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# Optical absorption coefficients of biological tissues obtained_by photoacoustic spectroscopy

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**ABSTRACT**

The knowledge of the optical absorption coefficient of biological tissues is very important when a light source has to be chosen in some therapies, as in the case of photodynamic therapy, or in a surgery in which a laser equipment is used, for example in ophthalmology or plastic surgery. In these cases, it is important to take the optical properties of the tissues into account; these properties can also depend on other parameters such as water content, colour and thickness, among others. In this article, an application of photoacoustic spectroscopy to obtain the optical absorption coefficient of rat, dog and rabbit skins is presented. The knowledge of these coefficients makes possible to select the most suitable wavelength to a specific medical application.

**Key Words:** Laser therapy, photoacoustic spectroscopy, optical absorption coefficient.

**RESUMEN**

El conocimiento de los coeficientes de absorción óptica de tejidos biológicos es muy importante, cuando una fuente de luz ha de ser elegida en algunas terapias, como es el caso de la terapia fotodinámica, o en cirugías en la cual un equipo láser es usado, por ejemplo en oftalmología o cirugía plástica. En estos casos, es importante tomar en cuenta las propiedades ópticas de los tejidos; esas propiedades pueden depender de otros parámetros, tales como su contenido de agua, color y espesor, entre otros. En este artículo, se presenta una aplicación de la espectroscopia fotoacústica al obtener los coeficientes de absorción de piel de rata, perro y conejo. El conocimiento de esos coeficientes hace posible seleccionar la longitud de onda, más aceptable para una aplicación médica específica.



**Palabras clave:** Terapia láser, espectroscopia fotoacústica, coeficiente de absorción óptica.

## INTRODUCTION

Biological tissues have characteristic optical absorption coefficients. This property characterizes them and allows the absorption of light in them in defined ranges, which depend on the predominant centre of absorption and the tissue water content. The optical absorption length, for typical biological tissues, is ranging from $10^{-2}$ to $10^{4}$ cm[1,2]. The importance of the knowledge of this

property comes from the variety of applications in which it can be used, for example, identifying tumor formations[3], evaluating morphological changes in lung tissue by measuring the spectral reflection of light transported by an endoscope at the bronchial mucous membranes[4]. It can also be used in image analysis for medical instrumentation, or in selecting laser equipment for medical applications[5].

By using a monochromatic light, the optical absorption coefficient of a homogeneous and isotropic sample can be obtained by means of the Beer-Lambert law[6]. Nevertheless, when a biological tissue is dealt with, it is necessary to take also into account the light absorption due to the light scattering in the tissue. Photothermal techniques could be useful to obtain the optical absorption coefficient in this kind of opaque materials, due to the fact that these techniques involve measurements of the heat produced as a kind of excitation of the relaxation by means of non-radioactive paths. These measurements depend on the sample thermal and optical properties. Among the photothermal techniques, Photoacoustic Spectroscopy (PAS) has been used to measure the optical absorption spectrum of opaque materials. The advantage of using PAS from other spectroscopic techniques is that PAS can be used for opaque, transparent, and highly scattering tissues. Besides, the samples do not require a special treatment or reagents, and it is possible to obtain a continuous absorption spectrum in a wished range.

In the PAS technique, a monochromatic modulated light beam impinges on the sample. The illuminated region produces heat due to non-radioactive relaxation. This heat flows from the sample, making the surrounding air layers to expand and contract at the monochromatic light beam modulation frequency[7,8]. As a result of this, an acoustic wave is produced, that is detected by a microphone; the amplitude of this wave is proportional to the absorbed density of energy. When the exciting light is scanned in wavelength, a similar spectrum of the optical absorption spectrum is obtained.

## EXPERIMENTAL

### Sample preparation

Samples of skin from de back of rats, rabbits and dogs were used: Wistar rats, New Zealand rabbits and Rotweiller and mongrel dogs with average weight of 350 g, 3 kg and 16 kg respectively were used. Each sample was kept into a 9% concentrated physiologic saline solution. The samples were shaven to prevent the hair from influencing the study. Afterwards, the samples were cut into 3 mm squares.

### Photoacoustic spectroscopy

The light emission was obtained from a 1,000 W xenon lamp, working to the 70% of its power for security, and the wavelenght was selected by means of an Oriel monochromator (77.325), in a range of 500-850 nm. The incidental light beam got in through the top at a frequency of 17 Hz, which is the chopper rate modulation (Chopper SR-540 Stanford Research Systems), and it was transferred to the study sample by means of optical fiber (24 in length, 0.316 in diameter, with a transmission rank 0.3-1.5$\mu$). Each sample was placed into the photoacoustic cell, which is a brass cylinder whose ends are quartz windows, and the condenser microphone (electret microphone, with response in a range of 12 to 20,000 Hz) is connected through a tiny hole in the wall. All the studies where made under atmospheric pressure and room temperature. The experimental setup is shown in Figure 1.

