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A NEW METHOD FOR PHOSPHORESCENCE MEASUREMENTS IN THE PRESENCE OF SCATTERED LIGHT

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Abstract – Phosphorescent sensors are usually based on measurement of light intensity or lifetime of sensor emitted light. This goal may be accomplished by measuring the phase-shift between the excitation and the emitted light. Scattered signals must be removed to perform the measurement; however, some of the scattered light due to fluorescence is impossible to remove when measuring phosphorescence decay times.

In this paper, a new method to measure lifetime in the presence of fluorescence light emitted by the phosphorescence sensor is presented. This method is based on the measurement of the phase-shift at two different frequencies. Theoretical aspects, the optimal frequencies and experimental results are discussed.

Keywords Phosphorescence, phase modulation, background fluorescence.

1. INTRODUCTION

In the last years, a lot of luminescent sensors have been developed to measure several interesting substances (oxygen, mercury, polycyclic aromatic hydrocarbons, etc.). Luminescence sensing (fluorescence and phosphorescence) is usually based on the measurement of the decay time of the light emitted by a chemical sensor. One important advantage of this technique is that optical fibers may be used to guide the light [1] [2] allowing examining places which would be otherwise difficult to access.

There are mainly two ways to determine the decay time. One of them consists in exciting the chemical sensor with a light pulse that is short compared to the luminescence decay time and fitting the observed decay with an exponential function [3][4][5]. The other method consists of exciting the sensor using a sinusoidal intensity-modulated light and calculating the decay time by measuring the phase-shift, α , of the emitted light. Assuming that light emitted by the sensor follows a monoexponential function, the relation of the phase-shift and the decay time, τ , is given by next equation [4],

$$\tan(\alpha) = 2\pi f \tau \tag{1}$$

where, f , is the frequency at with the measurement of the phase-shift is performed. Several authors have proposed methods for frequency optimization [6]. The classical

optimal frequency f_{opt} is that where the sensitivity of the decay time reaches its maximum value.

$$f_{opt} = \frac{1}{2\pi\tau} \tag{2}$$

When using practical devices with considerable noise level, the optimal frequency, f_{opt-n} , is [6]:

$$f_{opt-n} = \frac{f_{opt}}{\sqrt{2}} \tag{3}$$

Practical phase shift measurements are also associated with instrumental errors [7], which are included in the measured phase and produce a deviation of calibration points from their real values. Electronic phase error is produced in current to voltage conversion and is added to the phase of luminescence signal.

Measurement of the decay time by measuring phase has been successfully applied in fluorescence measurements [8][9]. However, in phosphorescence measurements, an important amount of the fluorescence emission usually overlaps measured phosphorescence even using optical filters. Although the emission peaks of fluorescence and phosphorescence are different, some of the light due to the fluorescence overlaps with the spectrum of the phosphorescence.

A method to solve the problem of scattered light is presented in [10]. This method is based on the measurement of the amplitude M , and the phase shift, ϕ , at two different frequencies.

In this paper, a new method based on the measurement of only the phase-shift at two frequencies is presented. The choice of the two optimal frequencies to make the calculus is also discussed. Experimental results are obtained by measuring the phosphorescence of Al-Fe in Sol-Gel [9] at different oxygen concentrations.

2. MEASUREMENT METHOD

Fig. 1 shows the vector diagram of the signal at the optical receiver. E_x , is the fluorescence light that overlaps with the phosphorescent emission; this light has no phase shift as respect to the excitation light at the frequencies of interest, because the fluorescence decay time is very small compared to the phosphorescence one. E_m is the light

emitted by the phosphorescent process. This light suffers a phase shift α (1), as respect to the excitation light. Thus, the measured phase-shift, φ , is different to the phase-shift between the excitation and the emitted light, α .

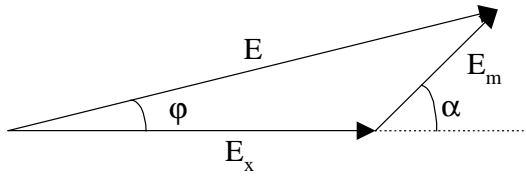


Fig. 1. Vector diagram of the signal.

(E_x : excitation and fluorescent light that arrives to the optical detector, E_m : phosphorescent light emitted by the sensor, α : phase-shift between excitation and emitted light, φ : measured phase-shift, E : measured amplitude).

