Dual wavelength measurement system for absorbance chemical sensing

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Abstract. A novel compact dual-wavelength measurement system for absorbance chemical sensing incorporating digital lock-in amplifiers is reported. Its main innovation is the introduction of a reference wavelength to correct the possible non-specific optical changes of the system. The performance of the intrumentation has been tested on a laboratory setup and with integrated optical absorbance sensors.

Keywords: Dual-wavelength measurement, Lock-in amplification, absorbance measurement, DSP - Digital Signal Processor

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1. Introduction

The interest in opto-chemical sensors has been growing rapidly in recent years. These sensors take advantage of the development of both optical communication technology and analytical chemistry techniques. One of these techniques is direct or chemically-mediated absorption spectroscopy [1, 2]. Direct spectroscopy measures the characteristic absorption spectra of an analyte, whereas chemically-mediated spectroscopy indirectly measures the spectral changes of a dye when it interacts with the analyte under investigation.

Chemically-mediated spectroscopy provides better sensitivity and selectivity than the direct technique [3]. In chemically-mediated spectroscopy, the working absorption wavelength is determined only by the spectral properties of the selected dye. Therefore, it is easier to directly substitute conventional and bulky spectrophotometers for simpler interrogators consisting of narrow-band optical sources. However, the use of a unique absorption band does not avoid the distortion of the signal due to non-specific absorptions (not related to the dye) or fiber bendings. These interferences can be reduced by using a reference signal, usually a second narrow-band light source, which is affected by the interferences as well and that is immune to the chemically induced absorption. This technique is called dual-wavelength spectrophotometry and it has been successfully used to compensate the absorbance interferences due to the Refractive Index (RI) of the sample [4] or simultaneously the RI and its turbidity [5] in Flow Injection Analysis (FIA) systems.

In this work, we report a novel compact dual wavelength measurement system based on digital lock-in amplification. It is designed for optical absorbance sensors and has been evaluated using an Integrated Waveguide Absorbance Optode (IWAO), which was previously developed in our research group [6]. This device consists of a chemically active polymeric membrane deposited over an optical integrated planar waveguide circuit and offers the advantages of integrated optics (miniaturization of the sensing system and reduction of costs and size) and those of chemically active membranes (increase of sensitivity of the sensor while reducing the response time).

The system has been successfully tested with some of these IWAOs, showing an adequate correction of undesired effects such as the membrane hydration.

2. Measurement system - description

The components of the opto-chemical dual wavelength measurement system and their connections with the absorbance sensor are shown in figure 1. The system includes two light sources with different wavelengths; one of them is used as the absorbance wavelength and the other is used as a reference signal for dual-wavelength spectrophotometry.

Light sources are Light Emitting Diodes (LEDs) modulated and controlled by a Digital Signal Processor (DSP). This DSP generates two sine waves to modulate each

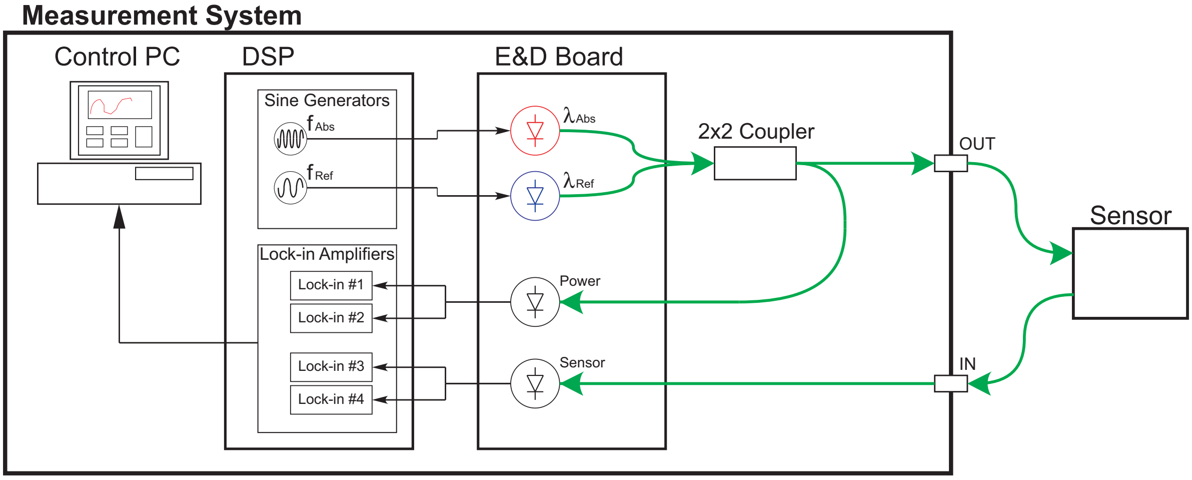

Figure 1. Scheme of the measurement system and its connection with the absorbance sensor.

LED at a different frequency. Light from both LEDs is injected into a multimode fiber using a 2x2 coupler. The first coupler output is connected to the Sensor Loop while the second is used as a reference for the LEDs output power (Monitoring Loop) to correct the possible emission fluctuations. Signals coming from the two loops are separately detected by two photodetectors and the DSP processes them using lock-in amplification.

