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# Diffusion of Methylene Blue in Phantoms of Agar Using Optical Absorption Techniques

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## 1. Introduction

Diffusion of substances in tissue is an extremely complex process. Various phantoms have been proposed as a model to simulate biological organs and to study physicochemical effects on the human body. Low concentration aqueous agar phantom systems are specially suited for this purpose (Madsen et al., 2005), because they resemble the desired tissue, and are inexpensive to prepare (Bauman et al., 2004). Recently, they have been suggested for the study of the treatment of neurodegenerative diseases of the central nervous system (CNS) by implantation of nanoreservoirs, for controlled drug release into the brain (Staples et al., 2006). A variety of experimental methods have been developed for the study of drug diffusion phenomena in such a complex system. Methylene blue can be used to monitor the diffusion processes inside a gel-like material to simulate the actual process that takes place in the living tissue, since the size of this molecule is similar to that of some chemotherapeutic drugs (Buchholz et al., 2008). Methylene blue is a heterocyclic aromatic chemical compound with the molecular formula  $C_{16}H_{18}N_3S^+Cl^-$ , a scheme of the molecule is shown in Figure 1. Additionally, methylene blue is a molecule that has played important roles in microbiology and pharmacology. It has been widely used to stain living organisms, to treat methemoglobinemia, and recently it has been considered as a drug for photodynamic therapy (Tardivo et al., 2005). This compound shows in-vivo activity against several types of tumors, when locally injected and illuminated with red laser light (Tardivo et al., 2005). Orth and coauthors have demonstrated that intratumoral injection of 1% methylene blue followed by illumination by an argon-pumped dye laser, was able to kill xenotransplanted tumors in animals and recurrent esophageal tumors in patients (Orth et al., 1998).

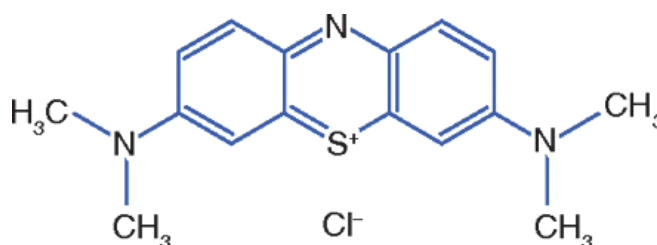


Fig. 1. Molecular structure scheme of the methylene blue.

Various techniques have been developed to study this kind of process using microscopy, optical techniques, electrical analysis, etc. (Bauman et al., 2004). For the experimenter it is always important to have access to new, simple, and reliable methodologies. Optical techniques have been also used successfully to study diffusion processes (Almond & Patel, 1996). These techniques are in general based in the study of light transmission at a fixed height of a sample column or illuminating the whole column to detect the change of the system. In this case, the results have been interpreted as a consequence of variations in the optical properties of the system. Photoacoustic effect has been demonstrated to be a useful tool for materials characterization, and in the study of diverse phenomena (Almond & Patel, 1996; Mandelis, 1993; Vargas & Miranda, 1988). Photoacoustics have been also used recently in the study of the evolution of dynamic systems, such as oxygen release in plants, blood sedimentation, evaporation of liquids, etc. (Acosta et al., 1996; Frandas et al., 2000; Landa et al., 2003; Martinez-Torres & Alvarado-Gil, 2007). The photoacoustic (PA) signal is not only directly related to the time evolution of the optical and thermal properties, but also with various physical processes leading to modulated heat and additional changes in the geometry of the sample (Bialkowski, 1996). The PA technique is based on the periodic heating of a sample illuminated with modulated optical radiation. In a gas-microphone configuration, the sample is in contact with the gas-tight cell. In addition to a steady-state temperature gradient, a thermal wave in the material couples back to the gas around the sample and this will result in a periodic fluctuation of the temperature of a thin layer of gas, close to the sample surface. This thin layer of gas will act as an acoustic piston, which will result in the production of a periodic pressure change in the cavity. A sensitive microphone coupled to the sample chamber can be used to detect this pressure fluctuation.

In this work the diffusion of an aqueous solution of methylene blue into an agar gel using a novel optical technique and photoacoustic spectroscopy are presented. The optic study was performed illuminating with a laser a transparent tube containing the sample of agar, simultaneously the data acquisition of the transmission is done using eight photodiodes. This technique allows measuring the diffusion of methylene blue into the agar as a function of the position and time. Additionally, the diffusion process is monitored applying the photoacoustic technique using a modified Rosencwaig photoacoustic cell (Fernelius, 1980; Quimby & Yen, 1980), in which the sample is illuminated with a modulated red laser beam at a fixed frequency (Teng & Royce, 1980; Wetsel & McDonald, 1977). For both techniques, simple theoretical analyses allow the determination of the evolution of the effective optical properties. The stabilization time of the process, is presented, and it is shown that the characteristic time, in which the dye diffusion process stabilizes, increases with the agar concentration.

## 2. Materials and methods

### 2.1 Materials preparation

Samples were prepared using agar powder (BD Bioxon hygroscopic bacteriologic agar) and 17.4 MΩ.cm of de-ionized water. The following agar powder concentration in water is used for the optical analysis [ $100 \times \text{mass of agar powder} / (\text{mass of agar powder} + \text{mass of water})$ ] and fixed at 0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 % mass/volume (w/v) and for photoacoustic technique measurement 0.01 % and 0.05 % mass/volume (w/v), were analyzed. This difference is due to the size of the agar column analyzed in each case. Optical measurements were made in containers much larger than the ones used in photoacoustics.

The mixture of agar in water was heated up to 80 °C and stirred during 4 min in such a way that all the agar powder is completely dissolved. The resulting solutions were deposited in containers, cooled to room temperature and the containers were sealed.

