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Chapter 10

Optical Fiber Sensors for Chemical and Biological Measurements

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Introduction to optical fiber sensors

When the light interacts with matter, some effects are produced that do not affect to electron levels of atoms, and consequently, they do not introduce changes in the light wavelength. Thus, the light is reflected, absorbed, scattered, and transmitted with the original wavelength (λ_1). Absorbed light can produce changes over the electron levels of some molecules, causing a new emission of light (luminescence), with larger wavelength (λ_2) than the original. All these phenomena are shown in Figure 1.

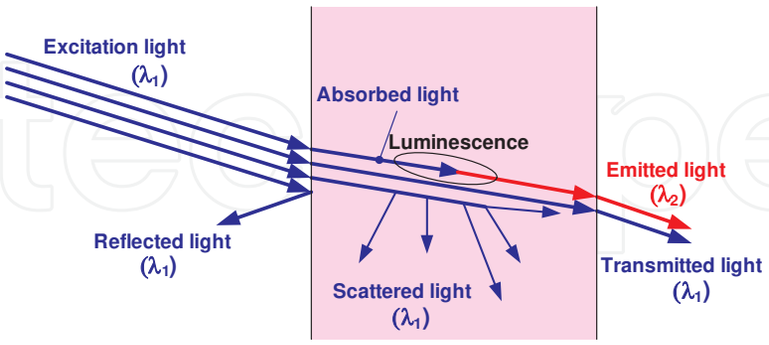


Figure 1. Some phenomena caused by the light-matter interaction.

In addition, other changes can appear, such as light polarization or modification of polarization angle of light. Thus, in a general way, the matter modifies the properties of light (direction, intensity, wavelength and/or polarization).

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When the modification of light properties depends on one of the characteristics of the matter, that change can be used to quantify this characteristic, obtaining an optical sensor.

Optical fibers guide the light from excitation source to the sensing area and from the sensing area to the optical detector. During this path, the light hardly suffers any attenuation, and the addition of other sources of optical noise is reduced. So, optical fibers produce a large improvement of Signal-to-Noise Ratio (S/N) in relation to optical sensors without optical fibers.

Besides, optical fibers guide the exciting, the reflected, the scattered, the emitted, and the transmitted light through examining places which would be otherwise difficult to access, making optical fibers quite useful in medicine or biology. It also avoids the need for equipment to be in the vicinity of substance to be measured, which is very interesting for remote operation [30,36,37,48].

Moreover, it is feasible to place several sensors (similar or different) in diverse places along the same optical fiber, obtaining a real sensor network. Several methods for multiplexing excitation signals and demultiplexing signals produced by sensors are available in the domain of time, frequency or light spectrum.

2. Principles of operation and optical fiber measurement systems

The operation of optical fiber sensors requires a light source for exciting the fiber system – including the optical sensor– and a photo detector to read the light emitted by sensing area that includes information about the X, the variable of interest. There are several options for the connection of light source and photo detector as is shown in Figure 2.

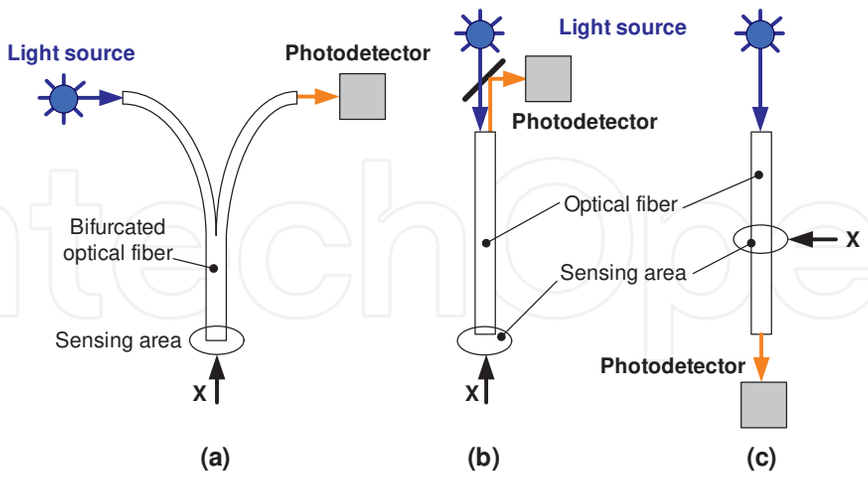


Figure 2. Schemes of possible connections between light source, sensing area and photo detector: (a) bifurcated optical fibers and sensor in the end of fiber; (b) individual fiber with semi-transparent mirror and sensing area in the end of the fiber; (c) individual fiber with sensing area inside the fiber.

Moreover, there are two different types of optical fiber sensor in function of the interaction between the variable, to be measured, and the light: intrinsic and extrinsic sensors.

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In intrinsic sensors, the fiber has two functions: first, it is the guiding for exciting and emitting light, and second, the fiber is the transducer. In this case, the variable to be measured modifies some properties of fiber, such as the refraction index or the absorption coefficient (Figure 3). Depending on the magnitude of that variable, the final change of the transmitted radiation will vary, as it happens in evanescence sensors. [13,28].

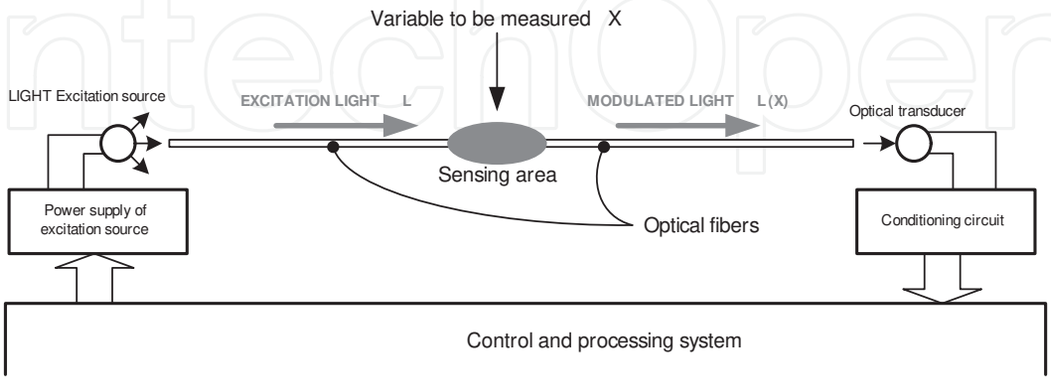


Figure 3. Diagram of the complete intrinsic optical sensor system.

Furthermore, extrinsic sensors use the optical fiber to guide the exciting light from the source to sensing area (outside the optical fiber), and the emitting light, from sensing area to the photo detector (see Figure 4).

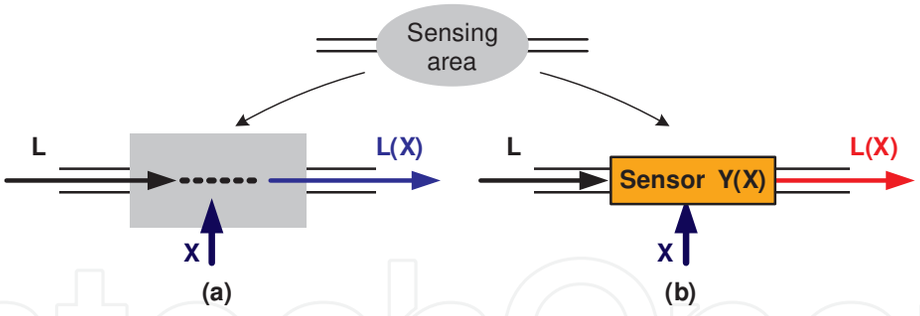


Figure 4. Types of extrinsic optical sensor based on modulation of the light technique: (a) the measured variable produces direct modulation of light; (b) the measured variable, X, produce a change in another variable Y of a sensor, and Y modulates the light.

Sometimes, the observed variable can modulate the light (Figure 4a) but, usually the interaction takes place by means of a specific sensor (Figure 4b) that acts as an interface between the variable X and the optical fiber [41].

For all the above cases, the resulting light contains information about the variable X, that is, the light has been modulated by X. The modulation can modify one or more light characteristic parameters, such as intensity, wavelength, polarization angle, phase or time delay. The type of modulation determines the light source, the photo detector, and the detection procedure [31].

2.1. Measurement based on light intensity

The light intensity is the simplest solution for most of optical fiber sensors and can be used for all cases of Figure 1. However, the use of light intensity introduces some problems in measurement processes because the light intensity is also sensible to other variables. This fact causes both perturbation and noise, and reduces the accuracy of measurement.

The effect of noise could be very important in extrinsic sensors (Figure 4) because the light must leave the optical fiber to reach the sensor, and return into the fiber. During the external path of light, optical noise could be added to the signal, reducing the S/N ratio. Optical filters between the fiber end and the photo detector can increase this ratio by reducing the presence of external sources of light. In addition, the use of a DC source for exciting must be substituted by a fixed frequency source and a narrow band-pass filter after the photodetection to reduce the bandwidth and to increase de S/N ratio. The use of synchronic switched-capacitor filters for both, excitation and received signals, improves the operation of the system because it provides large stability of central frequency [21]. All these solutions are shown in the block diagram of Figure 5.

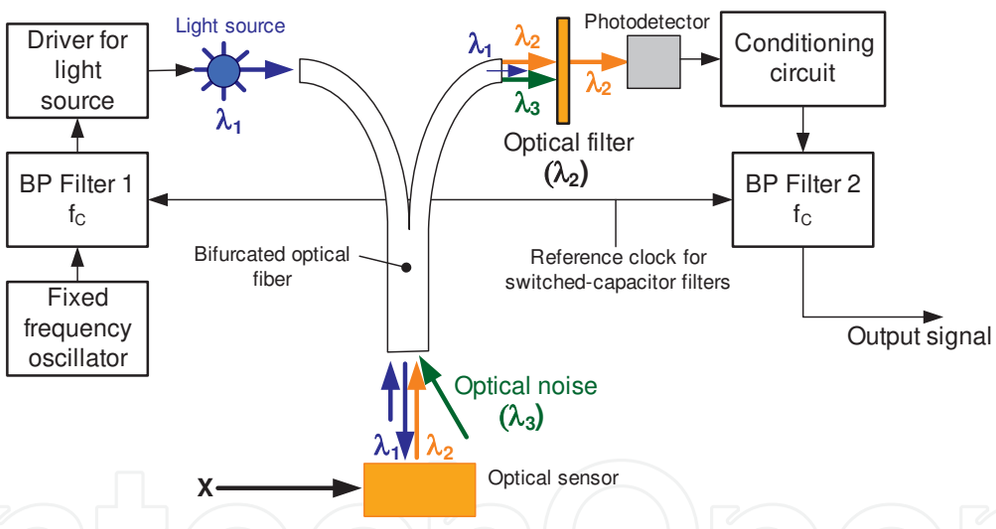


Figure 5. General idea of a light intensity measurement system based upon an optical fiber extrinsic sensor with bifurcated fibers. In case of sensors without modification in wavelength, the emission of sensor has the same wavelength that exciting light ($\lambda_1 = \lambda_2$).