2.1. Lock-in amplification process

Lock-in amplification is one of the traditional techniques for frequency-selective detection [7]. It uses synchronous demodulation to measure the signal level at a fixed frequency, removing noise and unwanted signals of different frequencies by acting as a tunable narrow band-pass filter. Lock-in amplification is mainly used in optical applications with a modulation frequency under 100kHz, thus allowing the recuperation of signals with a very poor signal to noise ratio (SNR). Besides improving the SNR, a Lock-In Amplifier (LIA) can be used as a frequency-selective detection technique to separate several wavelengths when using only one photodetector. In our system, four LIAs have been implemented to separate the two wavelengths in each loop. These dual-phase LIAs are based on a previous work from our research group [8, 9]. While basic lock-in amplification results in a phase-sensitive detection, dual-phase lock-in amplifiers provide separate measurements of the amplitude and the phase of the signal [7]. This not only results in a phase-independent measurement of the signal level, but also allows some noise correction using the information of the phase

In dual-phase lock-in amplification (figure 2), the input signal S is demodulated by multiplying it by two reference signals in quadrature (I and Q). Then, the products V_X and V_Y pass through low-pass filters (LPF) to remove non-DC components. The resultant signals X and Y are directly related to the magnitude M_S and phase θ_S of

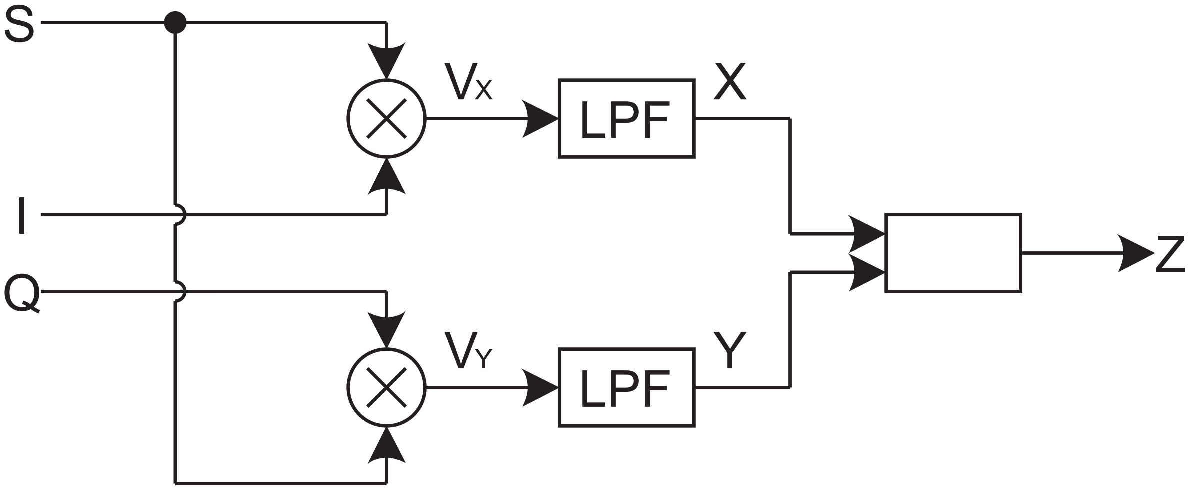

Figure 2. Dual-phase lock-in amplifier block diagram.

the signal S,

$$M_S = \sqrt{X^2 + Y^2}$$
$$\theta_S = \arctan\left({}^{Y}\!/_{X}\right).$$

The bandwidth of the LPF determines the SNR improvement, according to the classic signal to noise improvement factor for the recovery of signals from white noise [7],

$$\frac{SNR_O}{SNR_I} = \frac{B_I}{B_O}$$

where B_I is the input noise bandwidth and B_O is the bandwidth of the filter.

3. Measurement system - implementation

All the components of our measurement system have been encapsulated into the control PC case, as shown in figure 3, resulting in a compact and portable piece of equipment

3.1. DSP implementation

The Digital Signal Processor is an ADSP-21161N from Analog Devices. It is embedded in an evaluation board (ADSP 21161 EZ KIT LITE) for digital audio applications. This board also contains the AD1836 96kHz 24bits Audio Codec, which provides 4 Analog-to-Digital Converters (ADC) as inputs and 8 Digital-to-Analog Converters (DAC) as outputs. These converters are used to communicate the DSP with the Emission and Detection Board (E&D Board), where the light sources and the detectors are placed. The DSP functions implemented in the evaluation board can be seen in figure 4.

- The first DSP process (1 in figure 4) generates the two sine waveforms. This process runs at 48kHz and generates two sine waves at 1200Hz and 480Hz, which are sent

