

## Photoacoustic monitoring of Methylene blue irradiated

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**Abstract.** The photoacoustic signal of methylene blue irradiated with red light (640 nm), by 1 minute, was monitored in time. It was observed that the PA signal increase with time, fact related with the diminution of the thermal effusivity of the methylene blue after irradiation. It was also observed that the signal undulates with periodicity of about 5 minutes. This behaviour is under study to better understand it.

### 1. INTRODUCTION

The photoacoustic (PA) signal is the result of the conversion of electromagnetic energy of modulated amplitude in modulated thermal energy, through non-radiative decays in the absorbing matter [1]. It depends on the thermal properties of the sample and the base on the sample rests, been one of the thermal properties the thermal effusivity, as the Rosencwaig-Gersho model shows [2]. If the thermal properties of the base change, the photoacoustic signal in the PA chamber also changes. This enables photoacoustic techniques to monitor photochemical reactions, as photopolymerization or photobleaching, as a function of time. Photobleaching has been studied using photoacoustic techniques. Yunus et al. [3] studied the photobleaching of methylene blue embedded in different solid matrixes. They monitored the PA signal as function of time, frequency and beam power. In their study they used a configuration of closed cell, where the matrix were irradiated with pulsed light inside the PA chamber. Another study on photobleaching was conducted by George et al. [4]. In that study the photobleaching of the lasing dye Rhodamine 6G embedded in a solid matrix was analyzed with photoacoustic techniques, measuring the PA signal as function of time at different frequencies. They also used a similar experimental configuration as in [3]. Different substances of medical interest present photoproperties that are important in phototherapies, in particular photodynamic therapy (PDT). Porphyrins, chlorins and bacteriochlorins are among the most useful photosensitizers for *in vivo* PDT, although other classes of porphyrinoids such as phthalocyanines and texaphyrins are also used [5]. Methylene blue is a dye used by its photoproperties in photodynamic therapy of cancer. Considering its physicochemical properties, methylene blue is hydrophilic and this determines many aspects of its pharmacology. In this report, the photoacoustic effect of irradiated methylene blue diluted at physiological serum was measured.

### 2. MATERIAL AND METHODS

The PA setup was composed by a Tungsten arc lamp (150 W), a mechanical chopper, a PA cell and a two phases lock-in amplifier. Light was chopped at 17 Hz. As figure 1 shows, the PA cell was one with two faces, where one face was closed with a glass window and the other was closed with an

## 2. METHODOLOGY

The volunteers that took part in this experiment were young women (21 to 24-years-old). Volunteers should not present any injury nor metallic implant in the region of the skin chosen for the experiment. Also, the volunteers should not present allergy to the drug employed, nor stomach disorders. Initially, PA measurements were performed in clean skin. After that, diclofenac resinate (0,4mg) was applied in the right hand of each volunteer through one of the following methods: a) manual massage (for five minutes); and b) phonophoresis (US in continuous mode, three minutes). PA measurements were performed again after each kind of application. The period of phonophoresis application was fixed in three minutes considering the surface of the skin receiving application (approximately 3 cm<sup>2</sup>).

The US equipment utilized in pre-treatment of the skin was operated at 3MHz. Preliminary tests did not show differences in treatment performed with intensity between 0,5W/cm<sup>2</sup> and 1,0W/cm<sup>2</sup>, therefore, we adopted 0,5W/cm<sup>2</sup> as the US intensity, in order to minimize heat generation during the US sessions.

The PA signal for the skin was measured and recorded as a function of time. For PA measurements, light from a 250W tungsten lamp was modulated with a mechanical chopper and directed to a PA cell closed in one side by a glass window and in the other side by an aluminum foil (thickness about 65µm). Measurements were performed at 17Hz (modulation frequency). Skin was pressed against the external face of the aluminum foil (Figure 1). With this configuration, it was possible to perform *in vivo* measurements, which is a pre requisite for this kind of study.