Perturbation of light intensity has a lot of causes: changes in light source, optical fiber couplings (source-to-fiber, fiber-to-sensor, fiber-to-photo detector), and changes in the attenuation of fiber due to curvature, optical fiber length, etc. To prevent the effect of unknown changes in the characteristics of light path in luminescence sensors, it is possible to use a reference signal such as part of the exciting light reflected in optical sensor. The final design is similar to the system in Figure 5, but it uses a tri-furcated optical fiber and two photo detectors, one for the optical sensor emission, and other one for the reflected light from exciting signal (Figure 6).

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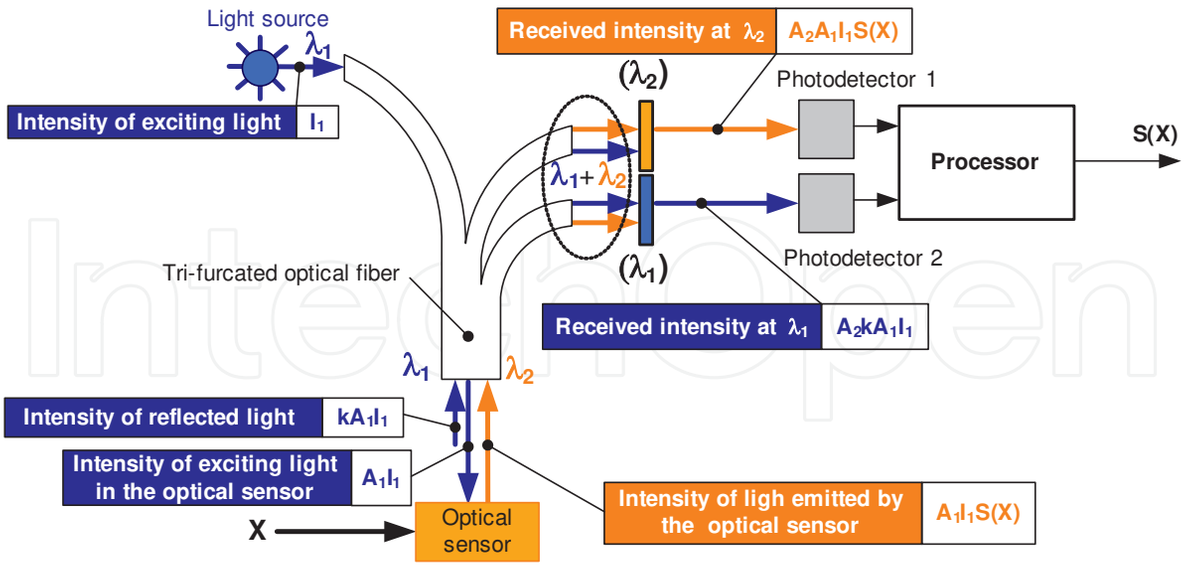


Figure 6. Diagram of the extrinsic optical sensor system with ratiometric measurement to avoid light interferences.

In Figure 6, I_1 is the intensity produced by the excitation source, A_1 is the attenuation coefficient from source to optical sensor, A_2 is the attenuation coefficient from optical sensor to each photo detector, and k is the reflection coefficient in the optical sensor. The processor can evaluate these coefficients by means of the reference signal at wavelength λ_1, and obtain the sensor response at wavelength λ_2, $S(X)$.

2.2. Time domain and frequency domain measurements

The responses of luminescence sensors produce two different measurable effects. The first one is the steady state value of intensity of emitted light that can be processed as is shown in the above point. However, the dynamic response of the optical sensor to a pulse excitation is similar to the plot of Figure 7a, where this response is characterized by the time constant of a mono-exponential decay (in a first approximation). This time constant τ, is dependent on the value of the variable of interest, X.

Light intensity and time constant can be used for measurement purposes, but the time constant has a better instrumental behavior [20,23] because the total uncertainty of the measuring instrument is considerably reduced.

However, the analysis is not simple because the emitted light has additional dependences such as the time constant of excitation pulse, the distortions of efficiency of optical sensor and the dynamic response of photo detector.

In the other hand, when the optical response of sensor has a dynamic behavior dependent on the input variable X through its time constant, that is, $\tau = f(X)$, this time constant can be evaluated in both, time and frequency domain, because the dependence can be obtained by means of the calculation of his time constant (Figure 7a), or by means of the phase delay in the frequency domain. In the last case, the excitation source is a light with DC + AC components

[1,36], where the alternate signal has a frequency around the one corresponding the time constant of the optical sensor.

The sensor response is a signal with the same excitation frequency, but with a phase delay, φ (Figure 7b) depending on emission time constant τ, by following:

$$\tau(X) = \frac{1}{2\pi f}\tan\left(\varphi\right) \tag{1}$$

Where f is the excitation frequency.

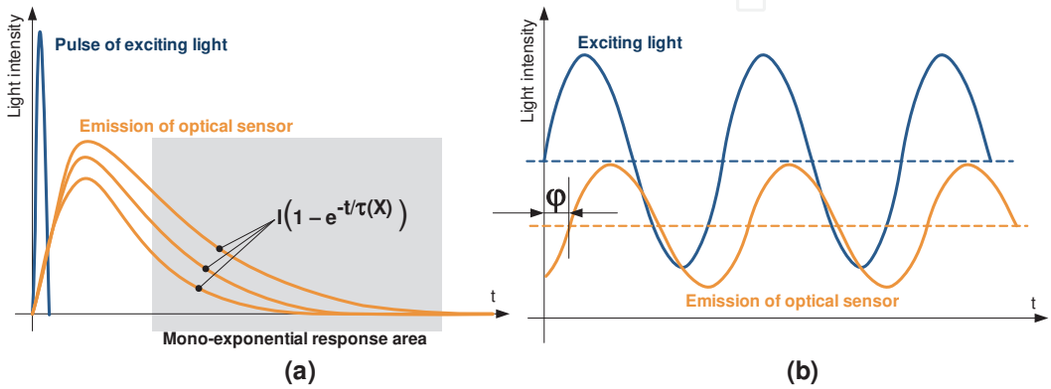


Figure 7. Responses of the luminescence sensor to: (a) a pulse light; (b) a sinusoidal light.

This measurement strategy is very useful for optical sensors with extremely short values of time constant (less than 1 ns), which is very interesting in some fluorescent sensors [17].

2.3. Design considerations of measurement systems based on optical fibers

There is not a universal solution for critical devices in the topologies of optical fiber sensors, because each type of measurement strategy forces the specifications and the requirements for those devices. The measurement system is constituted by the source for exciting light, the optical fibers, and the photo detectors. All these devices must be selected for matching the wavelength spectra of involved phenomena, and according to the measurement strategies. Thus, excitation source must cover the excitation band of the optical sensor; the optical fibers should introduce low attenuation in the involved wavelengths; and the photo detector device has to process all the light emitted by the optical sensor.

Optical filters could be included in the design to guarantee removing the excessive band pass, and to ensure enough noise reduction without decrease the signal power. In the case of optical sensors with wide spectrum sensitivity, too narrow optical filters allow us a heavy reduction of optical noise, but the use of them implies the decrease of the total light power, resulting in a poor S/N ratio.

The choice of source for exciting light depends on measurement type (intensity, and time or frequency domain). For intensity and frequency domain measurement, the source must

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produce a DC+AC light signal. The operation frequency of AC component does not have important restrictions in the case of intensity, but must be properly selected for frequency domain operation, according to the expected time delay produced by the sensor response. LEDs and laser diodes (LDs) are excellent solutions for these applications. Pulsating sources are the right selection for time domain measurement; in this case, the total energy of pulse and its duration are the most important parameters that must be taking into account in the design process. Pulse lasers are the best choice for this kind of measurement, because it is possible to obtain extremely short pulses. Other solutions, such as short-arc pulse lamps (Xe, H_2, etc.) could be used in a design [4], but they have some inconvenient: they cannot concentrate the light into the fiber tip and, consequently, need additional –and expensive– optical systems (parabolic mirrors, lenses…) to do it. Moreover, pulse lamps are used to produce wide emission spectrum, forcing the addition of optical filters to reduce the complete spectrum, and to adequate it to the wavelength band.

The photo detector is the device that provides an electrical signal in function on received light signal; its choice is quite similar to the selection of excitation source, because it must have a spectral response including the emission spectrum. Too wide spectral response would include undesirable optical noise, and narrow spectral response reduces the total power of desirable signal; in both cases, the effect becomes negative for S/N ratio.

A common solution for photo detector is the photodiode, a low-volume, low-cost, and versatile device valid for most of applications. However, photodiodes have high noise generation, large dark current, poor sensitivity, and parameter dependence on temperature. Solutions such as avalanche photodiodes (APDs) increase the sensitivity [2], but include additional noise (avalanche noise) and increase the sensitivity dependence on temperature. Sometimes, photodiodes and APDs should be refrigerated to keep a constant temperature by means of Peltier cells and control closed loops for temperature [39]. When the emission level is low (power signal is similar to noise equivalent power (NEP), photodiodes do not have enough sensitivity or introduce intolerable noise level. In these cases, a photomultiplier Tube (PMT) must be used, to guarantee a good behavior of light to electrical signal conversion. In the past, PMTs are complex, expensive; they have a large volume and need high voltage power sources. But, in the present, they are compact solutions, with low voltage supply (5 or 12 V), and reasonable cost. PMTs provides low dark current, produces low noise, and have high sensitivity, being an excellent solution for most of optical fiber sensor based on luminescence phenomenon.