At a frequency, f_1 , the measured phase shift is given by next equation (Fig. 1):

$$\tan(\varphi_1) = \frac{E_{m1} \sin(\alpha_1)}{E_x + E_{m1} \cos(\alpha_1)} \quad (4)$$

Assuming phosphorescence is a first order process, the light emitted by phosphorescence, is:

$$E_{m1} = \frac{E_{m0}}{\sqrt{1 + (2\pi f_1 \tau)^2}} \quad (5)$$

where E_{m0} is the light emitted by the sensor at 0Hz. If a second measurement of the phase φ is performed at a frequency f_2 , the phase shift is:

$$\tan(\varphi_2) = \frac{E_{m2} \sin(\alpha_2)}{E_x + E_{m2} \cos(\alpha_2)} \quad (6)$$

where E_{m2} is given by:

$$E_{m2} = \frac{E_{m0}}{\sqrt{1 + (2\pi f_2 \tau)^2}} \quad (7)$$

From equation (1),(4),(5),(6) and (7) it is easy to obtain that the decay time, τ , is (when $k \geq 1$):

$$\tau = \frac{1}{4\pi f_1 k (M_1 - M_2 k)} \left[M_1 M_2 (1 - k^2) - \sqrt{M_1 M_2 (k^2 - 1)^2 - 4k^2 (M_1^2 + M_2^2) + 4M_1 M_2 (k + k^3)} \right] \quad (8)$$

where,

$$M_1 = \tan(\varphi_1) \quad (9)$$

$$M_2 = \tan(\varphi_2) \quad (10)$$

$$k = \frac{f_2}{f_1} \quad (11)$$

When $k \leq 1$, the decay time is:

$$\tau = \frac{1}{4\pi f_1 k (M_1 - M_2 k)} \left[M_1 M_2 (1 - k^2) + \sqrt{M_1 M_2 (k^2 - 1)^2 - 4k^2 (M_1^2 + M_2^2) + 4M_1 M_2 (k + k^3)} \right] \quad (12)$$

Moreover, the ratio m between the scattered light and the light emitted by phosphorescence,

$$m = \frac{E_x}{E_{m0}} \quad (13)$$

may be also be obtained from equations (1),(4),(5),(6) and (7) and it is equal to:

$$m = \frac{2\pi f_1 \tau - M_1}{M_1 [1 + (2\pi f_1 \tau)^2]} \quad (14)$$

When fluorescence light is present, the phase shift measured may be relatively small. Thus the optimization of the frequencies at which phase shift measurement should be performed, may be critical.

First optimal frequency, f_1 , to make the measurement of the phase-shift φ_j , is that where the sensitivity of the phase as respect to the decay time reaches its maximum:

$$\frac{d}{df} \left(\frac{d\varphi}{d\tau} \right) = 0 \quad (15)$$

Solving this last equation, the first optimal frequency obtained is:

$$f_1 = \frac{-1 - 2m + \sqrt{1 + 8m + m^2}}{4m\tau\pi} \quad (16)$$

Another solution is:

$$f_1 = \frac{1 + 2m + \sqrt{1 + 8m + m^2}}{4m\tau\pi} \quad (17)$$

However, only equation (16) will be considered along this paper.

The second optimal frequency, f_2 , has been obtained by evaluating the error propagation of an error h in measuring the second phase-shift. The problem consists of finding the frequency where the sensitivity of measured lifetime as respect to the error, is minimum. It may be found solving next equation.

$$\frac{d}{df} \left[\lim_{h \rightarrow 0} \left(\frac{d\tau}{dh} \right) \right] = 0 \quad (18)$$

Results have been obtained by using MathCad 6.0 and are shown in fig. 2. When the ratio between scattered light and phosphorescent light is greater than 2, the second optimal frequency is:

$$f_2 \approx 4.5 \cdot f_1 \quad (m \geq 2) \quad (19)$$

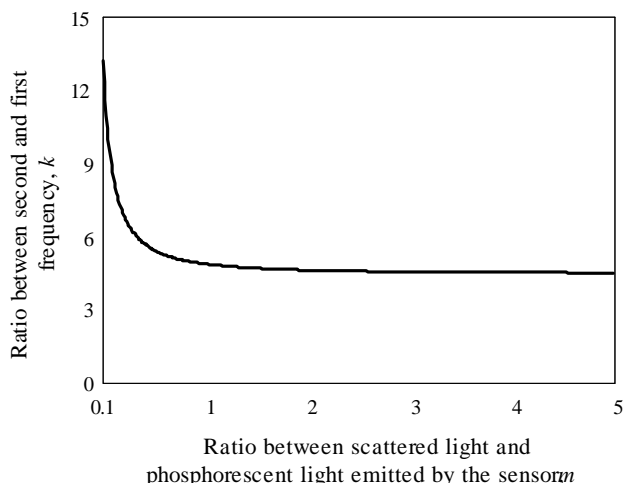


Fig. 2. Ratio between the second and first optimal frequencies in function of the ratio of scattered light.