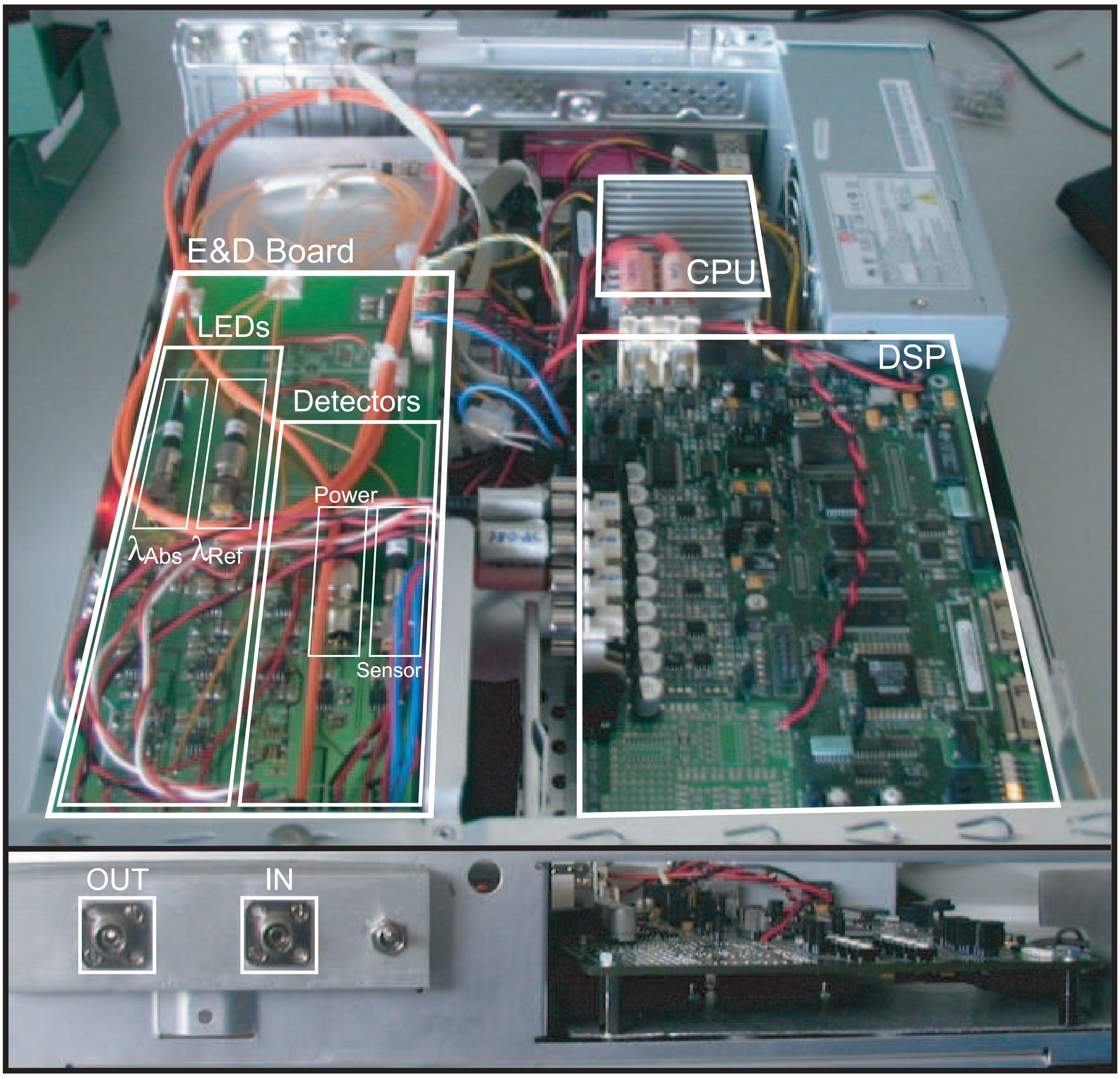

Figure 3. Measurement system photograph. All elements are enclosed in a 32×10×42cm commercial PC-case.

to the AD1836 and are converted to analog signals for driving the LEDs on the E&D Board. The bias points of these LEDs are coded in the amplitude of a third signal at 12000Hz. This third sine wave is necessary because the evaluation board can not output DC signals.

- The second task of the DSP (2) is the processing of the signals detected by the photodetectors. First, the AD1836 passes the signals through antialiasing filters (with a cutoff frequency of 24kHz) and digitizes them with a sampling frequency of 48kHz. Then, the digital signals S_S and S_P are decimated using antialising filters (4th order Low Pass IIR Butterworth Filters with 2.4kHz cutoff frequency) and downsampling them by 10 to obtain 4.8kHz sampled signals S_S' and S_P'. This downsampling is very important, as it allows the implementation of the four needed

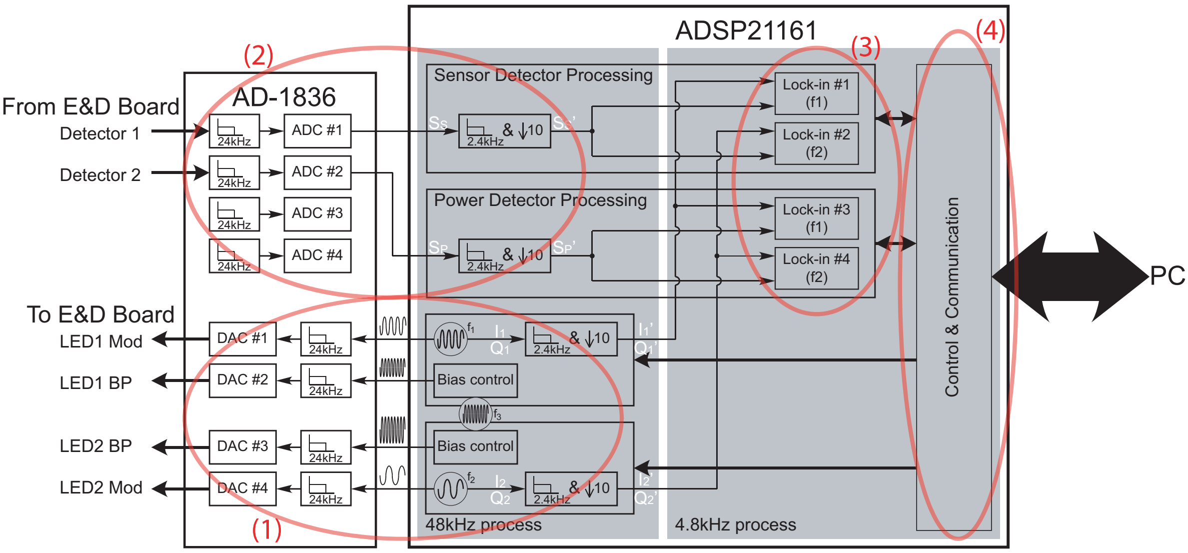

Figure 4. DSP Board scheme.

LIAs in the same DSP which would be impossible if the lock-in processes ran at 48kHz. The limit of downsampling, and so the number of LIAs, is the Nyquist criterion: the sampling frequency can not be smaller than twice the highest LED modulation frequency.

- The four LIAs implemented are therefore dual-phase lock-in amplifiers running at 4.8kHz (3). The downsampled signals S_S*'* and S_P*'* are multiplied by the reference signals I*'* and Q*'*, which in turn are obtained by downsampling the sine waves which were generated in the first 48kHz-process (I and Q). The multiplied signals are then low pass filtered to increase the SNR, by passing them twice through the same 2nd order Low Pass IIR Butterworth filter. The two Monitoring Loop LIAs use 32 bit low pass filters with a fixed integration time of 30 ms, while the two Sensor Loop LIAs use 64 bit low pass filters with a configurable integration time (10 ms, 30 ms, 100 ms, 300 ms or 1000 ms). The output signals of the LIAs are the amplitude and phase of each of the four optical signals.
- The last task of the DSP (4) is handling the communication with the PC via RS-232 interface [10]. The PC sends control commands through this connection to the DSP to change the LIAs configuration and the LEDs operation, while the DSP sends the output signals from the LIAs to the PC.