3. Chemical sensors that uses optical fibers

A chemical sensor is a device that can be used for measurement the activity or concentration of chemical specie (analyte) in a sample. It is constituted by two stages [24]. The first stage indentifies and interacts with analyte, and the second one is a transducer, coupled to the first stage (Figure 9).

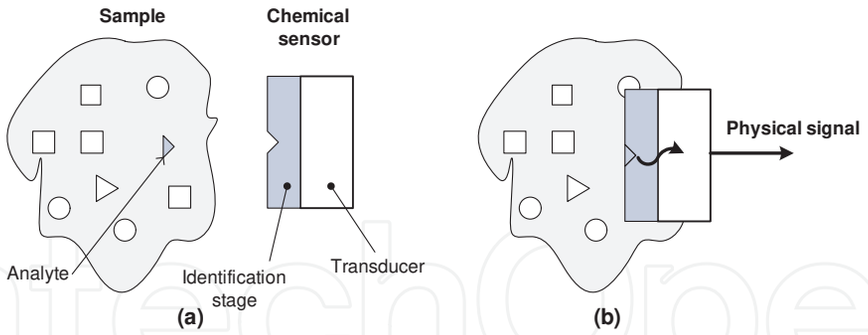


Figure 8. A chemical sensor: (a) sample and sensor; (b) the chemical sensor identifies the analyte, and generates a physical signal.

When the identification stage interacts with the analyte produces changes in its properties (emission and/or absorption of light, electrostatics changes, vibrations, chemical reactions, etc.), that is detected by transducer stage to generate an analytical signal [25,26].

Optical sensors are a type of chemical sensors that provides an optical response depending on analyte concentration in a sample, and they can classify in function of the optical property that has been measured: absorbance, reflectance, fluorescence, phosphorescence, luminescence, Raman dispersion, evanescence, refraction index, etc. When optical fibers are added to these sensors, it is possible to use the fibers for light signal transmission, obtaining an optrode [32].

3.1. Absorbance, transmittance, scattering and reflectance measurements

Light to matter interaction has been above explained (Figure 1), founding various phenomena that modify the properties of exciting (incident) light without changes in its wavelength. For several cases, the behavior of the light in this interaction depends on some characteristics of matter and, consequently, it could be used to identify those characteristics. Thus, the measurement of the light reflected, absorbed, scattered or transmitted is a way for detection or quantification of a property which is able to produce a change in the light.

In transparent media, absorbance and transmittance measurements are closely related because the rest of effects are negligible; consequently, they produce similar results. Absorbance can be used to identify some substances (atoms or molecules) in a medium, because each substance has a specific absorption spectrum. However, a simple quantification in any environment becomes very complex, because there will be more than one chemical specimen in the medium. So, a valid identification and/or quantification require a detailed study of a portion of spectrum. Absorption spectrometry is the technique that can identify and/or quantify the causes of the resulting spectrum, and it involves complex mathematical process and statistical analysis [45].

But, optical sensors based upon absorption are designed for specific analysis, usually in a particular and controlled medium. Hence, these sensors use a small number of wavelengths (even, one specific wavelength), and quantify the change on light intensity when the incident light runs through the sample [7]. By a similar way, reflectance sensors are also designed for specific analysis in opaque or low transparent substances (Figure 10).

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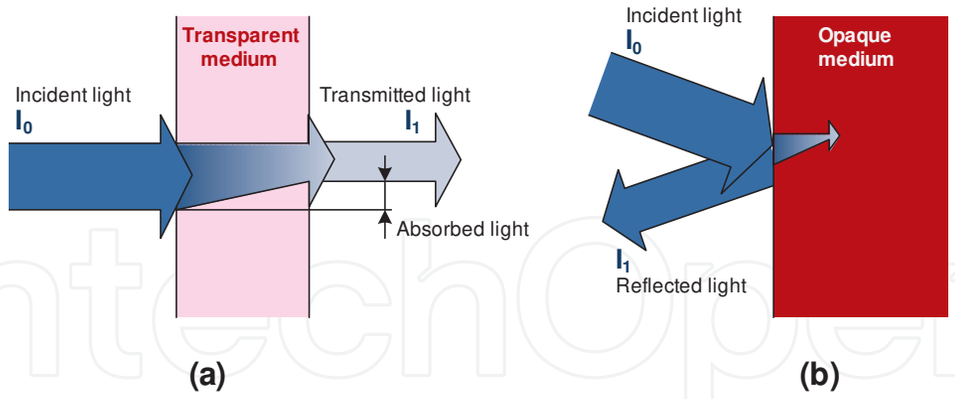


Figure 9. Abortion, transmission and reflection performance of the light: (a) in a transparent medium; (b) to face opaque medium.

In the case of absorbance, the relationship between incident and transmitted light at a specific wavelength can be expressed by means the absorption coefficient, A_λ,

$$A_\lambda = ln \frac{I_0}{I_1} \tag{2}$$

When this coefficient A_λ is a function of a chemical or physical parameter of medium, it is possible to use the change in intensity to quantify it, obtaining an absorbance sensor. Usually, that function is not simple and the instrumental design requires an empirical procedure to reach the static transfer curve. A_λ depends on length of optical path through the sample; this fact can be used for adjusting the instrumental sensitivity according to the excitation source and photo detector device.

In the case of reflectance, the hemispherical coefficient of reflectance, ρ_λ, for a wavelength λ, is defined as follows,

$$\rho_\lambda = \frac{I_0}{I_1} \tag{3}$$

This coefficient depends on obvious physical parameters, and sometimes also includes information about the presence of quantity of a specific substance. Thus, the reflectance can be used as an instrumental parameter in the design of a sensor for that substance. As the previous case, a large number of variables can affect the value of reflectance coefficient and an experimental calibration process must be carried out to obtain the static transfer curve.

Scattering light is only used for detection of some physical parameters, such as liquid turbidity [38] or smoke detection, and it is not usual in neither chemical nor biological measurements.

3.2. Fluorescence and phosphorescence measurements

Fluorescence and phosphorescence are two of processes of a photo-luminescence molecule. It absorbs UV or visible radiation to increase the energy level from a fundamental singlet state

S_0 to excited electronic singlet states S_1, as is shown in the Jablonsky diagram of Figure 11. Some low energy changes can occur from this new fundamental state S_1 to near energy levels produced by vibrational relaxation, without radiation emission. When the molecule returns to the original singlet state S_0, can emit a radiation with a longer wavelength than the absorbed radiation; this emission is known as fluorescence. But, the molecule can also return to the original state S_0 through non-radiant transition (vibrational relaxation, internal conversion, external conversion, and intersystem crossing). The most likely path to the fundamental state S_0 will be one that minimizes the mean timelife of the excited state.

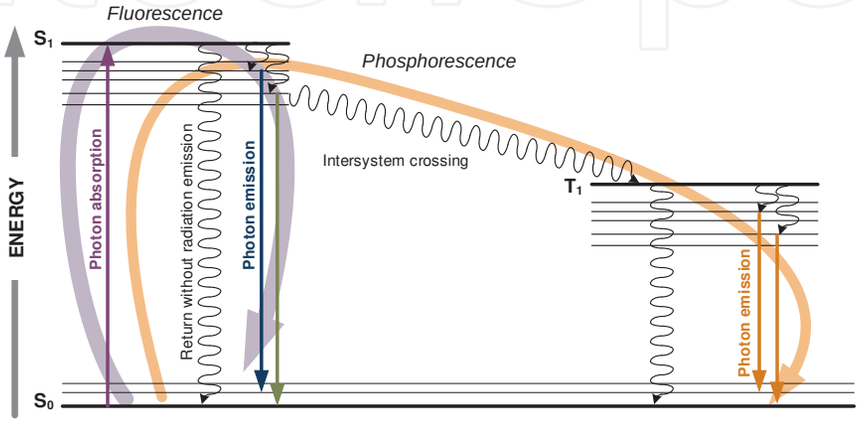


Figure 10. Jablonsky diagram for luminescence processes. Thick lines are fundamental states and fine lines correspond to vibrational states associated to a fundamental state.

Intersystem crossing is an unusual phenomenon that increases the spin multiplicity of electron and drives it to a triplet state (T_1). From this state the molecule returns to its original unexcited state by means an emission of radiation (phosphorescence) or without radiation emission. The phosphorescence phenomenon is longer in time than fluorescence one, and produce longer wavelength. In addition, due to the low probability of the phosphorescence, the total intensity of radiation is very low compared to the fluorescence process.

For both cases, fluorescence and phosphorescence, the kinetic of process can be represented by a first order equation:

$$\frac{d[M^*]}{dt} = -k \cdot [M^*] \tag{4}$$

Where [M*] is the concentration of molecules in excited states and k is a constant that represents the speed of process and depends on the molecule properties. By integrating,

$$[M^*]=[M^*]_0 e^{-kt} \quad \rightarrow \quad [M^*]=[M^*]_0 e^{-\frac{t}{\tau}} \tag{5}$$

Where $[M^*]_0$ is the initial concentration of excited molecules, and $\tau = 1/k$ is known as the medium lifetime of the excited state. As the emission intensity is proportional to the concen-

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tration of excited molecules, the previous equation can be rewritten in terms of light intensity, as follows,

$$I = I_0 e^{-\frac{t}{\tau}} \tag{6}$$

In some cases, the deactivation of excited states can be produced by a non-radiant external conversion way due to the interaction of photo-luminescent molecules with external molecules. This implies an energy transfer that reduces the concentration of excited molecules and, consequently, the intensity of light emission decrease. This effect is known as quenching and can be used to determine the concentration of these external molecules (quenchers). This effect can be quantified by means the Stern-Volmer equation,

$$\frac{I_0}{I([Q])} = \frac{\tau_0}{\tau([Q])} = 1 + \tau_0 k_b [Q] \tag{7}$$

Where I_0 and τ_0 are the intensity and medium lifetime of light emission without quencher, I[Q] and τ[Q] are the intensity and medium lifetime in presence of a concentration of quencher [Q], and k_b is the bimolecular constant of quenching. The product $\tau_0 k_b = K_{SV}$ is known as the Stern-Volmer constant. This constant is actually modified by diffusion process and depends on the diffusion coefficients of photo-luminescent and quenchers molecules [3,18]. The Stern-Volmer equation establishes a linear but not-accuracy relationship, due to heterogeneity of chemical sensor. It is possible to correct this relationship, and it must be done. [1,14,15,20,23,46].

