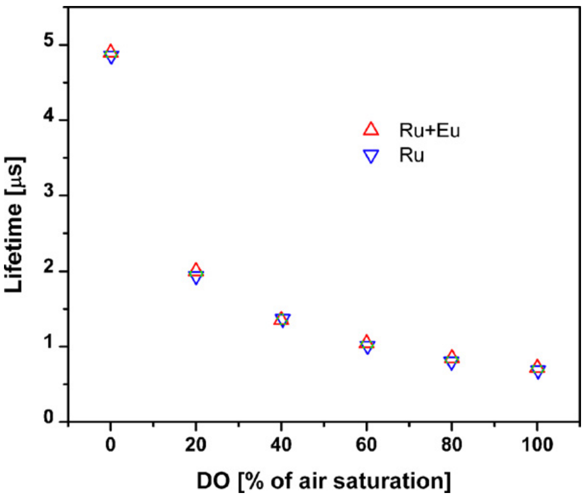

Fig. 7. Responses of the dual sensing probe (Eutdap + Rudpp) and the oxygen sensing probe (Rudpp only) to different oxygen concentrations. The responses of the two probes are virtually identical. The relative error of measurement is lower than one per cent. This proves the modulated luminescence of Eutdap is efficiently filtered out.

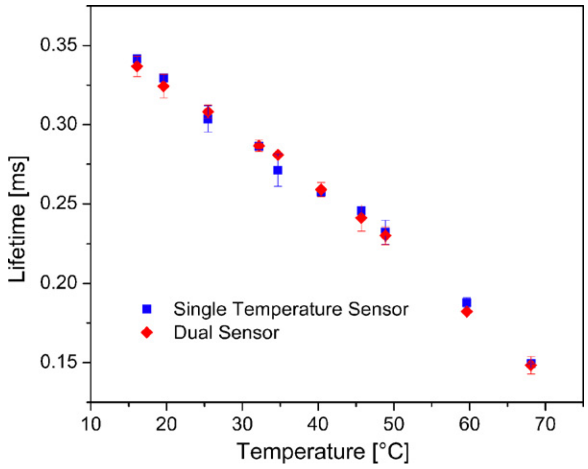

Fig. 8. Response of the dual sensing probe to temperature. Within the range of 16 and 70 °C the temperature dependency can be described by a second order polynomial.

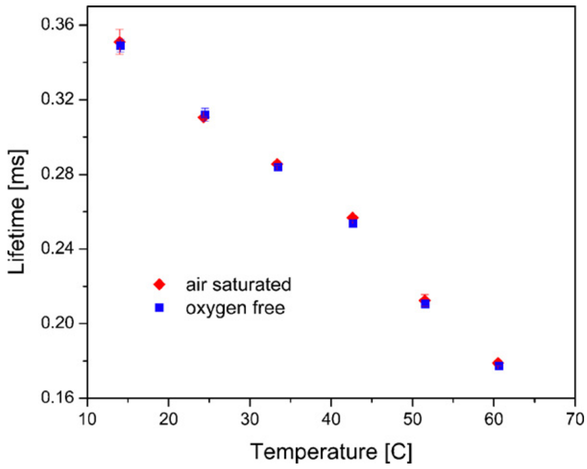

Fig. 9. Responses of the temperature sensing layer in the dual sensing probe in oxygen free and in air-saturated water at different temperatures. The lifetime of the temperature sensing layer in both environments is unchanged at each given temperature. No cross sensitivity to oxygen can be seen.

with a nonlinear temperature dependence between 15 and 70 °C. The statistical analysis (paired *t*-test, $t = 0.485$, $p = 0.639$) confirms that the results obtained by the two probes are the same within the margin of errors. Within this temperature range the correlation between lifetime and temperature can be described by a second order polynomial function $a_0 + a_1x + a_2x^2$ with $a_0 = 0.372$, $a_1 = -0.00188$ and $a_2 = -2.18\text{E}-5$. The adjusted R^2 is 0.9997. Like other europium complexes Eutdap has been found to be slightly quenched by oxygen. Thus, a sufficient oxygen barrier is important to limit cross sensitivity. Polymers with low oxygen permeability have been proven to limit cross sensitivity efficiently. Klimant et al. reported that their temperature probe comprising of a europium complex embedded in a 8 μm thick poly(vinylidene chloride-co-acrylonitrile) showed no cross sensitivity to oxygen [22]. In our experiment we used the cheaper PMMA as the matrix for Eutdap. Although PMMA (permeability $0.065 \times 10^{-13}\,\text{cm}^3\,\text{cm}\,\text{cm}^{-2}\,\text{s}^{-1}\,\text{Pa}$) is about 20-fold more permeable to oxygen than poly(vinylidene chloride-co-acrylonitrile) [21], the greater thickness of our matrix (~20 μm) and the additional double sided adhesive layer and the Mylar support on top of the temperature sensitive layer provided sufficient protection. Measurements in air saturated and in oxygen free environment confirmed no significant difference in response (Fig. 9). The paired *t*-test analysis proved that the results of the two measurements are essentially the same ($t = 1.04878$, $p = 0.31897$).