3. EXPERIMENTAL RESULTS

3.1 Experimental set-up

To evaluate the proposed method, a measurement system was built as can be seen in Figure 3.

Al-Fe in Sol-Gel support [11] is the phosphorescent sensor. Its absorption peak is 380-390 nm. The fluorescence emission peak is 480nm and its decay time is 8 ns (measured with a dye laser ORIEL 79110). The emission peak of the light emitted by phosphorescence is about 590nm and its decay time depends on the oxygen concentration. Maximum decay time is several hundreds of microseconds at 0% of oxygen.

Phosphorescence lifetime and intensity depends on the temperature. Fluorescence intensity also depends on the temperature. All the experimental results were obtained at 20°C by using a cooled incubator Memmert MMICP400.

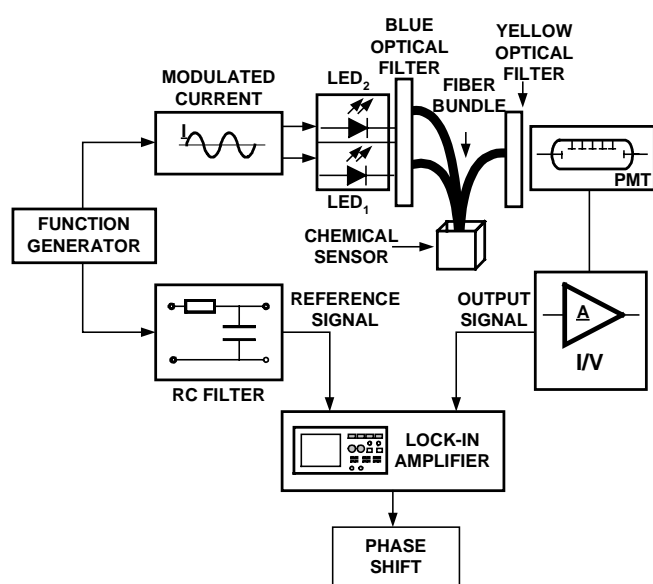


Fig. 3. Block diagram of the experimental set-up.

Intensity modulated light was obtained from ultraviolet LEDs (Nichia NSHU590) that have been excited by using a signal generator (Sony-TEK AFG310). Their peak wavelength is 370nm which may be considered as appropriate to excite the sensor although its absorption peak of the sensor is at a slightly larger wavelength. Anyway the absorption spectrum is wide. Two LEDs have been used in order to increase the light emitted by the sensor. The modulated light emitted by the two diodes and luminescence sensor response were guided by a fiber bundle (Cuda-Quartz 1mm diameter-SMA connector),

The ratio between the phosphorescence signal and the other interference light sources was improved employing two optical filters. A blue additive filter (Edmund A52-530) has been used to eliminate the infrared component of the light emitted by the UV LEDs and a yellow filter (Melles-Griot GG495) has been used to remove the 470 nm fluorescence peak.

The signal emitted by the phosphorescent process has a very low intensity and a poor signal-to-noise ratio. A photomultiplier (Hamamatsu-R928 PMT) was used as photodetector.

Phase delay was measured by using a lock-in amplifier (Stanford Research Systems-SR830 DSP). The signal provided by the signal generator was used as the reference signal. However, delay introduced by electronic circuits is a source of phase shift error in frequency domain lifetime measurement. Current to voltage converter is the circuit that introduces the most significant delay. In order to cancel this delay, lock-in amplifier reference signal was passed through a single-pole low-pass filter.

3.2 Results

Decay time of the phosphorescence light emitted by the sensor was measured at 0% of oxygen by exciting it with a light pulse with the same experimental setup. An argon stream was passed continuously through the sensor to remove oxygen. Fig. 4 shows the emitted light after averaging 512 times. This signal was fitted to a monoexponential function, and the decay time obtained was 496µs.

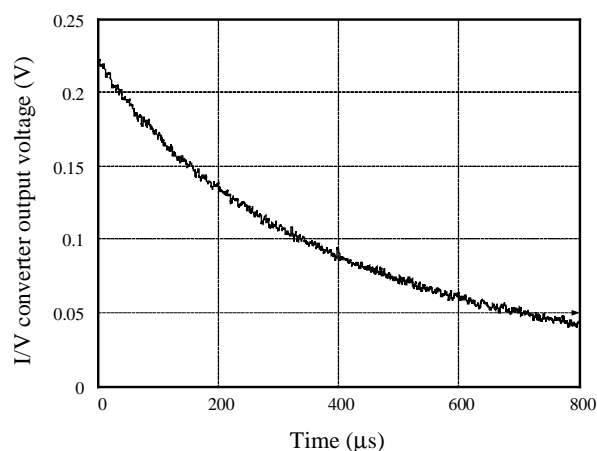


Figure 4. Phosphorescence signal when the chemical sensor is excited with a light pulse (averaging: 512 times).