3.3. Implementation of chemical sensors with optical fibers

Most of chemical sensors that use optical fibers are extrinsic, because the inclusion of reactive substances inside the fiber (necessary for intrinsic sensor) will increase the response time of recognition stage (Figure 9), due to the slow diffusion process of analyte through the fiber. Hence, most of optical fiber chemical sensors use bifurcated fibers (Figure 2a) or a single fiber with a semi-transparent mirror (Figure 2b). In both cases, the chemical sensor (or the sample to analyze) is placed near or in the end of fiber, depending on fiber type and measurement strategy (Figure 12).

In luminescence sensors, the fiber tip can be shaped to reduce the reflection for exciting wavelength and to prevent the presence of exciting light in the photo detector as a noise. It could include selective membranes to improve the selectivity of sensor (Figure 13); but the membrane increases the sensor settling time due to the diffusion process through it.

The complete sensor includes the source for excitation and the photo detector device. Table 1 shows some consideration about the selection of these systems, taking into account the type of chemical sensor. The most critical specifications for the light source and photo-detector device are for time domain measurements in fluorescence, due to the usual short time response of chemical sensor that forces the selection of extremely short pulse sources and high speed

detectors; the low intensity produced by phosphorescence sensors force the use of high sensitivity photo detectors in all cases.

Chemical Sensor	Light source	Photo-detector	Considerations
Absorbance	LED, Laser, LD	Photodiode	AC+DC signal Intensity measurement
Reflectance			
Fluorescence	Pulsating lamps, LED, LD, lasers	Photodiode, APD, PMT	Short time response Time-domain measurement
	LED, Laser, LD		AC+DC signal Frequency-domain measurement
			AC+DC signal Intensity measurement
Phosphorescence	Pulsating, lamps, LD or lasers	APD, PMT	Medium-large time response Time-domain measurement
	LED, Laser, LD	Photodiode, APD, PMT	AC+DC signal Frequency-domain measurement
			AC+DC signal Intensity measurement

Table 1. Light signal, excitation sources and photo detector devices for chemical sensors.

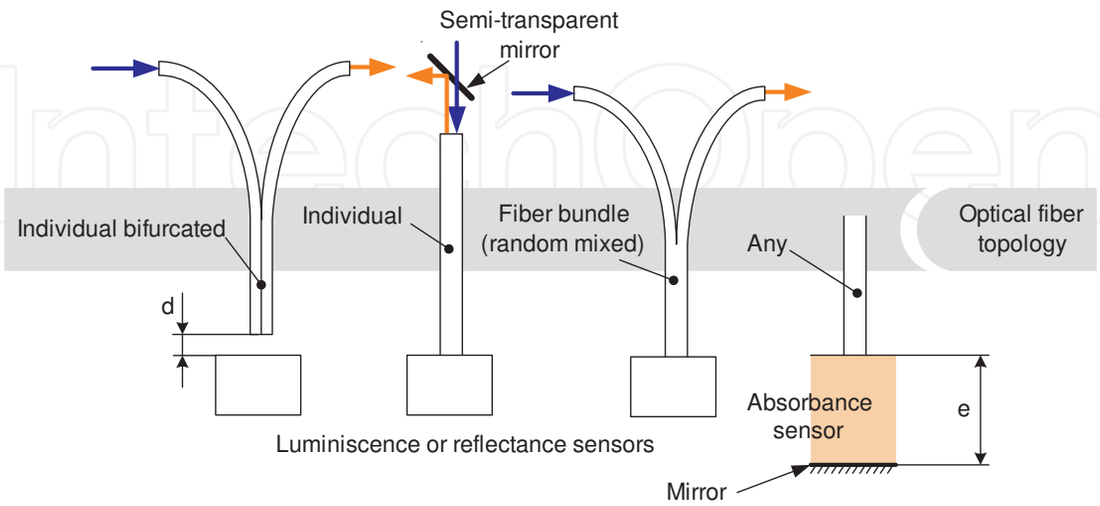


Figure 11. Situation of chemical sensor in the end of fiber considering the optical fiber topology: the parameters *d* and *e* must be calculated to obtain an optimal sensitivity.

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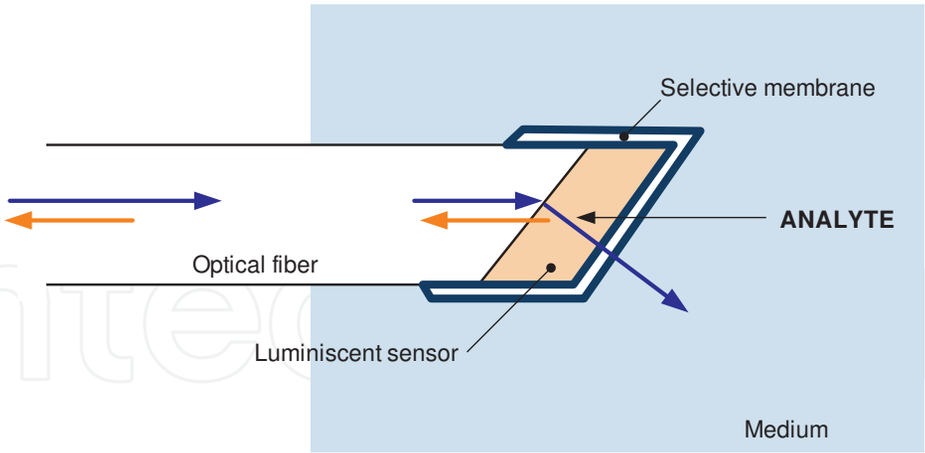


Figure 12. Chemical sensor placed into the fiber tip with a selective membrane.

4. Examples of optical fiber sensors for chemical measurements

4.1. Frequency domain analysis for the fluorescence of ruthenium chemical sensor

The measurements are commonly based in analysis of the time domain or the frequency domain, as it is explained in the above section 2. Time domain measurements have practical difficulties. This method requires a big number of points of the signal response to obtain the time constant, and this is a limitation because of the small size of the sampling period. Due to the characteristics of the physical phenomenon and/or the high cost of the system, it is more efficient using the frequency domain to measure the fluorescence emission, whose lifetime is in a range limited by nanoseconds and a few microseconds [22,47]. In this method, the lifetime is obtained from the phase shift between emission signal from the chemical sensor and the excitation signal used. Currently, some analytical instruments that enable the measurement of a large number of analytes such as pH, carbon dioxide, or oxygen, are known. This section deals with a brief description of the main components of fluorescence sensors, focusing on a sensor for measuring dissolved oxygen concentration.

The system consists of a DC+AC light source which excites the Ruthenium sensor. When this chemical sensor is energized, it produces a fluorescence excitation with a wavelength around 470 nm, and the following fluorescence emission wavelength is near to 600 nm in the case of oxygen measurements.

In fluorescence analysis is not necessary to employ a high intensity light source, but a correct generation of the excitation waveform is very important because this waveform will be used in the final processing. Thus, the best device for been implemented in the emission sub-system (Figure 14) is a LED [11,12,27].

This LED must emit a light with a wavelength close enough to the excitation one (470 nm), and as optic fiber is used to transfer the light, its viewing angle must be small enough to

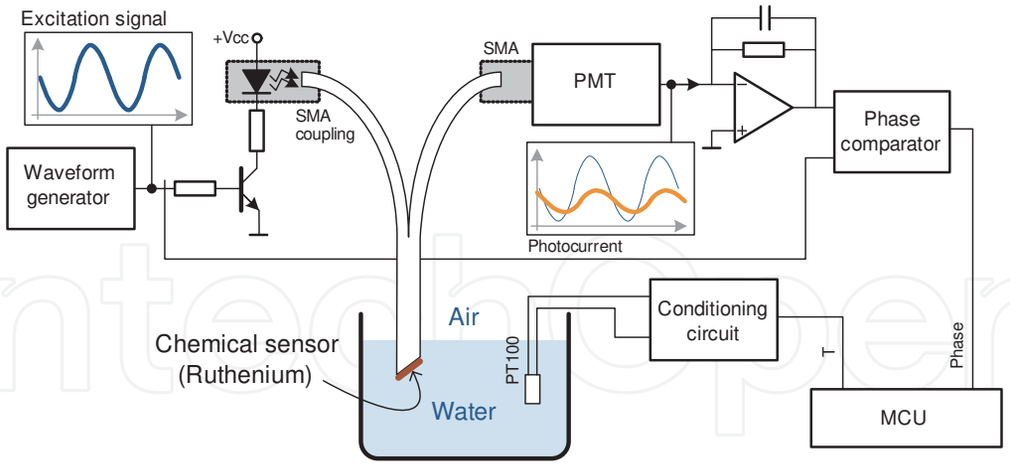


Figure 13. Optical fiber sensor for D.O. based on Ruthenium chemical sensor. It operates with phase detection and temperature correction.

improve the directionality of the emission. So, as LED OVL-5523 also has the intensity needed to excite the Ruthenium sensor, it can be a good solution for the light source of a frequency domain fluorescence system (fluorimeter).

The PIN photodiode is a common photo detector employed in a lot of digital communication systems with optical fiber because it has a good reliability and a quite wide bandwidth. But, considering the disadvantages, it can be mentioned, that it introduces a large noise, it needs an external system to establish its temperature, and its bandwidth is above our specification range. Other interesting photo detector is the APD, it does not have the disadvantages said previously, but in this case, its internal gain is intrinsically unstable. These devices are cheaper and have a smaller volume than PMT, which needs a special enclosure to obtain a correctly amplification of the output current. Nevertheless, their instrumentation characteristics make of this last photo detector, the best option to take part in the fluorescence based system.

In Figure 14, it is possible to appreciate the block diagram of the fluorimeter. The system generates a sinusoidal signal with a DC component for LED excitation. The light is transferred to the Ruthenium sensor by low-cost bifurcated optic fiber (gradual index plastic optical fiber with a diameter of 1 mm). The chemical sensor where the fluorescence phenomenon takes place is in contact with the sample. The fluorescence emission generated goes through the fiber to the PMT. The photo detector output signal (current) and the sinusoidal excitation signal are processed to obtain the frequency response of the fluorimeter.

The data produced by this system can be modelled by a Stern-Volmer equation, but in this case it is better to use a multivariable regression because the influence of the temperature is quite high.