3.2. E&D Board

Emission and detection electronics are implemented on the same printed circuit board (E&D Board). Emission electronics consists of two LEDs and their control circuits. In the first stage the LEDs bias points from the amplitude of the third sine wave are obtained by a Precision Full-Wave Rectifier. Then, in a second stage, the modulating signal (previously generated sine waveforms of 480Hz or 1200Hz) is added to the bias voltage. Finally, a third stage drives the LEDs with this composite signal. The emission maximum of the absorbance LED is at 780nm, which matches the absorption maximum of the active membrane used in the sensor. On the other hand, the reference LED is located at 850nm, out of the absorption bands of the membrane and as close as possible to the absorbance wavelength in order to improve the dual-wavelength spectroscopy.

Detection electronics consists of two photo-detectors (one for each loop) and their amplification stages. These are Silicon-PIN-photodiodes and the amplification is achieved by transimpedance stages.

3.3. Control PC

A Microsoft Visual C# based Graphic User Interface (GUI) has been implemented in the Control PC. It includes all the control commands and some post-processing and visualization options for the acquired data. The main control command is the selection of the LIAs integration time, which determines the bandwidth of the internal low pass filters (longer integration times mean narrower low pass filters).

The first data processing is the correction of the electrical noise of the same frequencies than the modulation signals by using the magnitude and phase information of the signal. The electrical noise phasor $\overrightarrow{N}$ is determined prior to the measurements when no optical signals are received by a calibration with the Sensor Loop open. This calibration is needed to correct the measured phasor $\overrightarrow{Z}$ by a simple phasor substraction to obtain the real value $\overrightarrow{O} = \overrightarrow{Z} - \overrightarrow{N}$.

The PC also performs the conversion of the captured signals into absorbance data. The voltage measured in the Sensor Loop is referred to the voltage measured in the Monitoring Loop, obtaining the dynamic absorbance for each wavelength. Then, the data of the absorbance wavelength is corrected with the data of the reference wavelength to obtain the corrected absorbance which correspons only to the chemical processes in the membrane.

4. Measurement system - evaluation

Prior to performing the IWAOs evaluations, the system response has been evaluated using the set-up presented in figure 5. This setup consists of two multimode attenuators: the attenuator #1 between the reference LED and the coupler, and the attenuator #2 between the coupler and the sensor detector. The purpose of this setup is to attenuate only one of the wavelengths with attenuator #1 and the two wavelengths at the same time with attenuator #2 in order to simulate non-chemical interferences.

The first characteristic of the system which has been evaluated is its linearity. The measured attenuation is recorded while the attenuation of the two wavelengths is changed using attenuator #2. The linearity of the system is almost perfect (figure 6) and its dynamic range is 50dB. The wavelength dependence of the attenuators is also noticeable and has been taken into account in the following evaluation.

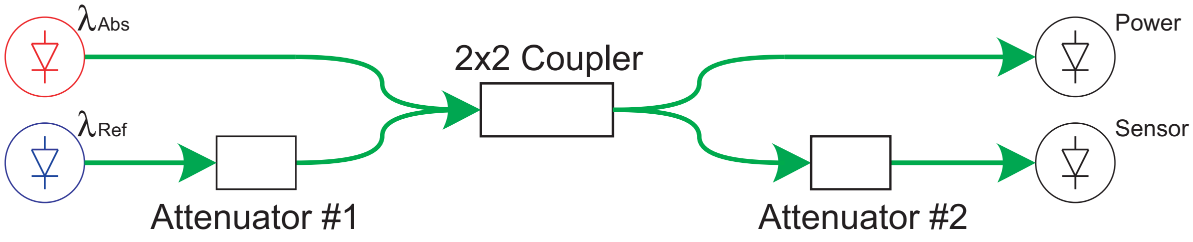

Figure 5. Laboratory set-up. Attenuator #1 varies only the reference signal, while Attenuator #2 affects both wavelengths.

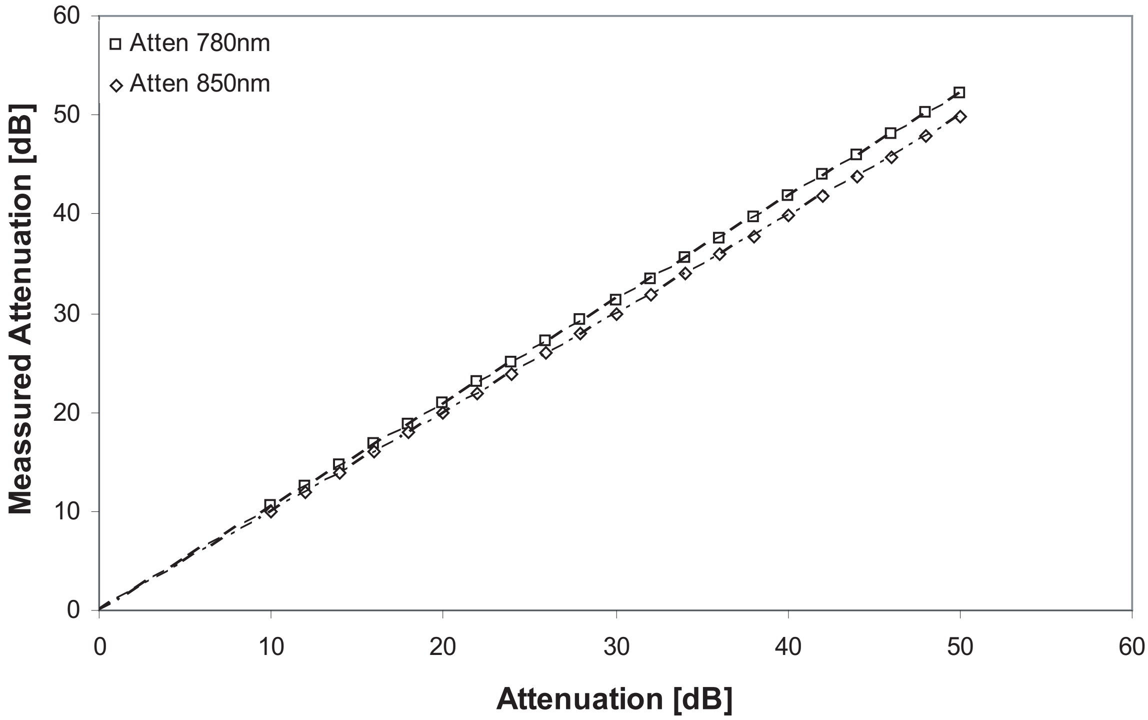

Figure 6. Response of the system to the attenuation of both wavelengths (Attenuator #2). The attenuator shows linear behavior but also a wavelength dependence which must be corrected.