## 2.2 Optical detection technique

In order to evaluate the diffusion processes, a simple optical system was developed. The experimental arrangement is shown in Fig. 2. In this case, the samples were contained inside glass tubes (10 cm long  $\times$  3 mm diameter). As the light source, a 635 nm and 4 mW laser diode with a uniformly opened elliptical spot, with an approximate area of 1.8 cm long and 3 mm wide, was used to illuminate the glass tube. The light transmitted through the sample is collected on the opposite side of the tube using a Judson PA-7: 16C detector (with a working range of wavelengths from 500 nm to 5.0  $\mu$ m). This detector consists of a linear array of sixteen photodiodes (Fig. 3), with a cross section of 1 mm<sup>2</sup> with a separation of 2 mm between two consecutive photodiodes. The detector output is connected to homemade electronics and from that to a National Instruments BNC-2090 device allowing the detection of eight simultaneous signals along the tube. The analog signals are captured using a data acquisition Analog-Digital card PCI-6035. This information is sent to a PC for storage and subsequent analysis.

The diffusion process was induced by adding 4 mL of methylene blue solution (0.0125 g.mL<sup>-1</sup>) on the upper side of the tube. As a consequence, the methylene solution starts to migrate downwards through the sample and the agar slowly changes color and becomes dyed by the methylene blue. The light transmitted through the sample changes when the dye absorbs the light and this is registered by the photodiodes array detector. In this way, the transmitted light is a direct measurement of the changes in concentration and provides the parameters associated with the kinetic diffusion process. The first photodiode was at 2 mm below the surface of the agar sample.

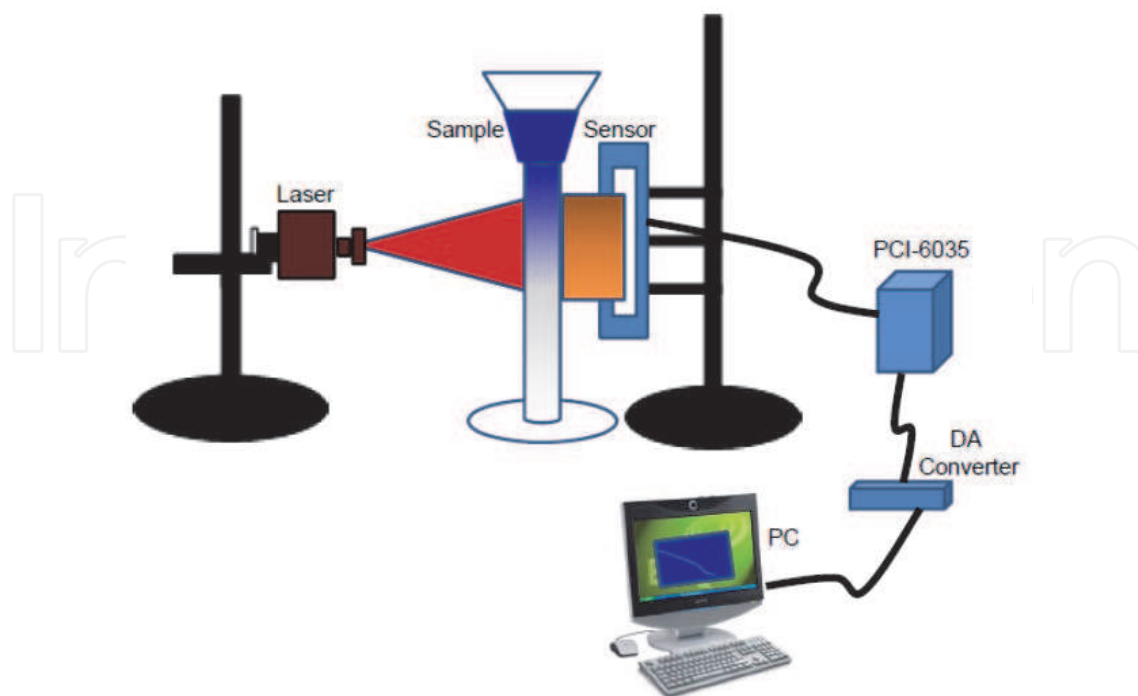


Fig. 2. Experimental arrangement for the light transmission measurement system.

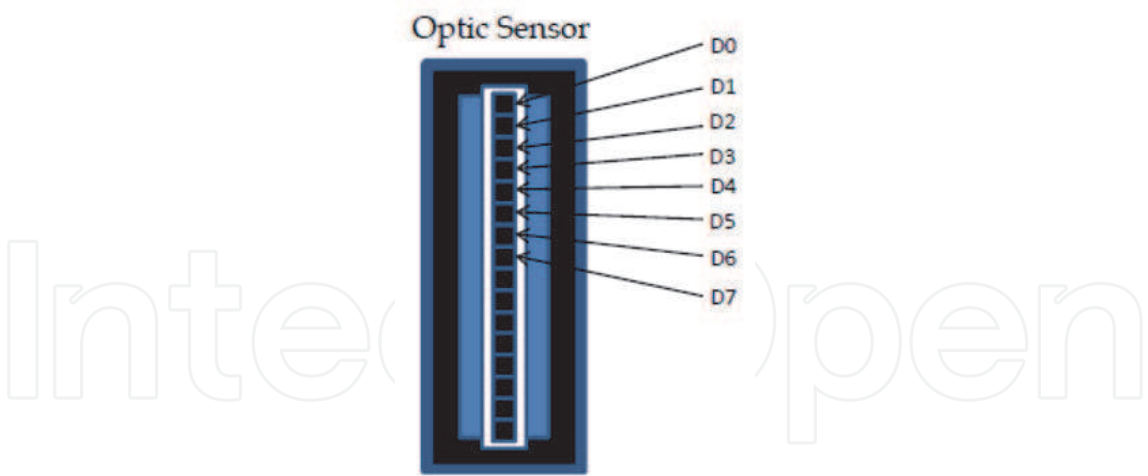


Fig. 3. Cross section of the optic detector, only the indicated upper eight photodiodes, was used.