The obtained model has a high correlation considering the phase shift and the temperature as explicative variables of oxygen concentration $[O_2]$. This model is almost linear with 0.9999 of correlation index as it is possible to see in Figure 15, where graphic points produce clearly a

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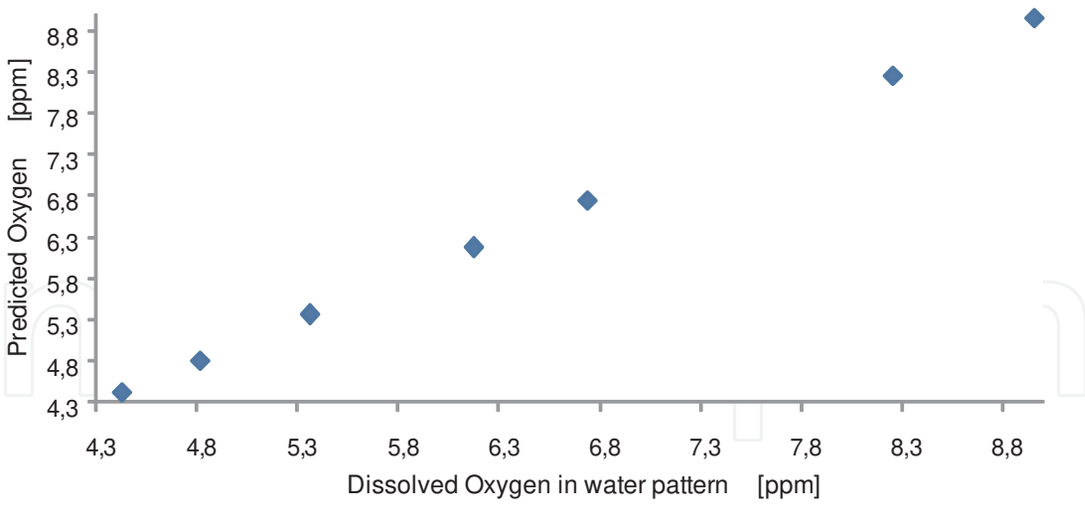


Figure 14. Relationship between the real values of D.O. in water patterns and predicted values from fluorimeter of Figure 14.

straight line. Furthermore, the maximum absolute errors that can be found in this kind of fluorescence systems round 2 ppb, with relative errors values of less than 0.05 %.

4.2. Time-domain analysis of phosphorescence of sol-gel Al-Ferron chemical sensor

Phosphorescence analysis in the domain of time is a well known procedure to carry out several important measurements of several analytes. Concentration of dissolved oxygen in water (D.O.), moisture level, pH value and other chemical parameters can be obtained by means of analysis of phosphorescence emission of a chemical sensor properly excited with light [5,9,18]. In this section, some considerations about main blocks of a time domain phosphorimeter will be discussed, including some improvements.

Light source must excite the chemical sensor that yields a phosphorescence emission with a wavelength quite far from excitation wavelength. In Al-Ferron Sol-gel chemical sensor [18, 48] used for oxygen measurement, excitation wavelength is placed from near UV to violet and the emission takes place around the green light wavelength.

An excitation with high pressure Xe pulse lamps (or similar short-arc lamps) produces a wide spectrum (white light) and high intensity pulses of light, requiring optical filters to reduce optical noise. In addition, these lamps need to include other optical accessories, like parabolic mirrors or lenses to concentrate the light into the optical fibers tip. The final cost of this kind of lamps and associated power and trigger circuits is very high, and these circuits introduce several critical subjects in cabling, housing, protection and/or EMC. Finally, an aging process takes place in arc lamps, reducing the lifetime of lamp, generally due to electrodes are worn out [6].

Laser light sources increase the intensity of pulses, reduce their narrowness, and avoid the use of additional optical systems such as filters and mirrors because the produced light is coherent. But they introduce the same problems in total cost, cabling protection and EMC. Final results

of laser-based time domain phosphorimeters are quite similar to results obtained with pulse lamps. The high concentration of power pulse becomes a problem for optical fibers connected to laser sources: the end of fiber has a progressive increase of attenuation by burning.

LD and UV-LEDs are other possible solution for light excitation. They facilitate the connection to optical fiber tip and reduce both, the total cost and the system volume, overcome most of inconvenient of arc lamps and lasers. Moreover, the MTBF of UV-LED is very high in comparison with lasers and lamps, reducing maintenance and replacement costs.

The excitation wavelength of chemical sensor (Al-Ferron immobilized in Sol-Gel) has a maximum peak around 390 nm and its emission spectrum has a peak value around 590 nm. Thus, UV LED like NSHU590 can be a balanced solution for the light source of a time domain phosphorimeter.

The detection of emitted light is critical in phosphorescence based system due to low level of Al-Ferron emission. The best solution –under the instrumental point of view– is the use of a PMT because of its high sensitivity. Moreover, it has low noise, low dark and non dependence on temperature. A comparison between APDs and PMTs results in similar instrumentation characteristics will be that the initial advantages of APD in volume are compensated with the presence of cooling systems [39] for holding constant temperature, and thus, avoiding sensitivity changes. Standard PIN-Photodiodes introduce large noise and need temperature stabilization [6].

Final design of phosphorimeter is shown in Figure 16, where the chemical sensor is included inside a flow cell for calibration purposes, by using a full-controlled gases mixture of argon and oxygen. UV LED output is a waveform that consists of narrow pulses widely separated from each other in order to guarantee tine enough for full extinguishing of chemical sensor emission between pulses. Resulting excitation waveform is shown in that figure.

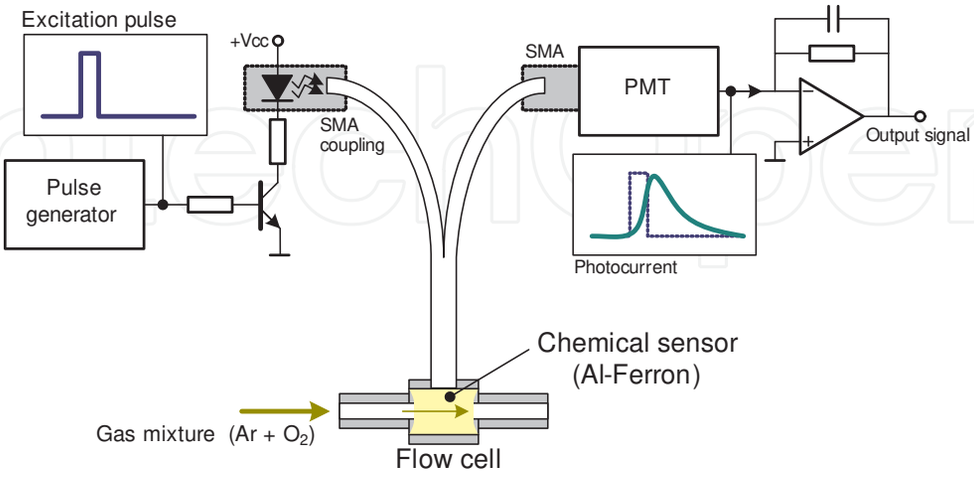


Figure 15. Design of an optical fibers time domain phosphorimeter with Al-Ferron chemical sensor. Bifurcated optical fibers are constituted by a bundle of 1500 borosilicate fibers, in contact with the chemical sensor powder. All optical filters have been removed for this design because the optical noise is not too important.

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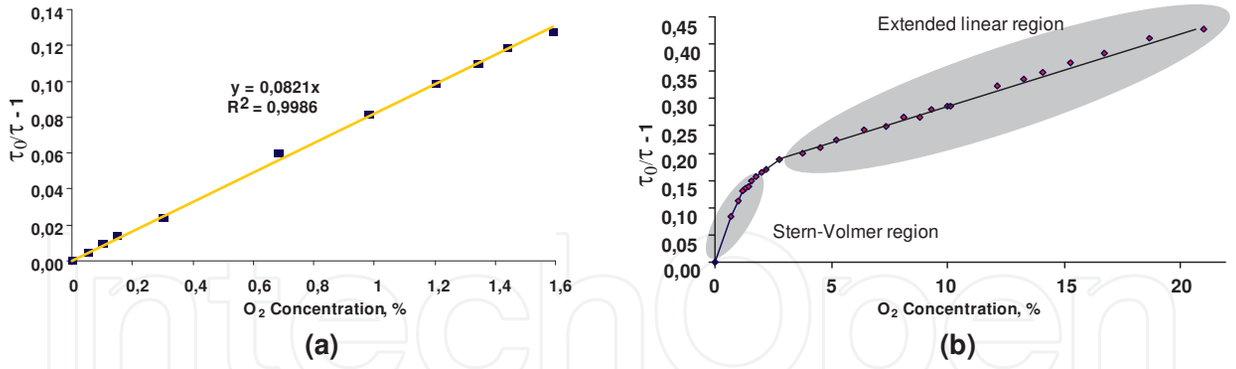


Figure 16. Experimental results of a phosphorimeter using Al-Ferron chemical sensor: (a) for low oxygen concentrations; (b) Extended results of Stern-Volmer relationship with two linear areas.

This system has an excellent behaviour for low level oxygen concentration, obtaining a good correlation coefficient for Stern-Volmer equation (see Figure 16a).

Stern-Volmer equation for low-level oxygen concentration is a well-know fact, but the behaviour of phosphorescence emission at large value of $[O_2]$ is usually described as a 'saturation process' in the chemical sensor. Thus, for $[O_2]$ less than 4%, Stern-Volmer equation can be experimental verified but becomes inexact above this point. However, there is not saturation process but a slope change in plot. In Figure 16b, an extended plot (from 0% to 21% of $[O_2]$) is displayed, showing two different slopes. The fact of slope change allows us to use phosphorescence lifetime analysis over the limitations of Stern-Volmer equation although the obtained sensitivity is lower. The obtained change in slope is a common question in phosphorescence analysis and it is present in both, medium lifetime analysis and intensity analysis as it has been described for other phosphorescence sensors.

5. Optical fiber sensors for biological applications

Optical fiber sensors can be applied for several biological measurements. However, in most of cases, the final sensor does not have a direct interaction with a biological parameter, but it has a chemical or physical operation principle. The general idea is similar to the exposed in Figure 9, an indirect interaction. In this case, a biological variable produces a chemical or physical change suitable for measurement by light modulation (absorbance, reflectance, luminescence, etc.). So, as a general conclusion, an optical fiber sensor for biological measurement is a type of above discussed solutions.