After this, the effectiveness of the dual-wavelength correction has been also evaluated. Results are shown in figure 7, where the measured attenuation for each wavelength and the corrected attenuation (calculated using the second wavelength) are plotted along with the real attenuations (introduced by the calibrated attenuators). During the first seven minutes the attenuator #2 is manipulated to affect the two wavelengths at the same time. The maximum error in the measured corrected attenuation is very small (0.05dB at 30dB of attenuation). After the 7th minute, the attenuation of the reference wavelength is varied using attenuator #1. The corrected attenuation matches the real values, with differences lower than than 1dB.

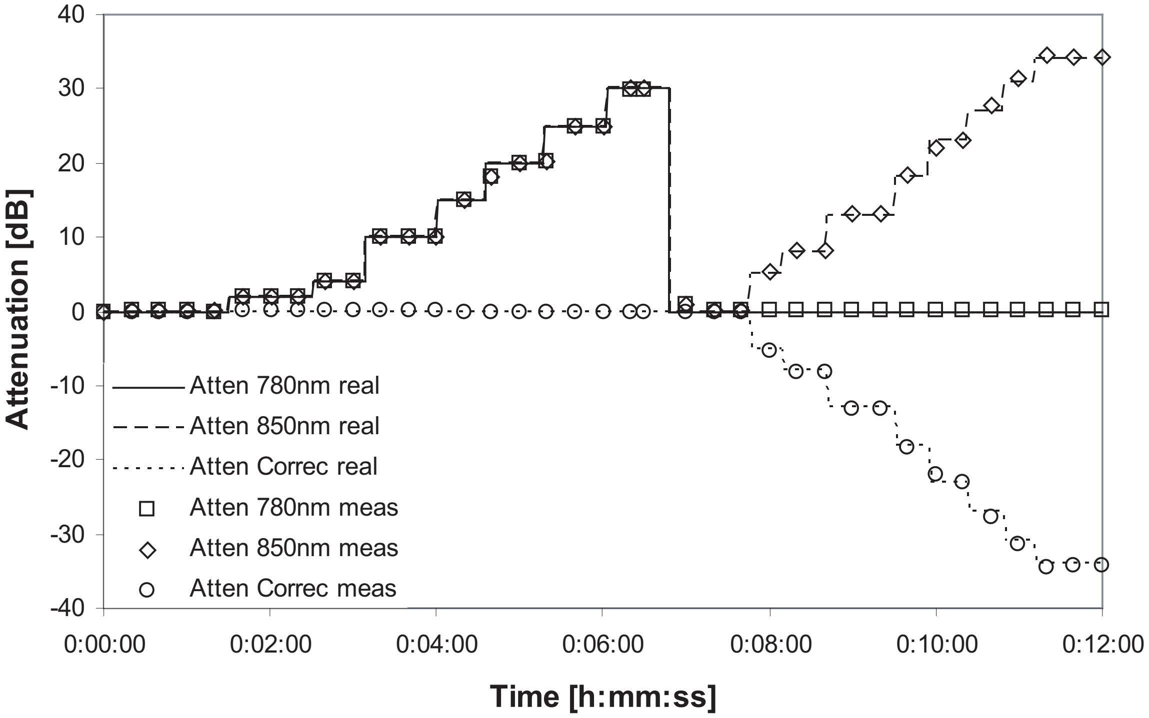

Figure 7. Measured and real attenuation for each wavelength.

5. Complete system evaluation

The chemical evaluation of the system response has been performed using two different ion-selective absorbance sensors. Calcium and mercury membranes have been chosen as optode models because they were previously used for the development of Integrated Waveguide Absorbance Optodes (IWAO) [6, 11]. The calcium sensor has been used as the initial approach because membrane characteristics, concerning the detection limit, selectivity and sensibility, can be known from the literature [12] as the membrane components are commercial, with the exception of the chromoionophore. After that, a mercury sensor has been employed as a first trial for a future environmental application, in order to take profit of the advantages of using the dual wavelength system. A FIA system has been employed as an automatic tool for membrane characterization by sequential injection of the discrete standard solutions into a continuously flowing stream (figure 8).

5.1. Absorbance sensor

The IWAO consists of a chemically active membrane deposited over a planar waveguide circuit defined by Complementary Metal Oxide Semiconductor (CMOS) compatible processes over a silicon wafer. Three main parts of the IWAO can be distinguished (figure 9): (1) the transducer based on a curved rib ARROW [13] planar waveguide