2.3 Photoacoustic technique

The diffusion process of methylene blue aqueous solutions in agar samples was also using the photoacoustic technique (PA). It consists of a conventional PA cell (Figs. 4 and 5), closed on one side by a transparent quartz window and on the opposite side by a transparent polyvinyl acetate foil, used as a backing material, with a thickness of 98  $\mu\text{m}$  (Vargas-Luna et al., 2002). On top of this foil, the agar gel sample was deposited. The polyvinyl acetate and the sample was illuminated through the quartz transparent window. An electret microphone is used, coupled to the cavity wall, to detect the pressure fluctuations in the PA chamber, generated by the periodic light beam of a 160 mW diode laser at 658 nm (ML120G21) modulated at a constant frequency. The microphone signal is fed into a lock-in amplifier (SR830), from where the output signal amplitude is recorded, as a function of time, in a personal computer. At the beginning of the experiment, 100  $\mu\text{L}$  of agar solution are deposited; when the signal stabilizes, 10  $\mu\text{L}$  of methylene blue solution (0.0125 g.mL<sup>-1</sup>) are added to the surface of the agar with a micropipette. Due to the methylene blue diffusion inside the agar, the PA signal changes in the subsequent stages. In order to get data independent of the microphone characteristics, the PA signal amplitude at any time was normalized dividing it by the maximum value of the PA signal amplitude for a given experiment.

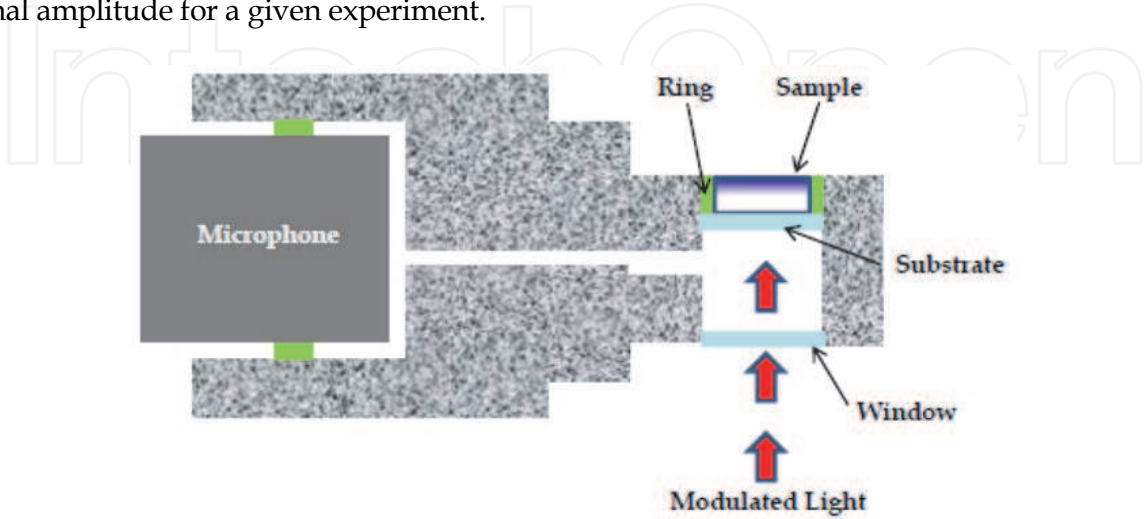


Fig. 4. Schematic cross-section of the used conventional PA cell.

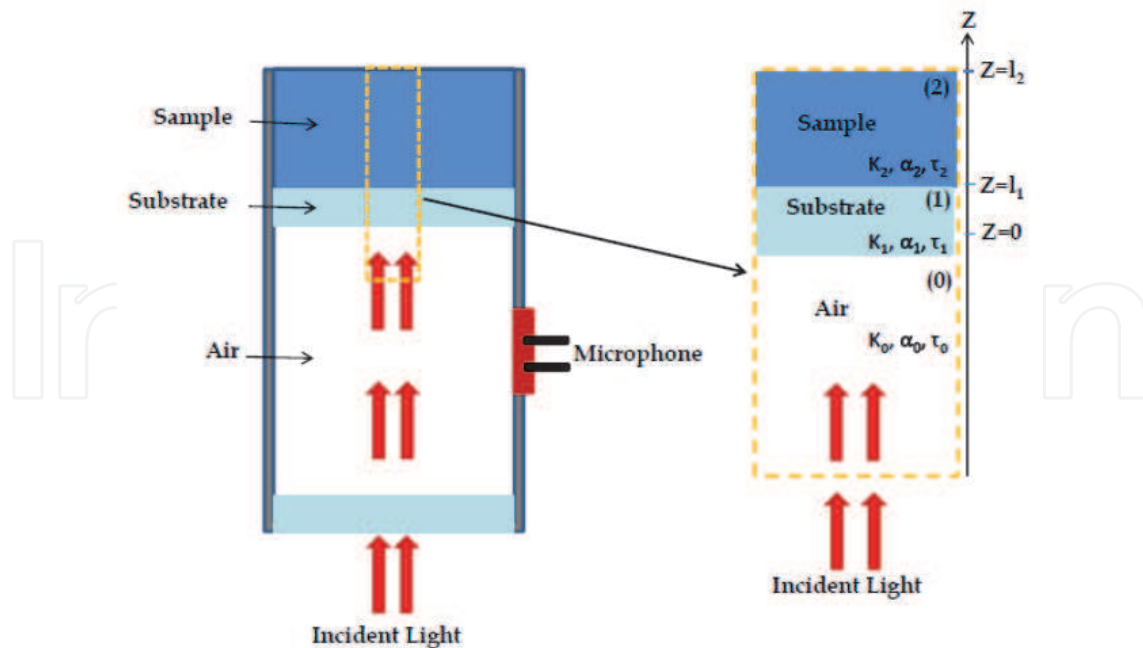


Fig. 5. Cross-section of the cylindrical photoacoustic cell, showing the positions of the sample, backing material, and gas column.