An example is the well known reaction to detect or determine the quantity of ATP (adenosine triphosphate), a coenzyme used in cell reactions by means luciferine,

$$ATP + Luciferine + O_2 \rightarrow Oxyluciferine + CO_2 + AMP + LIGHT$$

The results of this reaction include adenosine monophosphate (AMP), and it emits light! The light intensity is proportional to the quantity of ATP. This phenomenon is known as biolumi-

nescence but, it could be called chemical-luminescence. There are a lot of applications of this test in the determination of quantity of cells or their activity in a sample.

The main restrictions imposed to the use of any sensor for biological applications are the bio-compatibility and the disturbance for in-vivo measurements; because this kind of sensors is applied in human and veterinary medicine, and in food industry, sectors with extremely restrict conditions and standards. For example, a catheter with a D.O. sensor for determining the oxygen saturation in blood could be a fluorescence sensor based on ruthenium chemical sensor, but it must have a complete bio-compatibility.

In next sections, some examples of sensors for biological applications are presented. In all cases the objective is the monitoring and control of food production.

5.1. Milk quality sensors based upon optical fibers

Daily measurement of nutritional milk parameters could be used for cow selection, cow feed tuning in order to increase economic efficiency, and milk differentiation to obtain predefined values of fat content, total protein or lactose in the farm outlet. Modern dairy farms include several control and automation systems, which are able to provide interesting data for farm management and to improve the economical results of exploitation [44]. NIR spectrometry has been used to estimate milk composition, but previous works are referred to dry milk, homogenised milk, high cost spectrometry equipment [43], or requires sampling and previous treatment of milk samples [16,49], avoiding a cow-side final implementation.

All spectrometry equipment consists of an excitation light source able to produce a continuous spectrum for all wavelengths and a photo-detection system for measuring the received light in the same light spectrum. The reduction of range of interesting light wavelengths simplifies the design of complete system and decreases the final cost because low-cost LEDs and photodiodes can be used for excitation and light detection. Moreover, photodiodes can be used without cooling systems or temperature controllers, keeping an enough S/N ratio.

To investigate the potentiality of VIS-NIR spectrometry, several milk samples has been taken from a farm during milking (along milking and from different cows). Each milk sample is divided into two similar sub-samples and preserved using refrigeration and bronopol (2-Bromo-2-nitro-1,3-propanediol). First sub-sample is sent to a certified laboratory for composition analysis, using standard procedures, obtaining reference values for fat (TG), total protein (TP) and lactose (TL) content; second sub-sample is analyzed by spectrometry. Finally, results of both analyses are compared in order to determine the capability of VIS-NIR spectrometry to estimate the milk composition.

The analysis of each milk sample by spectrometry is carried out using a low-cost VIS-NIR spectrophotometer from Ocean Optics, able to provide 1236 values in the 400.33 to 949.59 nm, resulting in a resolution of 0.444 nm. Three different spectra are obtained by means of custom-designed analyzing cell connected to spectrophotometer and light source using several optical fibers as we can see in Figure 17. When an appropriate excitation lamp is used, this system is able to provide orthogonal spectrum (M90) caused by scattered light, transmittance spectrum

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(TR) and reflectance spectrum (RE). All these values are corrected by ratiometric techniques to reduce uncontrolled attenuation and disturbances [7].

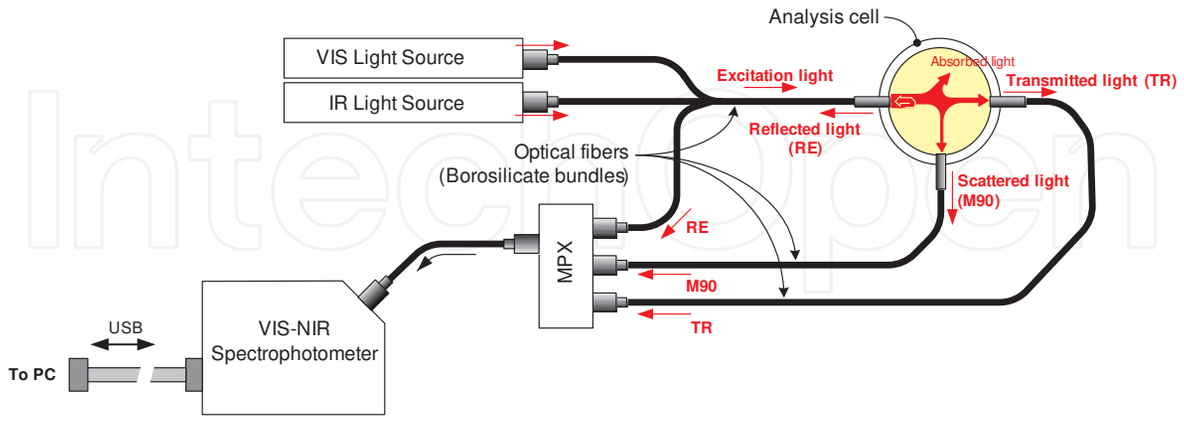


Figure 17. Three spectra analyzer for fresh milk

Spectral data has been smoothed by applying iterative local linear polynomial fit with tricubic weighting [8] to redraw smoothed spectra with low resolution, 20 nm. Thus, the total number of input variables for statistical treatment is reduced and, the problem simplified, without significant data lost. Regression-based methods are used for prediction, using TG, TP and TL as dependent variables and smoothed spectra M90, TR y RE, with 20 nm of resolution as independent variables. For each value of three smoothed spectra, square and cubic terms are generated such as additional input variables to include non-linear behaviour of model. Hence, model includes 504 input variables (56 × 3 ×3), 56 values of each spectrum, and its square and cubic terms).

Total number of input variables is lower than number of observations. So, a multivariate technique for dimensional reduction must be applied, the traditional Principal Component Regression (PCR) or the useful PLS (Partial Least Squares) in univariate response (PLS-1) [29]. Both, PCR and PLS-1 methods are based on calculation of orthogonal components from a linear combination of original variables to reduce the total number of variables. The objective of PLS-1 is to extract the components from correlations between original independent variables and dependent variable. In our case, to choice the final components number, the average squared error of predicted values is calculated for all cases, by means of leave-one-out cross-validation. The use of R statistical environment simplifies these calculations and procedures [40]. Table 2 shows the optimum number of used components for both methods and the percentage of explained variance. The results are quite simple: fat content in milk can be obtained with only one excitation wavelength!

Based on this idea, a low-cost optoelectronic sensor has been developed for working in the NIR region of light spectrum. The developed sensor shown in Figure 18 is a reflectance optical fiber sensor that consists of a stainless steel tube, optical fibers for light conduction from a light emitter to the milk to a light receiver, and circuits for the signal treatment and control unit.

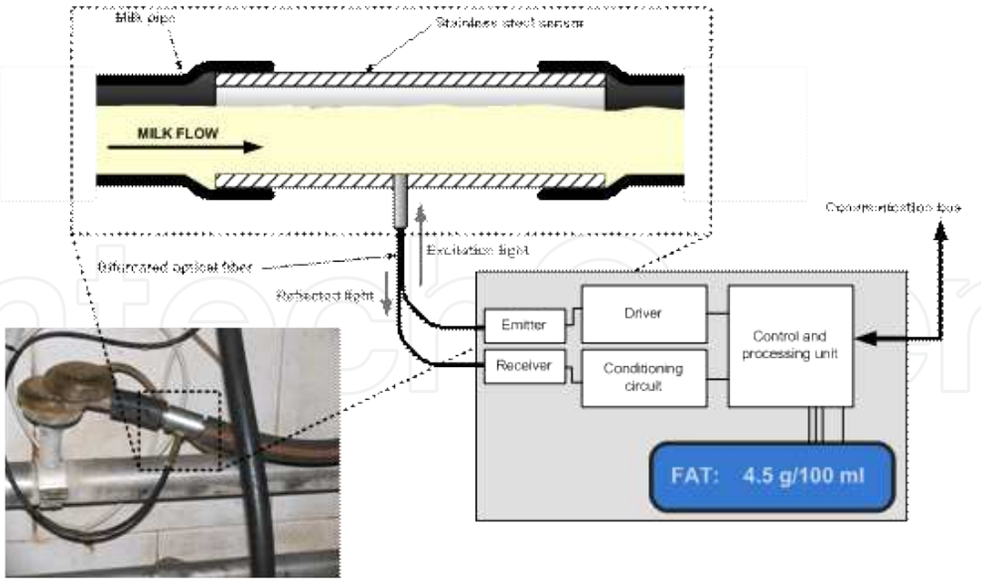


Figure 18. On-line optical fiber sensor for the estimation of fat content in milk. Picture shows an in-farm implementation of this system.

Variable	Number of components		Explained variance (%)
	PCR	PLS-1	
Fat content (TG)	1	1	82
Lactose content (TL)	11	8	62
Total protein content (TP)	2	2	17

Table 2. Comparison of PCR and PLS-1 results in prediction of milk composition. An overall interpretation could establish an excellent behaviour for prediction of fat content (it uses only one component and can explain a high percentage of variance); results are interesting for lactose content, although using many components.

The operation of the system is as follows: the light proceeding from an infrared LED comes into contact with the milk, where part of the light is reflected and then, detected by a photodiode. Due to the fact that the reflected light depends on milk fat, the value of fat can be calculated by a control unit. Figure 19a shows the real behaviour of this sensor for homogenized milk samples, and Figure 19b, for raw milk during milking process. In both cases, the output signal is the voltage produced after conditioning circuit.

5.2. Optical fibers colorimeters in food quality control: Wine and consumption oil

Colour contributes to organoleptic attributes and quality parameters of food. Moreover, it can be used in the production process: to determine the maturation level of fruits and vegetables, in the identification of origin and adulteration of consumption oils, in the fermentation process of grape juice for winemaking or other fermentation process (beer, cider, etc.). In all these cases, colour determination is used to make decisions during the production processes.