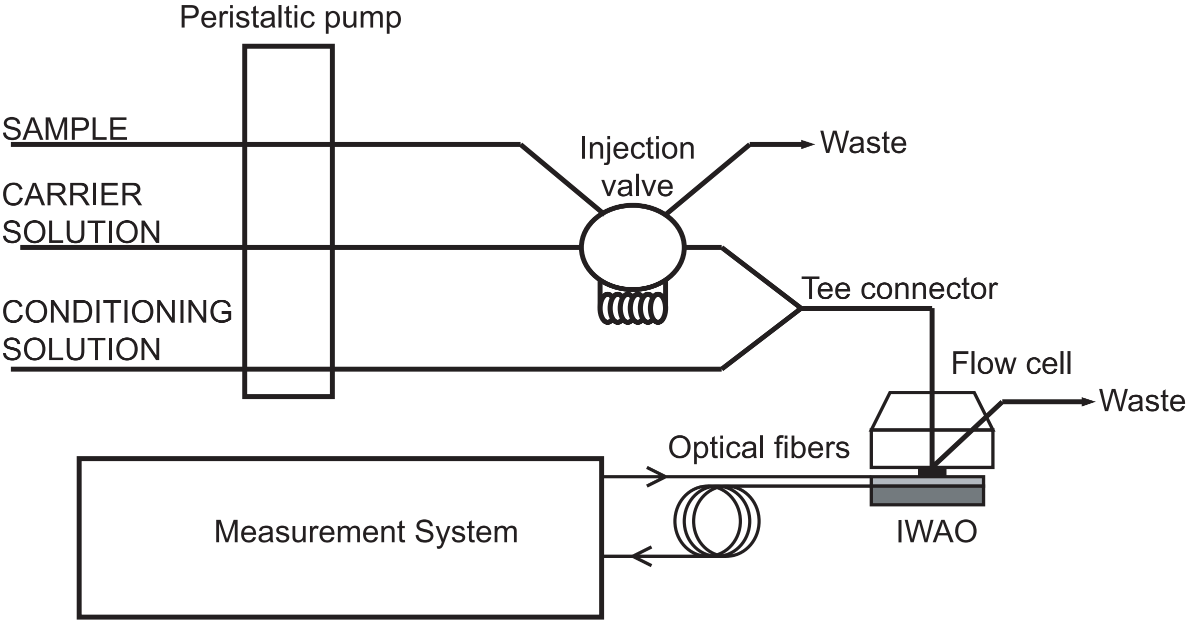

Figure 8. Scheme of the employed FIA System. A certain volume of the standard solutions (calcium or mercury nitrates) in the sample channel are injected in a carrier solution (double distilled water), which is continuously directed to the detector. This stream is mixed previously to the detection with a conditioning solution, which maintains constant the pH value. The carrier solution determines the base line (fully protonated chromoionophore) and an absorbance decrease is recorded when the analyte reaches the optode.

with the active chemical membrane, (2) the V-grooved auxiliary support, where the optical fibers from and to the measurement system are fixed, and (3) the steel mounting platform, which allows the alignment of the waveguides to the optical fibers.

The active plasticized-PVC membrane contains an ion-selective ionophore, a lipophilic ionic salt to maintain the electroneutrality, and a chromophore (a pH indicator/dye). The analyte interacts with the ionophore, and the chromophore releases protons to maintain the electroneutrality in the membrane. As a result, changes in the spectral properties of the dye when deprotonating can be monitored [14]. The composition of the membranes consists of approximately 61-68% of PVC as the polymer support, 29-30% of Tris-2-(etthylhexyl)phosphate as the plasticizer, 2% of Bis[4-(1,1,3,3-tetramethylbutyl)phenyl] phosphate calcium salt or 7% of a thiourea as the calcium and mercury ionophores respectively, 0.4% of the Potassium tetrakis(4-chlorophenyl)borate as an anionic additive and 0.4% of ketocyanine 5ce [15] as the chromoionophore

Ketocyanine 5ce presents an absorption maximum centered at 770nm as shown in figure 10, matching the wavelength of the absorbance LED, and provides high capacity to differentiate between little pH variations (0.49 A.U./ΔpH) as a result of its high molar absorptivity (2.4×10^5 $\mathrm{l}\times\mathrm{mol}^{-1}\times\mathrm{cm}^{-1}$). The reference wavelength (fixed at 850nm) is out of the absorption bands and as close as possible to the absorbance wavelength to maximize the dual-wavelength spectroscopy correction.