In order to understand the evolution of the PA signal, a theoretical methodology is used, in which it is considered that the system has homogeneous optical and thermal properties at any given time (Vilca et al., 2010). The formalism consists in finding the temperature of the layered system shown in Fig. 5. Using the heat conduction equation with a modulated heat source at modulation frequency  $f$  (Carslaw, 2005; Almond & Patel, 1996):

$$\frac{\partial^2 T(z,t)}{\partial z^2} - \frac{1}{\alpha} \frac{\partial T(z,t)}{\partial t} = -\frac{1}{k} F(z) \left( \frac{1 + \cos(\omega t)}{2} \right), \quad (1)$$

where  $z$  is the spatial coordinate,  $t$  is the time,  $T$  is the absolute temperature,  $\alpha_j(k_j)$  is the thermal diffusivity (thermal conductivity) of layer  $j$ ,  $\omega = 2\pi f$  and  $F(z)$  is the spatial distribution of the deposited energy over the sample, per unit volume and unit time. Under these conditions, the temperature at any point inside the sample ( $z \geq 0$ ) is given by

$$T(z,t) = T_{\text{amb}} + T_{\text{dc}}(z) + T_{\text{ac}}(z,t), \quad (2)$$

with  $T_{\text{amb}}$  being the ambient temperature.  $T_{\text{dc}}(z)$  and  $T_{\text{ac}}(z,t) = \text{Re}[\theta(z)e^{i\omega t}]$  are the stationary raising and periodic components of the temperature, due to the first and second terms of the heat source, respectively. From now on, the operator will be omitted, taking into account the convention that the real part of the expression must be taken to obtain physical quantities. We will focus our attention on the oscillatory part of the temperature, since it is the quantity of interest in lock-in and similar detection techniques.

It can be shown that when the layers  $T_{\text{amb}}$  have an ideal perfect thermal contact (Pichardo & Alvarado-Gil, 2001), and considering that layer 2 is sufficiently thick, to avoid the presence of thermal waves traveling in the  $-z$  direction inside it, the following result is obtained for  $z \leq 0$ :



$$\theta(z) = \Theta \frac{\eta_1 r_1 \left( \frac{\varepsilon_{21} + 1}{r_1 + 1} e^{\sigma_1 l_1} - \frac{\varepsilon_{21} - 1}{r_1 - 1} e^{-\sigma_1 l_1} + 2 \frac{\varepsilon_{21} - r_1}{r_1^2 - 1} e^{-\beta_1 l_1} \right) + 2(1 - R_2) \frac{\eta_2 r_2}{r_2 + 1} e^{-(\beta_1 + \beta_2) l_1}}{(\varepsilon_{01} + 1)(\varepsilon_{21} + 1) e^{\sigma_1 l_1} - (\varepsilon_{01} - 1)(\varepsilon_{21} - 1) e^{-\sigma_1 l_1}} e^{\sigma_0 z} \quad (3)$$

Where  $\Theta = (1 - R_1)I / (2\varepsilon_1(1 + i)(\pi f)^{1/2})$ ,  $\sigma_j = (1 + i)(\pi f / \alpha_j)^{1/2}$ ,  $\varepsilon_{mm} = \varepsilon_m / \varepsilon_n$ ,  $\varepsilon_j = k_j / (\alpha_j)^{1/2}$ , and  $r_m = \beta_m / \sigma_m$ , with  $\beta_j$  the absorption coefficient,  $\eta_j$  the efficiency at which the absorbed light is converted into heat,  $R_j$  is the reflection coefficient, of the corresponding layer  $j$ , with  $j = 1, 2$  (Almond & Patel, 1996).

Taking into account that under our experimental conditions, layer 1 can be considered as thermally thick and optically transparent ( $\mu_1 \ll l_1 \ll 1/\beta_1$ ),  $R_2 \approx 0$ , which is a reasonable assumption for layer 2 (agar combined with methylene blue),  $\eta_1 \approx \eta_2$  as usual (Almond & Patel, 1996), and  $\beta_2 l_1 \ll 1$ ; therefore Eq. 3 takes the form of,

$$\theta(z) \approx \frac{\eta_1(1 - R_1)I\beta_1\sqrt{\alpha_1}}{4\pi i\varepsilon_1 f} (1 + T_{21}\sqrt{\alpha_{21}}\beta_{21}e^{-\sigma_1 l_1}) e^{\sigma_0 z}, \quad (4)$$

where  $T_{21} = 2/(1 + \varepsilon_{21})$ ,  $\beta_{21} = \beta_2/\beta_1$ , and  $\alpha_{21} = \alpha_2/\alpha_1$ . It will be assumed that the thermal properties of layer 1 are constant along the entire experiment and assuming that only the optical absorption coefficient  $\beta_2$  of layer 2 is changing appreciably, during the process of diffusion of the methylene blue into the agar. This last assumption is valid for low concentrations of methylene blue only; it is convenient to define the normalized signal  $\Omega$  as follows:

$$\Omega = \frac{\theta(z, \beta)}{\theta(z, \beta_0)} = \frac{(1 + T_{21}\sqrt{\alpha_{21}}\beta e^{-\sigma_1 l_1})}{(1 + T_{21}\sqrt{\alpha_{21}}\beta_0 e^{-\sigma_1 l_1})}, \quad (5)$$

where  $\beta_0 = \beta_{21}(t=0)$  is the normalized optical absorption coefficient at the beginning of the diffusion process and  $\beta = \beta_{21}(t)$  is the normalized optical absorption coefficient at some subsequent time  $t > 0$ . Expressing Eq. 5 as a complex function in its polar form, it can be shown that its amplitude  $A(f)$  is given by