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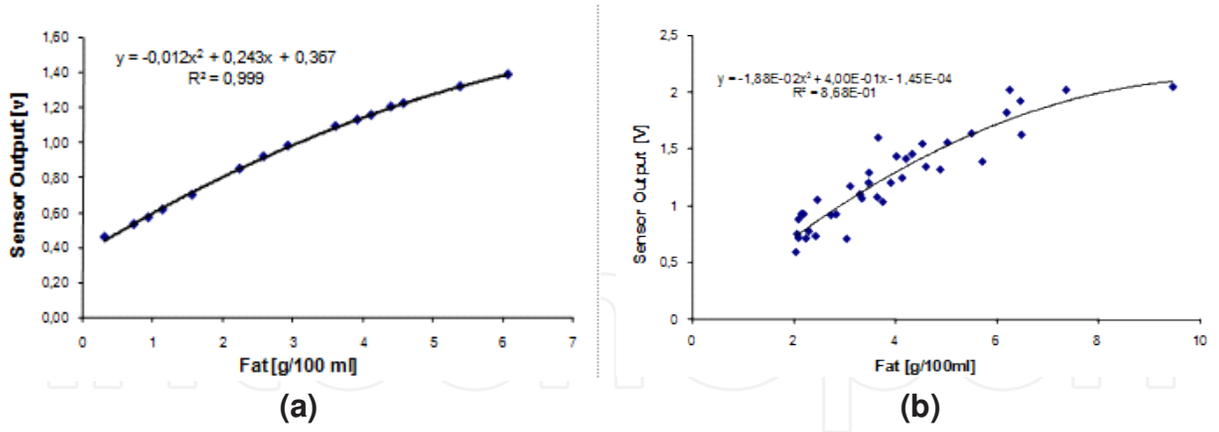


Figure 19. Analysis of 38 samples of fresh un-homogenized raw milk. Actual fat values are provided by a certified laboratory and have and uncertainty less than 2%.

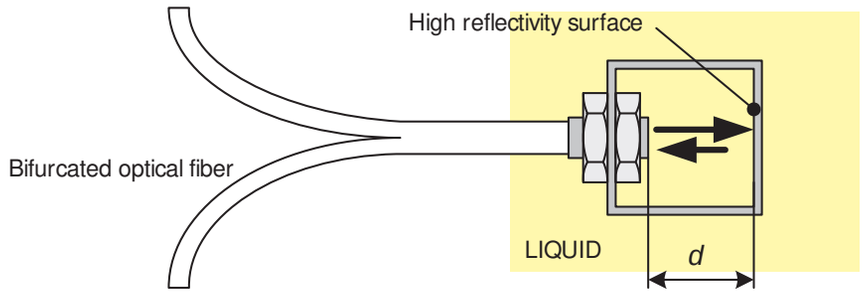


Figure 20. Optical fiber colour probe for liquid foods. The distance d is a design parameter and it depends on liquid transparency. All materials of sensor must accomplish with food industry standards.

In some traditional food industries, the colour is provided by experts, but this introduces subjectivity and uncertainty, and increases the processing time. The final results are a lost of repeatability, reproducibility and quality, and an increase of final cost. Expert estimation of colour can be substituted by a colorimeter that produces on-line results, improves instrumental parameters and reduces cost. A complete colour estimation includes an analysis of reflected (for solid foods) or transmitted/absorbed (for liquid foods) light spectrum in visible wavelengths (400 to 700 nm), but it is usual the reduction of analysis to a short set of wavelengths according to food type and the property that we like to know.

A colour analysis for solid foods such as vegetables, fruits or meat does not require optical fiber sensors and can be carried out by CCD cameras and image analysis; however, sensors for colour estimation of liquid foods can take advantage of optical fibers to reach any measurement place during production process. Figure 20 shows a colour probe with bifurcated optical fibers that uses a transmittance/absorbance measurement.

In wine industry, colour depends on some parameters such as the grape composition, winemaking techniques and several reactions that take place during wine storage. The composition of wine colour changes continuously during winemaking and storage, with associated changes in sensory characteristics. Usual colour analysis for grape juices and wines

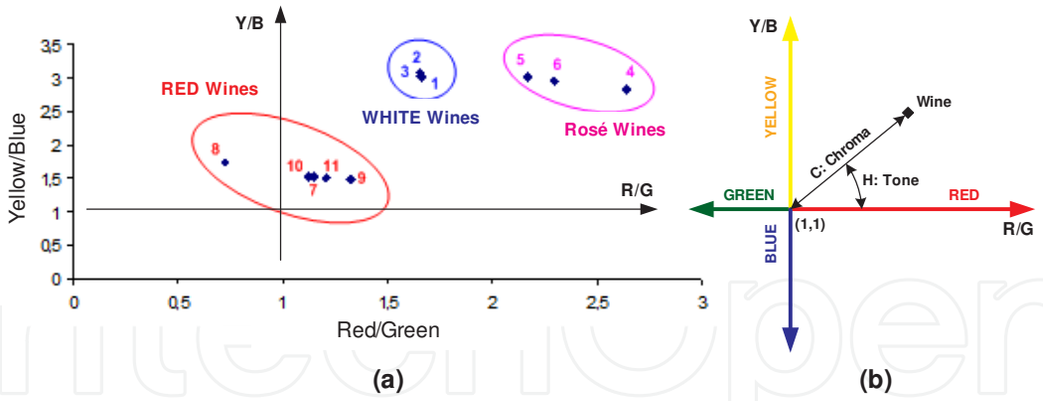


Figure 21. (a) Wine classification in Y/B-R/G coordinates system; (b) Definition of chromaticity parameters of a wine.

is made by measurements at three wavelengths in blue, green and red spectrum areas: 420, 520 and 620 nm [19], but there are several methods to measure the chromatic parameters in all wines types, such as the method based on the CIE [33] or the OIV [34] method to determine the wine colour. These methods use two very similar processes to obtain colorimetric values of wine samples because the wine absorbs the radiation incident, or transmits the one that not absorbed. In both cases, the objective of each method is to obtain three colorimetric values to situate each wine in one point of the specific colour space [34]. Both methods have quite similar characteristics, including their high cost, because they use spectrometers, very expensive and delicate equipment, and other subsystems like special illuminants.

In addition, final colour read-out involves a complex procedure, not allowing on-line operation; this limitation reduces the use of these colorimeters in winemaking process.

On-line requirements and low-cost condition force to explore new methods of colour measurement, that is able to provide on-line chromatic values without punishing the cost, that is: they can be used within the control system of winemaking processes [10]. A new design with RGB colour space simplifies the sensor and reduces the cost of illuminant because a halogen lamp is able to provide enough power excitation in the three selected wavelength. To simplify the fiber topology and connector system it is possible to use a RGB photodiode as photodetector.

The results from this RGB optical sensor can be plotted in the traditional diagram used for wine colour classification (Figure 21a) [42]; thus, the chromaticity values (tone, H and chroma, C) can be derived from measured values (Figure 21b) by,

$$C = \sqrt{(YB - 1)^2 + (RG - 1)^2} \qquad H = arcsin\left(\frac{YB - 1}{C}\right)$$

where YB and RG are, respectively, the Yellow-to-Blue and the Red-to-Green ratios,.

The use of a colorimetric optical fiber probe has a lot of applications in food industry. Another interesting case is the colour determination of consumption oil, because it can be used to identify the type of oil, even the olive type and the acidity level. Figure 22 shows a diagram block of a RGB colorimeter, applied to oil colour characterization. It includes a full controlled

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illuminant (white light emitter) with a feedback of emitted light to avoid long term and temperature derives.

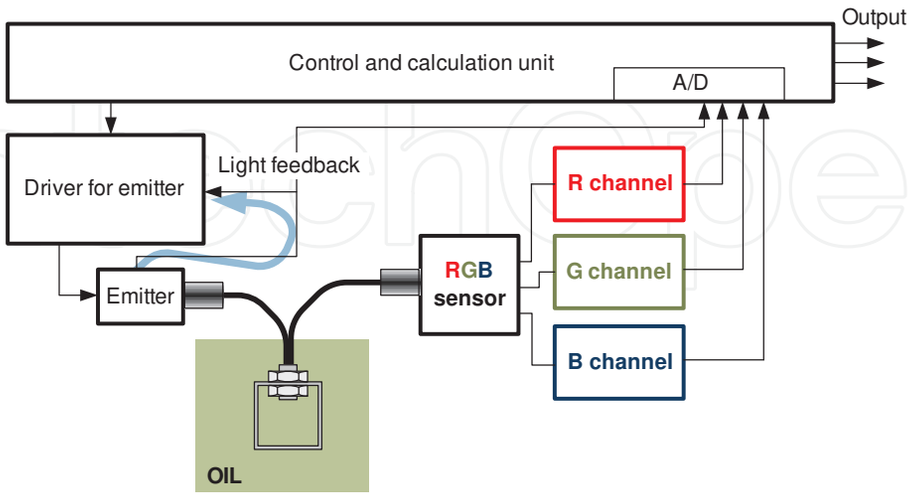


Figure 22. Optical fiber RGB colorimeter applied to oil colour characterization.

As we can see in Figure 23, oil colour can be used to identify the origin of oil, even with only two wavelengths: red (620 nm) and green (540 nm), reducing the blocks of block diagram of Figure 22. A more precise identification needs the value of blue (420 nm) channel and could provide additional knowledge, such as adulteration of oil with dye or the evolution of properties during cycling use for deep frying.

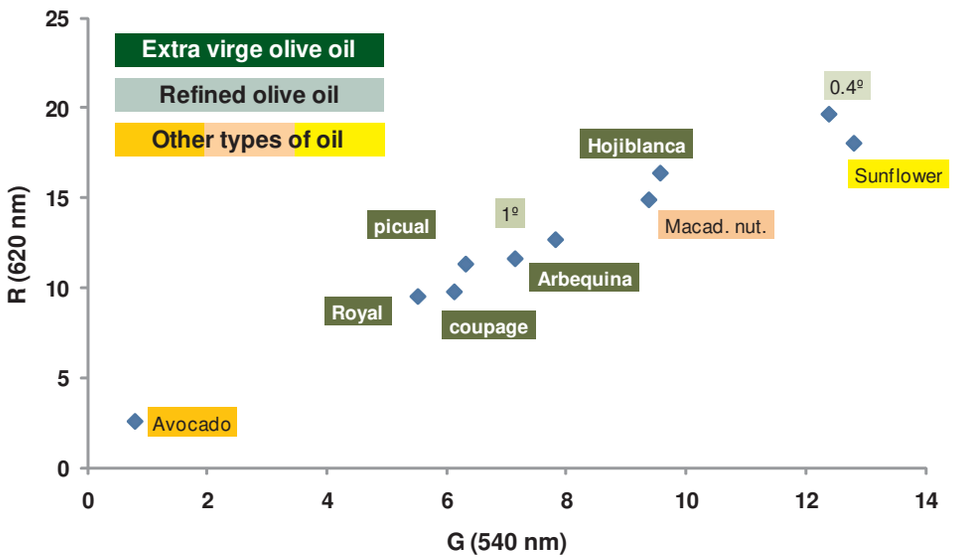


Figure 23. Differentiation of several types of consumption oils by means the values of green (abscissas) and red (ordinates), using arbitrary units.