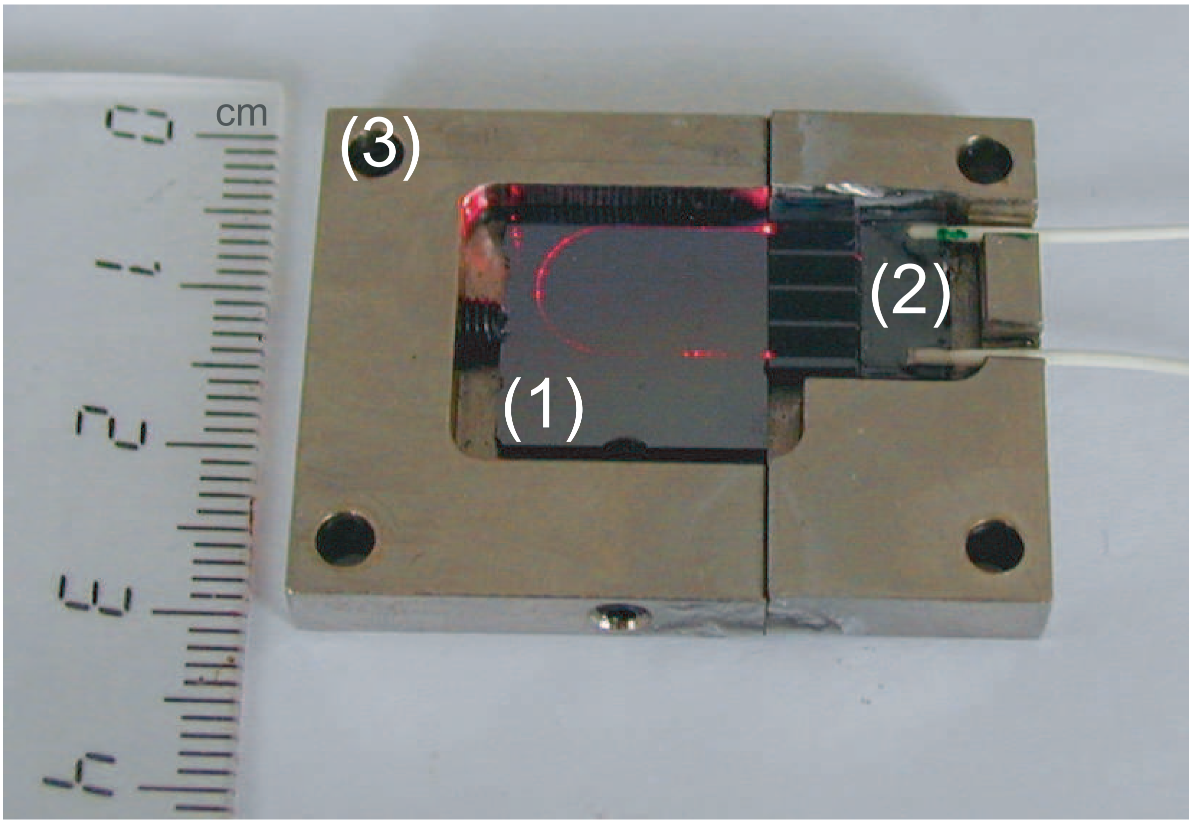

Figure 9. Photograph of an IWAO: (1) Transducer; (2) V-grooved platform; (3) connection platform.

5.2. Calcium measurements

The first formulized optode is a calcium-selective membrane. For this purpose a commercial calcium ionophore has been used, the selectivity, sensitivity and detection limit of which was previously evaluated in a conventional spectrophotometer. Figure 11 shows the recorded signals with and without the reference wavelength correction when three standard solutions of the same concentration are injected in the FIA System. One of the problems of the plasticized-PVC membranes is the change of its absorbance while it is hydrating at the beginning of the experiments. This effect can be directly corrected using the reference wavelength (as shown in figure 11), because the guiding properties of the membrane for both wavelengths vary in the same magnitude. Also, our system improves the good features of the IWAOs, mainly their higher sensitivity and similar detection limit using the same membrane in a conventional configuration. With the direct signal the sensitivity obtained is S=0.127 A.U./decade and the D.L.=1.41×10^{-4} M, with the corrected one S=0.139 A.U./decade and D.L.=3.8×10^{-4} M and compared to those of a conventional spectrophotometer, S=0.011 A.U./decade and D.L.=1.17×10^{-4} M.

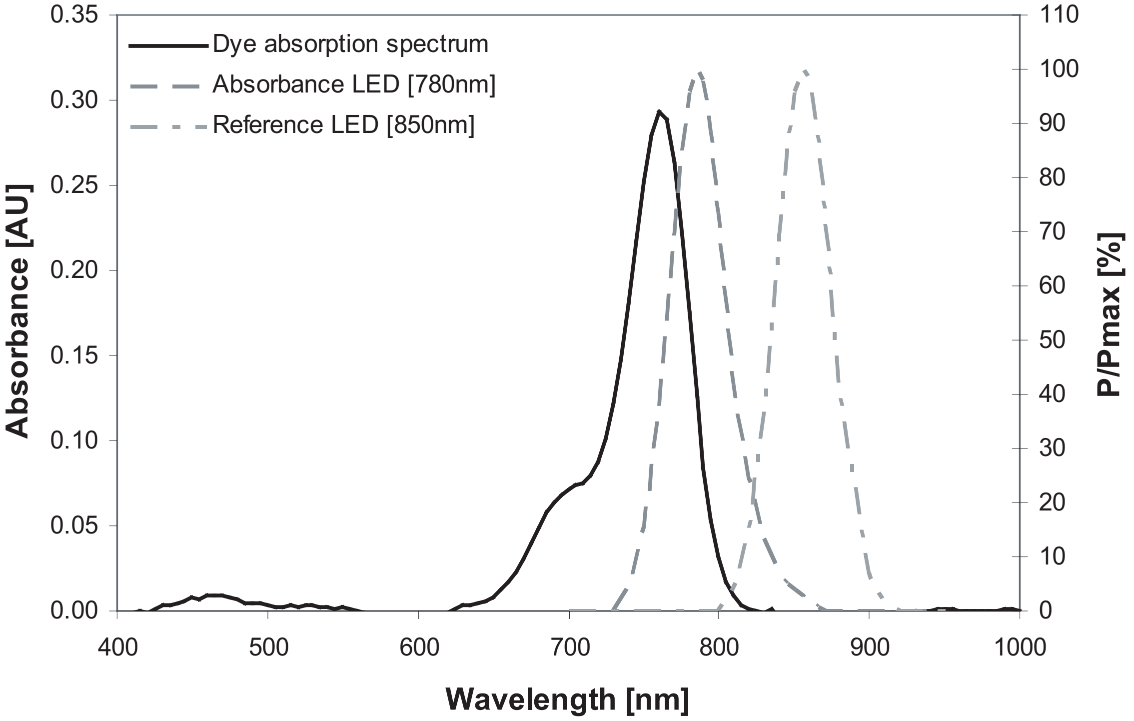

Figure 10. Localization of the absorption bands of the dye and the LEDs.

5.3. Mercury measurements

A mercury-selective membrane with a newly synthesized mercury ionophore based on a thiourea has also been used to test the system performance. Mercury is one of the most toxic metals, and its severe negative health effects [16] have promoted the development of analytical methods to determine it. The goal of the work presented in this paper is to prevent the signal deviation during the continuous monitoring of the analyte.

Figure 12 shows the direct and corrected signals, while different mercury standard solutions are injected by triplicate in the FIA system. The membrane shows the same problem of initial hydration as the previous one, which is corrected with the new measurement system while maintaining the sensor sensitivity and detection limit. A slight negative drift in the base line signal during the calibration can be noticed. This effect can not be corrected with the dual wavelength because it is ascribed to the loss of components due to photochemical processes by the continue irradiation of the light sources. The improvement of the dye stability by modifying their structure or adding stabilizing agents to the membrane is under study.

6. Conclusions

A rugged, compact, portable, low cost and automated dual-wavelength measurement system has been developed for sensing applications using absorbance chemical sensors.