$$A(f) = \sqrt{\frac{1 + (T_{21}\sqrt{\alpha_{21}}\beta)^2 e^{-2\sqrt{f/f_c}} + 2T_{21}\sqrt{\alpha_{21}}\beta e^{-\sqrt{f/f_c}} \cos(\sqrt{f/f_c})}{1 + (T_{21}\sqrt{\alpha_{21}}\beta_0)^2 e^{-2\sqrt{f/f_c}} + 2T_{21}\sqrt{\alpha_{21}}\beta_0 e^{-\sqrt{f/f_c}} \cos(\sqrt{f/f_c})}}, \quad (6)$$

where  $f_c = \alpha_1/\pi l_1^2$  is the cut-off frequency of layer 1. In this way, after determining experimentally the normalized amplitude given in Eq. 6, by means of a fitting procedure, the relative optical absorption coefficients  $\beta$  can be determined for a fixed time during the diffusion process, if the thermal diffusivity and effusivity of layers 1 and 2 are known.

### 3. Results and discussion

#### 3.1 Optic technique

The signals for the eight photodiodes are presented in Fig. 6, for the five studied agar concentrations. As can be observed from this Figure, all the measurements show similar behavior as a function of time. The transmitted light signal shows small changes in the first

seconds, after some time it exhibits a strong decrease and in the last stage the rate of change of the signal slows down. For a fixed concentration, the shift of the curve is higher when the measurement is made further away from the top of the glass tube. An additional displacement is observed for a fixed photodiode when the agar concentration increases. In particular, for the lowest concentration (0.1%), the first photodiode (D0) signal reaches the stabilization after 20 h, and for the lower sensor (D7) the signal reaches a constant value after 55 h. In contrast for a higher concentration (0.5%) the first photodiode shows a constant value after 80 h and the last sensor shows a stable signal after 140 h.

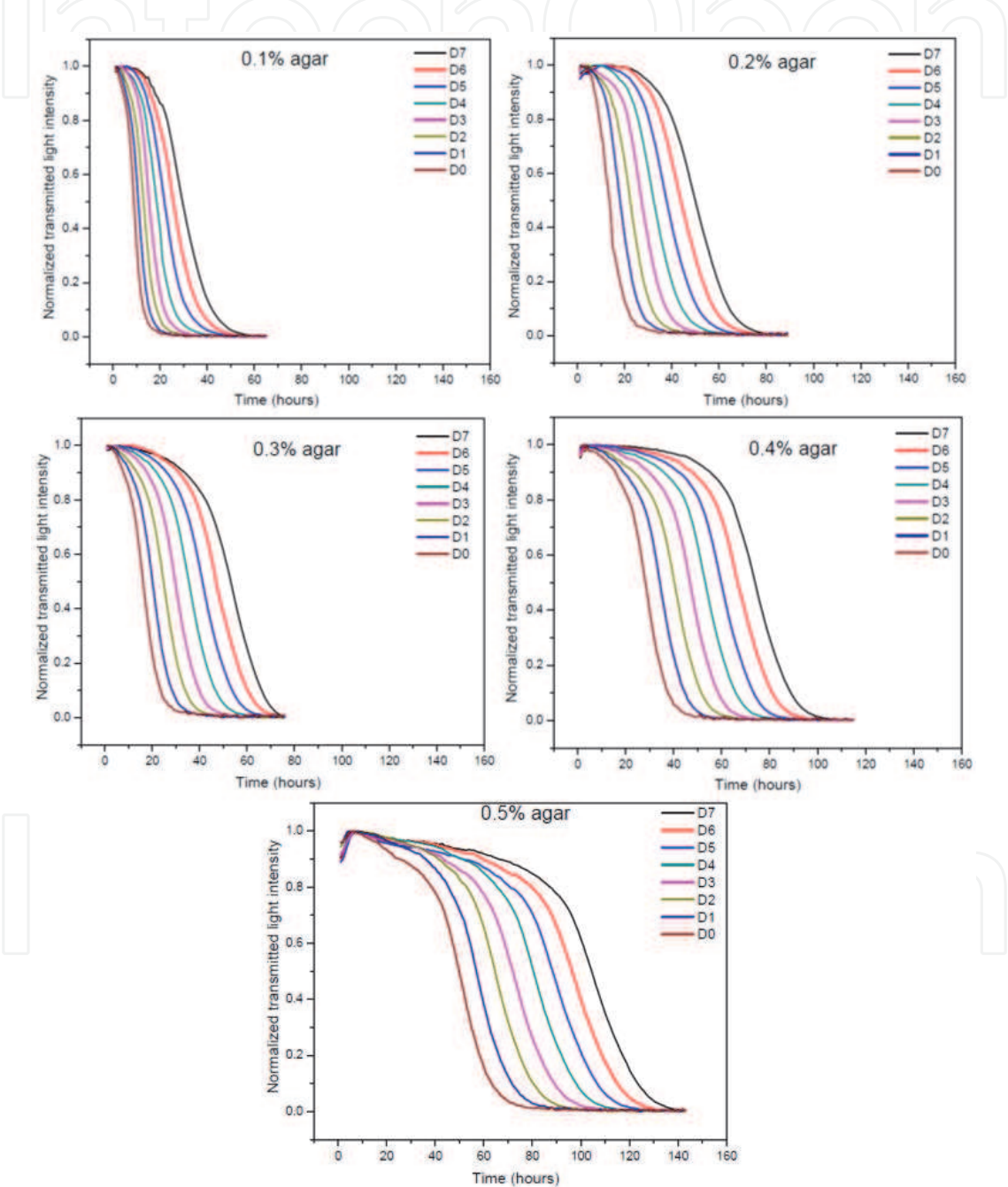


Fig. 6. Light transmission measured with eight optical photodiodes in the linear array for different concentrations during methylene blue diffusion on 0.1, 0.2, 0.3, 0.4 and 0.5 % w/v of agar concentration.