6. Conclusions

Optical fiber sensors are widely applied for a lot of measurement processes because they have important advantages such as the high noise immunity and the use for remote and multi-position measurement. In particular, the use of optical fibers in combination to chemical sensors increases the potentiality of these sensors and extends their applications.

In above sections, we have presented several operation principles (absorbance, reflectance and luminescence), data processing strategies, and the potential use for measurement purposes by means of some real implementation and the consequent discussion about experimental results. For all these systems, we have taken into account some restrictions and conditions of associated devices such as light excitation sources, photo detector devices and, of course, the design conditions of optical fiber systems and sensors.

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References

[1] Altamirano, M, Senz, A, & Gasponer, H. E. Luminescence quenching of tris (2,2-bi-pyridine) ruthenium(II) by 2,6-dimethylphenol and 4-bromo-2,6-dimethylphenol in sol-Gel processed silicated thin films. J. Colloid Interface Sci, (2004). , 270, 364-370.

[2] Barragán, N. A, Pérez, M. A, Campo, J. C, & Alvarez, J. C. Photo detection of low level phosphorescence produced by low level oxygen concentrations using optical system based on fiber optical and avalanche photodiode. Proc. IEEE-ISIE Puebla (Mexico), (2000). , 596-601.

[3] Basu, B. J, et al. Optical oxygen sensor coating based on the fluorescent quenching of a new pyrene derivative. Sensors and Actuators, (2005). , 104, 15-22.

[4] Campo, J. C, Pérez, M. A, Mezquita, J. M, & Sebastián, J. Circuit-design criteria for improvement of xenon flash-lamp performance (lamp life, light-pulse, narrowness, uniformity of light intensity in a series flashes). Proc. IEEE-APEC, Atlanta (USA), (1997). , 1057-1061.

[5] Campo, J. C, Pérez, M. A, González, M, & Ferrero, F. J. Measurement of air moisture by the phosphorescence lifetime of a sol-gel based sensor. Proc. IEEE-IMTC, Baltimore (USA), (2000).

[6] Campo, J. C, Barragán, N, Pérez, M. A, & Álvarez, J. C. A comparison between different excitation/detection systems for luminescence lifetime based instrumentation. Proc. IEEE-IMTC, Anchorage (USA); May, (2002).

[7] Carleos, C. E, et al. On-line estimation of fresh milk composition by means of Vis-NIR spectrometry and partial least squares method (PLS). Proc. IEEE-IMTC, Victoria (Canada), (2008).

[8] Cleveland, W. S. Robust locally weighted regression and smoothing scatterplots. J Amer Statist Assoc 74: (1979). , 829-836.

[9] Costa, J. M, et al. A critical comparison of different solid supports to develop room-temperature phosphorescence sensing phases in air moisture", Sensors and Actuators B, (1997).

[10] Cozzolino, D, et al. Chemometrics and visible-near infrared spectroscopy monitoring of red wine fermentation in a pilot scale. Biotech. and Bioeng. D01.1002/bit. 21067, (2006).

[11] De Graff, B. A, & Demas, J. N. Luminescence-based oxygen sensors. Reviews in fluorescence. Springer Science, (2005). , 2, 125-151.

[12] Demas, J. N, Degraff, B. A, & Coleman, P. Oxygen sensors based on luminescence quenching. Anal. Chem. (1999). , 793A-7800.

[13] Dessy, R. Waveguides as chemical sensors". Anal. Chem. Nº 19, Oct. (1989). , 61, 1079-1088.

[14] Douglas, P, & Eaton, K. Response characteristics of thin films oxygen sensors, Pt and Pd octaethylporphyrins in polymer films. Sensors and Actuators, (2002). , 82, 200-208.

[15] Draxler, S, et al. Effect on polymers matrices on the time-resolved luminescence of a ruthenium complex quenched by oxygen. J. Phy. Chem., (1995). , 99, 3162-3167.

[16] Eshkenazi, I, et al. A Three-cascade enzyme biosensor to determine lactose concentration in raw milk. J. Dairy Sci. 83, (2000). , 1939-1945.

[17] Fisher, R. P, & Winefordner, J. D. Pulsed source-time resolved phosphorimetry. Analyt. Chem. Nº 6, May, (1972). , 44, 948-956.

[18] Gewehr, P. M, & Delpy, D. T. Optical oxygen sensor based on phosphorescence lifetime quenching and employing a polymer immobilised metalloporphyrin probe. Part 1: theory and instrumentation. Medic. & Biol. Eng. & Comp. Jan (1993). , 2-10.

[19] Gil, R, Gómez, E, Martínez, A, & López, J. M. Evolution of the CIELAB and other spectrophotometric parameters during wine fermentation. Influence of some pre and postfermentive factors. Food Research Internat. (1997). , 30(9), 699-705.

[20] Gillanders, R. N, Tedford, M. C, Krilly, P. J, & Bailey, R. T. A composite Sol-Gel fluoropolymer matrix of dissolved oxygen optical sensing. J. Photochem. Photobiol. (2004). , 163, 193-199.

[21] Grillo, G. J, Pérez, M. A, & Florencias, A. E. Synchronic filter based on switched capacitor filters for high stability phase-detectors systems". Proc. IEEE-IMTC, Sorrento (Italy), April, (2007).

[22] Hercules, D. M. Fluorescence and phosphorescence analysis. J. Wiley and Sons, N. York, (1966).

[23] Hurtubise, R. J, Ackerman, A. H, & Smith, B. W. Mechanistic aspects of the oxygen quenching of the solid-matrix Phosphorescence of perdeuterated phenanthrene on partially hydrophobic paper. App. Spectros. (2001). , 55, 490-495.

[24] Janata, J, & Berzegh, A. Chemical sensors. Analyit. Chem. Nº 12, June, (1988). , 60, 62R-69R.

[25] Janata, J, Josowicz, M, & Devaney, D. M. Chemical sensors. Analyt. Chem. Nº 12, June, (1994). , 66, 207R-228R.

[26] Janata, J, & Josowics, M. Chemical sensors. Analyt. Chem. Nº 12, June, (1998). , 70, 179R-208R.

[27] Kneas, K. A, Xu, W, Demas, J. N, & De Graff, B. A. Dramatic demonstration of oxygen sensing by luminescence quenching. J. Chem. Ed., 696, (1997).

[28] Lechuga, L. M, Calle, A, & Prieto, F. Optical sensor based on evanescent field sensing. Part I: surface Plasmon resonance sensors". Analyt. Chem. suppl 1), Jan, (2000). , 19, 54-60.

[29] Martens, H, & Naes, T. Multivariate calibration". Wiley. Chichester. (1989).

[30] Martin, M. J, Wickramasinghe, Y. A. B. D, Newton, T. P, & Crowe, J. A. Fiber-optics and optical sensors in medicine" Med. Biol. Eng. Comp. (1987). , 25, 597-604.

[31] Mills, A. Response characteristics of optical sensor for oxygen: models based on a distribution on t_0 or Kq", Analyst, (1999). , 124, 1301-1307.

[32] Narayanaswamy, R. Optical chemical sensors: transduction and signal processing. Analyst, April, (1993). , 118, 317-322.

[33] OIVOeno Resolution 1/(2006). Determination of the chromatic characteristics according to CIElab. Certificate as. Paris, July, 28th 2006.

[34] OIVCompendium of international methods of wine and must analysis. International Organisation of Vine and Wine. Edition (2006).

[35] Ogurtsov, V. I, & Papkovsky, D. B. Selection of modulation frequency of excitation for luminescence lifetime-based oxygen sensors. Sensors and Actuators, (1998). , 51, 337-381.

[36] Pawlowsky, M, & Wilson, D. F. Monitoring of the oxygen pressure in the blood of live animals using the oxygen dependent quenching of phosphorescence" Adv. Esp. Med. Biol, (1992).

[37] Pérez, M. A, Campo, J. C, González, M, & López, P. Design of a multi-parameter substance analyzer", Proc. IEEE-IMTC, Baltimore (USA) (2000). , 3, 1584-1589.

[38] Pérez, M. A, & Muñiz, R. Optical fibre turbidimeters. Ch. 8 of Optical Fibre. New developments, Intech, Dec. (2009).

[39] Prieto, M, Braña, E, Campo, J. C, & Pérez, M. A. Thermal performance of a controlled cooling system for low-level optical signals. App. Thermal Eng. March (2004). , 24, 2041-2054.

[40] R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, (Austria). 2007.

[41] Seitz, W. R. Chemical sensors based on fiber optics". Analyt. Chem. Nº 1, Jan. (1984). , 56, 16A-34A.

[42] Torre, C, Muñiz, R, García, B, & Pérez, M. A. A new, low-cost RGB colorimeter for wine industry based on optical fibers. Proc. XIX World IMEKO Conf. Lisbon (Portugal), (2009).

[43] Tsenkova, R, et al. Near-infrared spectroscopy for dairy management: measurement of un-homogenized milk composition. J. Dairy Sci. 82: (1999). , 2344-2351.

[44] Tsenkova, R, et al. Near-infrared spectroscopy for biomonitoring: cow milk composition measurement in a spectral region from 1100 to 2400 nm. J. of Animal Sci. 78: (2000). , 515-522.

[45] Tsenkova, R. Visible/near-infrared spectrometry and its device. US Patent 7570357, Aug (2009).

[46] Weiss, G. H, Bendler, J. T, & Schlesinger, M. F. Continuous-side model for Langmuir gas sorption in glassy polymers. Macromol. (1992). , 25, 990-992.

[47] White, C. E, & Argauer, R. J. Fluorescence analysis. CRC Press, Oct. (1970).

[48] Wolfbeis, O. S. Fiber-optic chemical sensors and biosensors, I & II. CRC Press, (1991).

[49] Young-Ha Woo et alDevelopment of a new measuring unit (MilkSpec 1) for rapid determination of fat, lactose and protein in raw milk using NIR transmittance spectroscopy. Appl. Spectros. (2002). , 5(56)

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