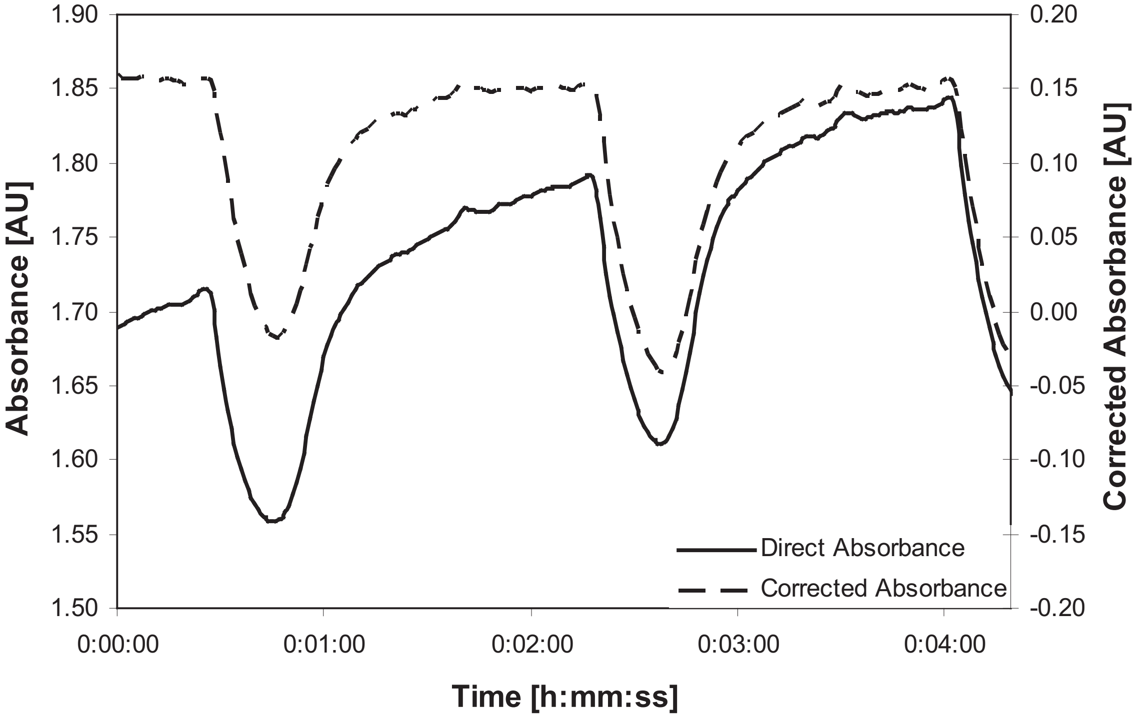

Figure 11. Recorded signals of the Calcium-selective optode showing the correction of the membrane hydration.

The system includes two different wavelengths, one as the absorbance wavelength and the other as a reference. The reference depends only on physical changes of the sensor, correcting the output signal from any undesired interference. To demonstrate the feasibility of the new measurement system, an Integrated Waveguide Absorbance Optode (IWAO) has been activated with two different ion-selective membranes and used for the determination of the concentration of calcium and mercury ions in water samples. The measurements show an excellent correction of the absorbance variations caused by membrane hydration, which constitutes one of the main problems of this kind of measurements. This new measurement system can be used with any absorbance sensor by simply selecting the adequate LEDs for the different absorption bands of the employed dyes.

Acknowledgments

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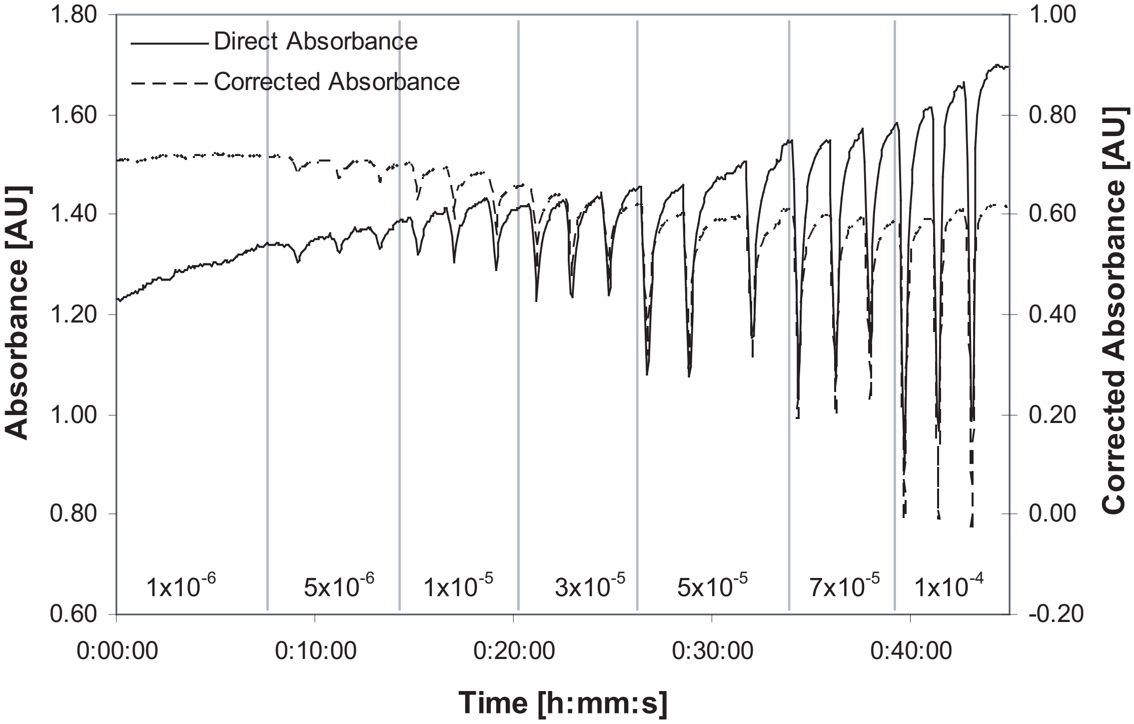

Figure 12. Recorded signals during Mercury-selective membrane calibration.

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