In order to get usable numerical parameters, the experimental data were analyzed using a sigmoidal fitting function applying the following equation,

$$I = I_0 + \frac{\Delta I}{\left(1 + e^{\frac{(t-t_0)}{\tau}}\right)}, \tag{7}$$

Where  $t$  is the time,  $I_0$  is the initial value for the normalized transmitted light intensity,  $\Delta I$  is the maximum change of the signal, and  $t_0$  is the time at which the sigmoidal process reaches its minimum derivative.  $\tau$  is the mean time in which the sigmoidal process occurs. In the particular case of 0.3% agar concentration and using the photodiode D2 (Fig. 7) the results were  $t_0 = 41.4$  hours and  $\tau = 6.02$  hours ( $r^2 = 0.99$ )

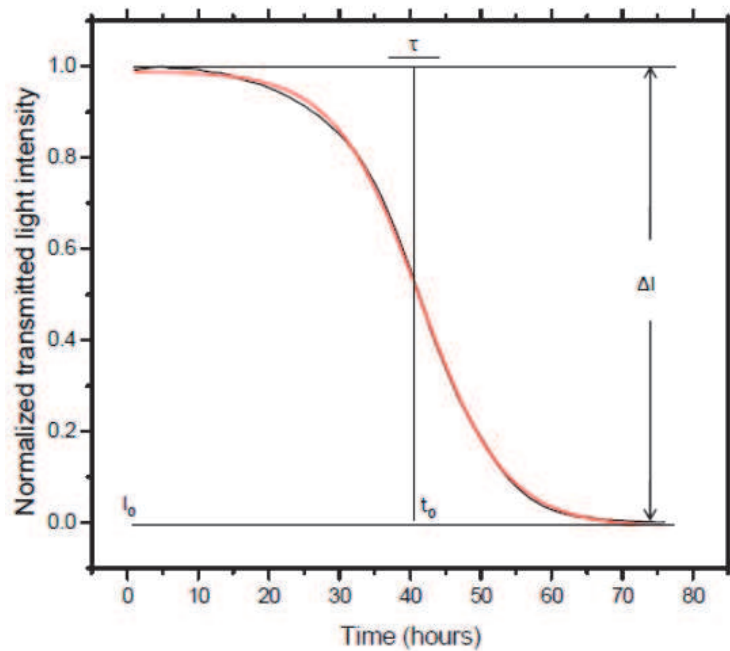


Fig. 7. Effect of the methylene blue diffusion into agar on the setting down time as a function of the distance, measured on the top surface of the phantom of agar column for five different agar concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 %w/v) of agar.

Studies of general diffusion processes, has been shown that a good approximation consists in considering the diffusion coefficient as, the relation between the cross section of the window through which the phenomenon is observed divided by the settle-down time (Crank, 1975). In this case the size of the window is 1 mm<sup>2</sup>. Following this procedure the diffusion coefficient can be estimated. In order to get comparative values a normalization process was performed. For each sensor the diffusion coefficient was normalized with respect to the coefficient of the lower concentration. In Figure 8 the normalized diffusion coefficient was calculated for all agar concentrations for D7 photodiode. The result show that the diffusion coefficient diminishes three times from the initial value when the agar concentration increases. The D7 photodiode was chosen because it is located far away from the methylene blue source and can be expected that provide a more realistic value of the diffusion coefficient.

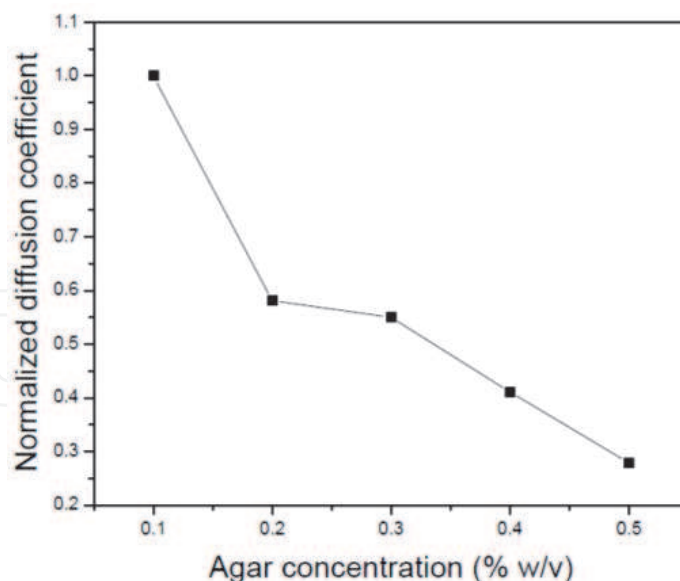


Fig. 8. Normalized diffusion coefficient behavior with the agar concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 %w/v), determined for D7 photodiode.

### 3.2 Photoacoustic technique

The results for the PA measurements for 0.01 % and 0.05 % w/v concentrations of agar phantoms are presented in Figs. 9a and 9b. It can be observed that in the first seconds, the PA signal diminishes gradually, due to the progressive diffusion of the dye that induces a decrease of the light absorption; and the signal for the sample with higher agar concentration shows a slower decay. Also, the low frequency option provides a better measurement due to a higher thermal diffusion length of the PA system. These effects have been studied for different frequencies indicating that thermal wave phenomena, is more sensitive when the thermal wave monitors the changes occurring through the column detector that contains the sample (Vilca et al., 2010).