The ratio between scattered light and phosphorescent light was measured by measuring the light emitted at 0% of oxygen and at 21% (the phosphorescence is completely deactivated at this last oxygen concentration). The ratio m found was equal to 5.3. Thus, first optimal frequency is 145Hz from equation (16). Anyway, a value equal to 150Hz was finally taken. Second optimal frequency is about 675Hz (eq. 19). Fig. 5 shows the expected phase shift plot and the optimal frequencies.

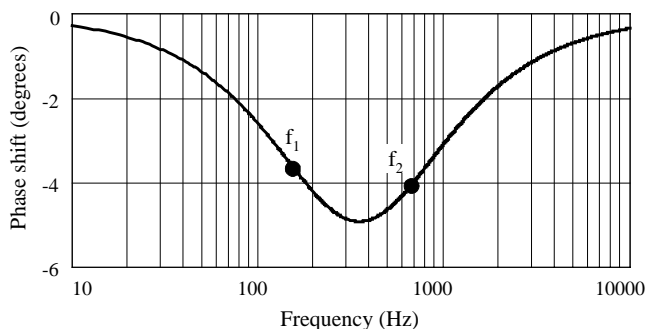


Figure 5. Expected phase shift and optimal frequencies f_1 and f_2 to perform the measurement.

Measuring the phase at the two previous frequencies, the decay time obtained from eq. 8 was 492 μ s. Fig. 6 shows the waveform of the light emitted by the sensor and the reference signal at 675Hz. As can be seen, the noise of the signal is significant; however the lock-in amplifier removes most of the noise.

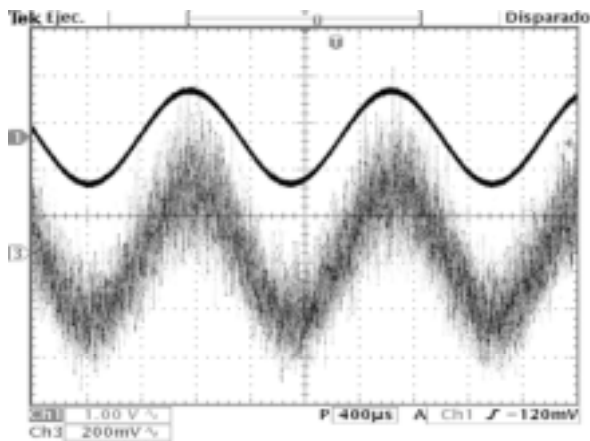


Figure 6. Upper trace: reference signal. Lower trace: AC component of the light emitted by the sensor at an oxygen concentration equal to 0%.

A Stern-Volmer plot was obtained by passing several streams of air+argon through the sensor using an exponential dilutor. Although the decay time varies with the oxygen concentration, all the experiments were performed with $f_1=150$ Hz and $f_2=675$ Hz. Fig. 7 shows the plot. The correlation coefficient is 0.99 which may be considered as relatively good.

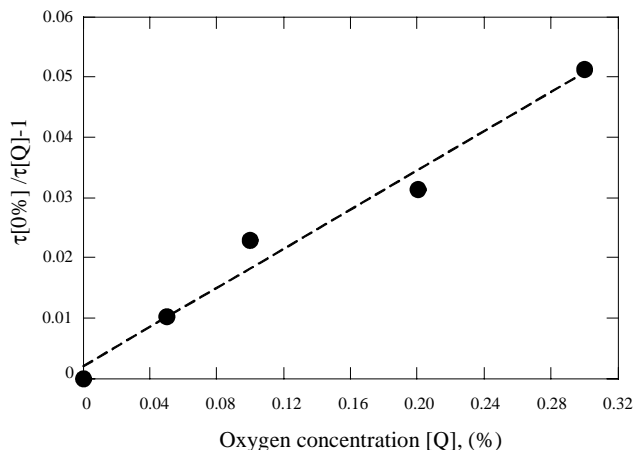


Figure 7. Stern-Volmer plot.

4. CONCLUSIONS

A new method to measure the decay time of luminescent sensor in the presence of scattered light has been presented. This method is suitable for phosphorescence measurements where this scattered light, basically fluorescence background, can be high compared to that of the phosphorescence. Theoretical aspects, best frequencies to make the measurements and experimental results have been discussed.

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