The advantage of the frequency domain/time domain (FD/TD) approach is that it allows for the optical system to be simplified. Instead of splitting the fluorescent signal into two and isolating the individual signatures from the two beams, our approach collects the full strength of the signal beam. Thus, a beam splitter, dual optical filters and dual photodetectors are not required thereby providing an enormous advantage in terms of costs and miniaturization.

One potential outcome of our results is the upgrading the capability of already successfully commercialized luminescence based sensors for oxygen, which employ ruthenium or platinum complexes, such as the Fluorimeter DO-1 used in our experiments. One drawback of these sensors is the temperature sensitivity of the fluorophores – for any given oxygen concentration the oxygen probe can show different fluorescence intensities and decay times at different temperatures. To enable the sensor to correctly measure the oxygen, temperature compensation is necessary. This process requires knowledge of the actual temperature and the correlation between temperature and the fluorescence intensity or lifetime of the oxygen sensitive dye. With our FD/TD approach we are able to extract all of the required information with the dual sensing probe. Fig. 10 shows the correlation between Rudpp's lifetime, temperature and oxygen using the dual sensing probe. As can be seen, the fluorescence lifetime of Rudpp decreases with increasing temperatures and oxygen concentration. Analysis of the data reveals that the dependency of the Rudpp lifetime to oxygen concentration and temperature can be expressed by the following equation:

$$\ln(\tau) = a + b \times T + c \times [O_2]^{0.5} \qquad (4)$$

where a, b and c are empirical coefficients with values 1.78, -$7.57 \times 10^{-3}\,{}^{\circ}\text{C}^{-1}$ and -4.89×10^{-1}, respectively. The correlation coefficient R^2 is 0.995. From this equation, one can easily determine the temperature-corrected oxygen concentration using the Rudpp oxygen probe.

In conclusion, we showed that the combination of the frequency domain and the time domain techniques is an elegant approach to resolve the fluorescence signals from different luminophores in a mixture. The technique is able to resolve luminescence signals without cumbersome mathematical estimations and expensive optical filters even when the luminescence spectra are completely overlapping. In addition, using established digital signal processing technology, the FD/TD approach can be easily realized in one

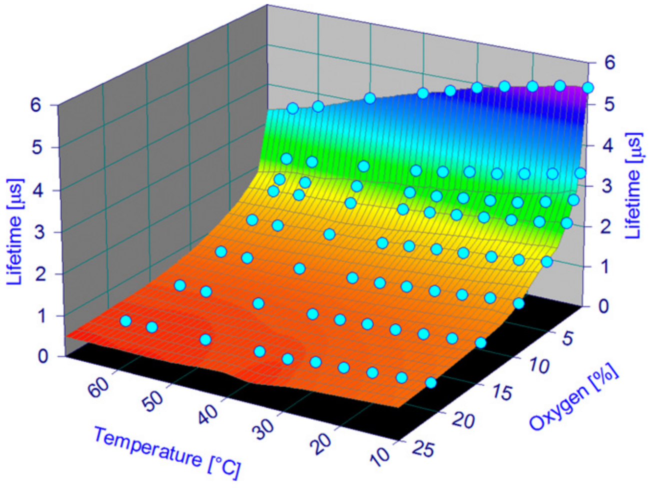

Fig. 10. Temperature and oxygen dependence of Rudpp luminescence. The measurement was carried out in the HTBR reactor with integrated temperature and gas controls.

miniature system comprising only of a photodetector, signal amplifier, analog-to-digital converter (ADC), excitation light source and a microcontroller. It should be noted that, theoretically, the lifetimes of two luminophores such as Rudpp and Eutdap can also discriminated using only the time domain (gated detection) technique [14]. However, this will require very fast and hence expensive electronics. For example, according to the Nyquist theorem [23] in order to measure 10 points of the decay curve with a lifetime of 1 μs, the ADC must be capable of taking 20 million samples/s. Consequently, the microcontroller has to cope with this sampling speed. This can lead to higher power consumption and decreasing the operation time of any battery powered sensor. In contrast, the FD/TD device will not require these fast electronics and should have low power requirements.

Acknowledgements

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