In Figs. 10a and b, the time dependence of the normalized signal amplitude is shown. These data were obtained dividing the PA signal by its maximum for the specific experiment. It can be observed that higher modulation frequencies are more sensitive to the changes induced by the diffusion process. It is important to mention that the normalization procedure is useful to obtain independent results of the specific characteristics of the microphone and substrate; this is desirable if we want to focus our attention on the changes of the optical properties of the sample. This method also cancels the  $1/f$  frequency dependence of the PA signal, leaving unaffected the frequency in the exponential terms. The effect of the normalization procedure magnifies the observation of the dye diffusion process, without affecting the settle-down time and the net change of the signal. From the point of view of thermal wave theory, the thermal diffusion length is mainly related to the exponential decay. In this way the normalization procedure is not eliminating the most important dependence on the frequency that represents the basic advantage of photoacoustic spectroscopy. In order to discard the effect of the evolution of the thermal properties in the photoacoustic measurements, the thermal diffusivities of the samples were measured using the thermal wave resonator cavity technique. The values for 0.01 % and 0.05 % w/v concentrations were  $1.460 \times 10^{-4} \text{ cm}^2\text{s}^{-1}$  and  $1.466 \times 10^{-4} \text{ cm}^2\text{s}^{-1}$ , respectively. These values are very close to the thermal diffusivity for pure water (Almond & Patel, 1996).

Additionally, the measurements of agar samples in which the dye solution was completely diluted did not show considerable differences with the samples without the dye, being  $1.453 \times 10^{-4} \text{ cm}^2.\text{s}^{-1}$  and  $1.455 \times 10^{-4} \text{ cm}^2.\text{s}^{-1}$  for 0.01 % and 0.05 % w/v agar concentrations, respectively. Using these values and considering the changes in the thermal diffusivity of agar due to the addition of the dye, an estimation of the effects using Eq. 4 was performed. It was found that the magnitude of the PA signal is not affected appreciably. Therefore, the influence of the dye solution and its diffusion inside the agar gel on the thermal diffusivity values can be considered negligible. Based on these results, the variation in the PA signal can be exclusively related to the optical properties changes of the sample and can be appropriately parameterized as an effective optical absorption coefficient  $\beta_{\text{eff}}$ , that would measure the light that is being converted into heat during the diffusion process. Experimental data shown in Fig. 10 were fitted with Eq. 6, considering the thermal diffusivity values measured using the thermal wave resonator for the agar and gel mentioned above, thermal effusivity is  $\varepsilon_2 = 1.588 \text{ W.s}^{1/2}.\text{cm}^{-2}.\text{K}^{-1}$ , and for the polyvinyl acetate is,  $\alpha_1 = 1.95 \times 10^{-4} \text{ cm}^2.\text{s}^{-1}$  and  $\varepsilon_1 = 0.0490 \text{ W.s}^{1/2}.\text{cm}^{-2}.\text{K}^{-1}$ . With this procedure, the values of the effective optical absorption coefficients are obtained, as shown in Fig. 11.

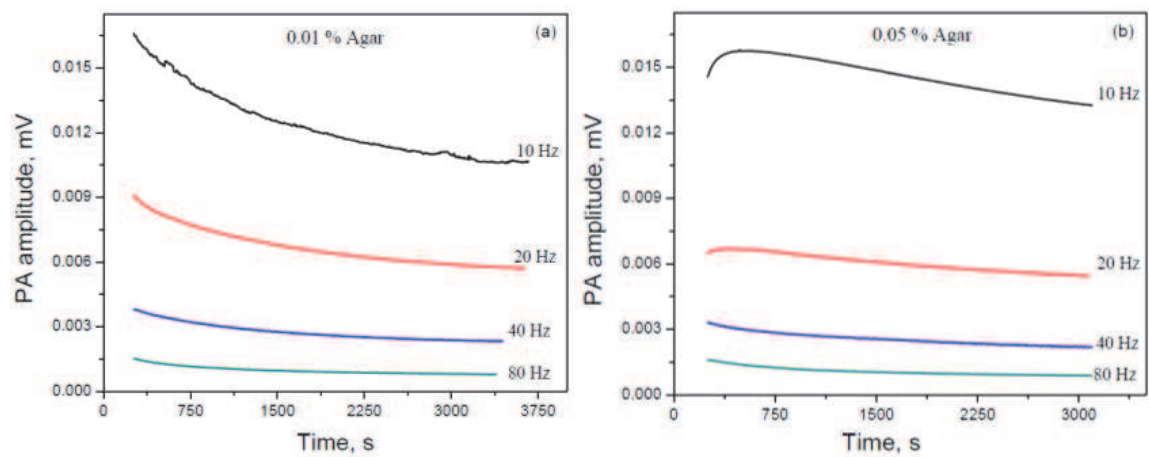


Fig. 9. PA signal behavior as a function of time during the diffusion processes through the solution, in (a) 0.01 % and (b) 0.05 % w/v, of agar phantoms after the application of the methylene blue solution.

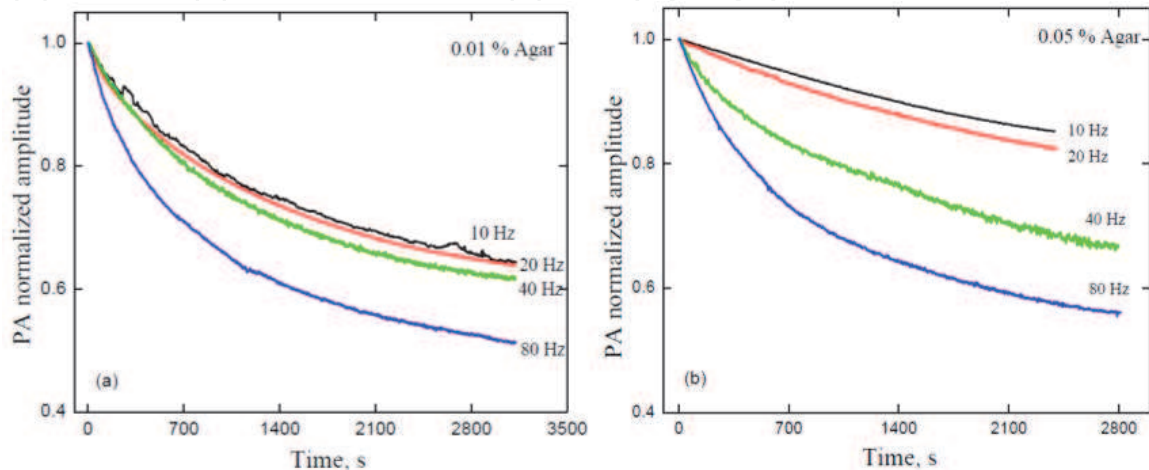


Fig. 10. Normalized photoacoustic signal for (a) 0.01 % and (b) 0.05 % w/v of agar.