The changes observed in the PA signal amplitude after each form of drug application were attributed to changes in the thermal effusivity of the system (skin + applied substance), due to penetration of the drug in the skin. Using the model proposed by Gutiérrez-Juárez et al [3], we associate these alterations in the thermal effusivity to changes in the relative concentration of the drug in skin. With the binary system composed by the applied substance and the skin, the relative concentration of the drug in skin is given by

$$X = \frac{\ln(\varepsilon_m / \varepsilon_p)}{\ln(\varepsilon_s / \varepsilon_p)} \quad (1)$$

where  $\varepsilon_m$  is the measured thermal effusivity,  $\varepsilon_s$  is the thermal effusivity of the applied substance and  $\varepsilon_p$ , the thermal effusivity of the clean skin.

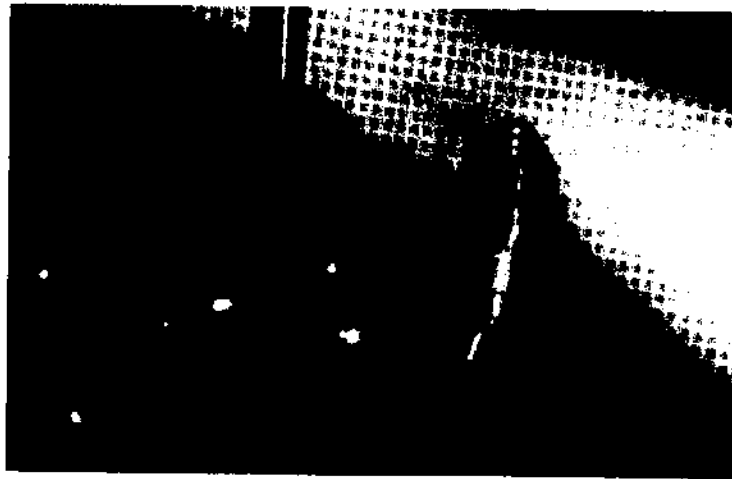


Figure 1. *In vivo* skin measurement (skin is pressed against the aluminum foil that closes the PA cell).

### 3. RESULTS AND DISCUSSION

Table 1 shows the results obtained for the relative concentration of the diclofenac resinate: a) after massage ( $X_{MASS}$ ); and b) after phonophoresis ( $X_{US}$ ).

**Table 1.** Relative concentration of the diclofenac resinate for each volunteer (average for a minimum of 8 measurements for application method, for each volunteer).

	Volunteer 1	Volunteer 2	Volunteer 3
$X_{MASS}$	0,111	0,042	0,158
$X_{US}$	0,136	0,051	0,194

$X_{MASS}$  – obtained after diclofenac application by manual massage.

$X_{US}$  – obtained after diclofenac application by US (phonophoresis).

PA data were analyzed statistically, comparing the PA amplitude level of the clean skin, skin after manual drug application and skin after phonophoresis application. Statistical significance of the reported differences was verified (ANOVA, 0.05 level). The results show that the relative concentration of diclofenac resinate in skin was higher after phonophoresis than after the conventional massage method. One can observe that the manual massage and the phonophoresis method were applied during different times (respectively, five and three minutes). These different application times reproduce clinical procedures adopted by physiotherapists. However, measurements (not shown) performed with different times (one and five minutes) for both methods allow us to conclude that the difference in PA signal amplitude should be attributed to the distinct application methods employed. The average PA amplitude is lower after US application, indicating higher concentration of the drug in the skin layer under study (stratum corneum, thickness between 30 and 40 $\mu$ m for the modulation frequency employed). Phonophoresis application promoted an average increase of about 20% in the relative concentration of the drug in the stratum corneum, immediately after treatment. This agrees with data found in literature about the role of phonophoresis in transdermal drug transport [4-6].

### 4. CONCLUSION

It was possible to detect differences in drug delivery between the specified physiotherapy treatments, indicating that phonophoresis indeed enhances drug absorption by tissue. Subsequent measurements have already been performed in order to determinate the typical time constants of drug delivery for each kind of treatment. Another perspective of work in this field is the investigation of which application mode (continuous or pulsed) of the ultrasound is the most effective in phonophoresis drug delivery. This is the subject of our present work.

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