The effective absorption coefficient shows a systematic decay on a time scale of 1000 s for both samples. In order to get usable numerical data, a fitting procedure can be performed using an exponential decay, parameterized in the form,

$$y = y_0 + A_1e^{-(t-t_0)/\tau} \tag{8}$$

where  $t$  is the time,  $y_0$  is the value of the absorption coefficient when the time is very large,  $A_1$  measures the size of the decay of the absorption,  $t_0$  is the initial time and  $\tau$  is the characteristic time decay of the process that measures the time interval needed in the process of dilution for the methylene blue solution in the agar sample to be stabilized. The characteristic decay times for 0.01 % and 0.05 % w/v agar samples are 1111 s and 1232 s, respectively. This can be understood taking into account that, when the concentration of agar grows the agar gel becomes harder; therefore, it is more difficult for methylene blue to penetrate the solution. These results show that the PA technique is sensitive and useful in the measurement of the decay time, and secondly, it provides the difference in time in which the methylene blue solution diffuses for two different agar concentrations. These differences supply important results for biomedical sciences in which agar gels are used as phantoms resembling some of the properties of living organs and tissues.

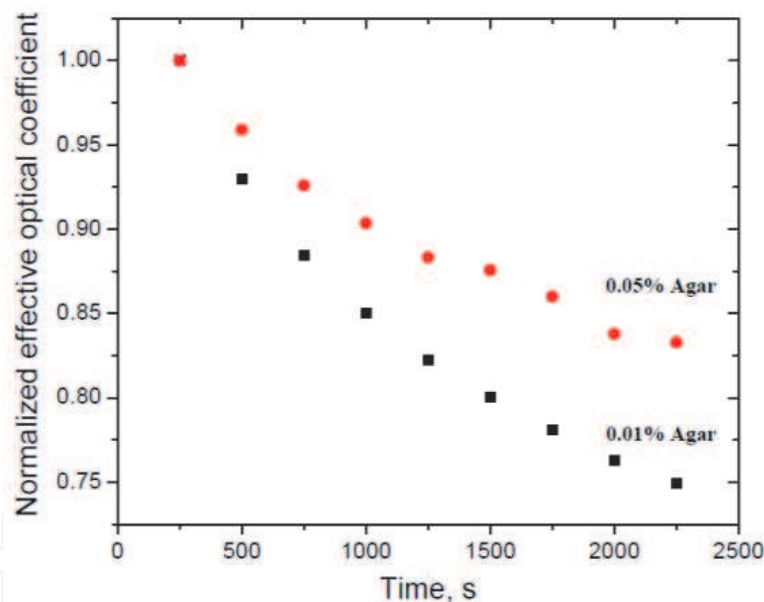


Fig. 11. Normalized effective optical absorption coefficient as a function of time, for two gel phantoms with concentrations of 0.01 and 0.05 % w/v of agar during the dye diffusion.

This work shows that increasing five times the concentration of agar in water, stabilization time only grows around 10 %; this behavior, is expected to occur only at low agar concentrations. At higher agar concentrations, stabilization of the processes would take longer time intervals. At these concentrations the link among the agar molecules generates a strong structure that is harder to penetrate by the dye.

From the optical and photoacoustic methodologies, it can be inferred that each option presented in this work, has its limitations and advantages. The optical experiment design provide a direct and position resolved measurement, having the possibility of studying in the laboratory the process of any substance applied on a given phantom, being highly useful

in the diagnosis and time that a given medication can reach the desired zone. The optical technique can also provide useful information on which wavelength of the illuminating laser must be used. In the simple case of methylene blue, one of the reasons that explain the good quality of the experimental data obtained is the fact that a red laser for monitoring, has been used. For any other substance the wavelength at which it absorbs must be known to choose the right illuminating source. After that, using this optic technique previous conjecture can be corroborated and applied to optimize the measurements. In contrast, the photoacoustic technique would be more useful in the analysis of fast process with low agar concentrations (tissues of low density) providing an average optical absorption coefficient. This would be highly useful when studying samples as living tissue in which the lateral profile of the optical measurements is not possible. In this case, these measurements could be helpful in designing instruments with applications for clinical diagnosis.

The use of both measurements allow to obtain an integrated analysis of the diffusion process in which the optical measurements provide crucial data, as the evolution of optical absorption coefficient that can be useful in the comprehension of the data obtained with the photoacoustic technique.

#### 4. Conclusions

The process of diffusion in methylene blue in phantoms of agar gels has been studied using two techniques, namely a novel optical methodology and photoacoustic spectroscopy using a conventional cell. Both techniques provide a useful analysis of the diffusion process. In both techniques it was found that an increase of the agar concentration slows down the methylene blue diffusion process. The optical measurement allows obtaining direct results and the monitoring of optical absorption coefficient as a function of the position. Given the close relationship of the optical absorption coefficient with concentration, we can infer that a direct measurement of the concentration of the dye as a function of time and position is possible. In contrast, the photoacoustic measurement would be more useful in the analysis of fast processes with low agar concentrations (tissues of low density) giving an average optical absorption coefficient. This would be highly useful when studying samples as living tissue in which the lateral profile of the optical measurements is not possible. In this case these measurements could be helpful in designing instruments with applications with *in situ* applications as in the case of clinical diagnosis.

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