The photoacoustic signal was sent to a lock-in amplifier (Stanford Research Systems SR-850), and also, by means of a serial port RS232 in a personal computer, it was processed. This photoacoustic signal was normalized to the emission of the xenon lamp spectrum. This spectrum was obtained by using graphite dust as a sample into the photoacoustic cell. The PA signal normalization was performed through the data acquisition software, and the software was developed with Lab View architecture. Finally, the obtained spectrum shows the normalized photoacoustic signal amplitude, referred to the spectrum of the xenon lamp. This signal is directly proportional to the total optical attenuation coefficient ($\mu_t$), which takes into account the optical absorption ($\mu_a$) and scattering ($\mu_s$) coefficients ($\mu_t = \mu_a + \mu_s$) that can be known from the equation 1[9]. Then, for thermally thick samples, it is possible to know the total optical attenuation coefficient based upon the Rosencwaig and Gersho theory of the photoacoustic effect.



$$\mu_t = \frac{a_s\left(q^2 + q\sqrt{2-q^2}\right)}{1-q^2} \quad (1)$$

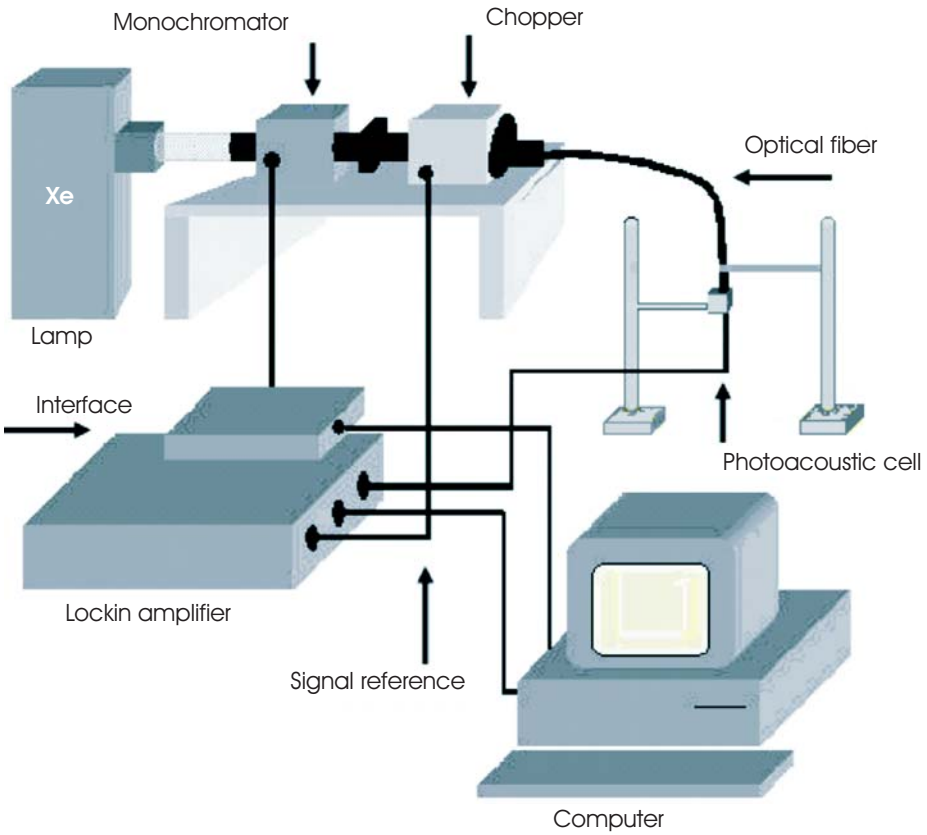


**Figure 1.** Experimental set-up for PAS studies.

where:

$$a_s = \sqrt{\frac{\pi f}{\alpha}} = \text{Thermal diffusion coefficient} \quad (2)$$

$\alpha$ = thermal diffusivity of the sample, typically of 1.4 x $10^{-3}$ $cm^2/s$

$f$ = modulation frequency of the modulated light beam; in this case 17 Hz

$q$ = normalized photoacoustic signal amplitude

For specialized literature, the value of thermal diffusivity was taken into account for the calculation of the optical absorption coefficient reported in this case[10].

The average thicknesses of the rabbit, dog and rat skins were 1.3 mm, 0.92 mm and 0.6 mm, respectively, which means that all of them are thermally thick: $a_s d >> 1$ where $d$ is the sample thickness. It is necessary to mention that equation (1) is valid when the sample is thermally thick as in this case[8].

## RESULTS AND DISCUSSION

Figure 2 shows the total optical attenuation coefficient ($\mu_t$), obtained by using equation 1 with the measured normalized photoacoustic signal amplitudes (q). From this figure, it can be seen that the optical absorption coefficient decreases as the wavelength increases for the studied skins. This behavior corresponds mainly to the optical absorption spectrum of melanin contained in these skins[11]. The different concentration of melanin and particular characteristics for each sample gives different color in the samples as a result. The optical attenuation coefficient depends on the skin color, as can be seen in Figure 2, where the dark dog skin shows the highest coefficient in the studied spectral range. On the other hand, the rabbit skin had the lowest absorption coefficient in the studied samples. In the case of the studied rat and rabbit skins, these were white, and the spectral differences could be due to their density and roughness. The results obtained are in the same magnitude order, with one factor out of two, than those obtained by Wai-Fung[12], whose article shows that the optical absorption coefficient values depend on the method used. Besides the behavior is similar, to values of the coefficients that decrease, in exponential form when increasing the wavelength.

Such is the case of Beek[18], who reports in his article the values of the optical absorption coefficients of diverse organs of dogs, rabbits, pigs and rats, including the skin, using the method of double integration spheres. Besides, he develops a comparative analysis with the findings of other authors and reaches the same conclusions.

Figure 3 presents the optical absorption length ($\mu$t-1) as a function of the wavelength, which shows the estimated depth at which light gets inside the studied skins. This figure is important because in medical applications, such as ophthalmology, it is very important to know $\mu_t^{-1}$ to avoid injury in the healthy tissues near the zone where some laser treatment will be performed. It is also relevant in dermatology, for example, in scars management it is important to know this optical absorption length in phototherapy, where the action is mainly on the

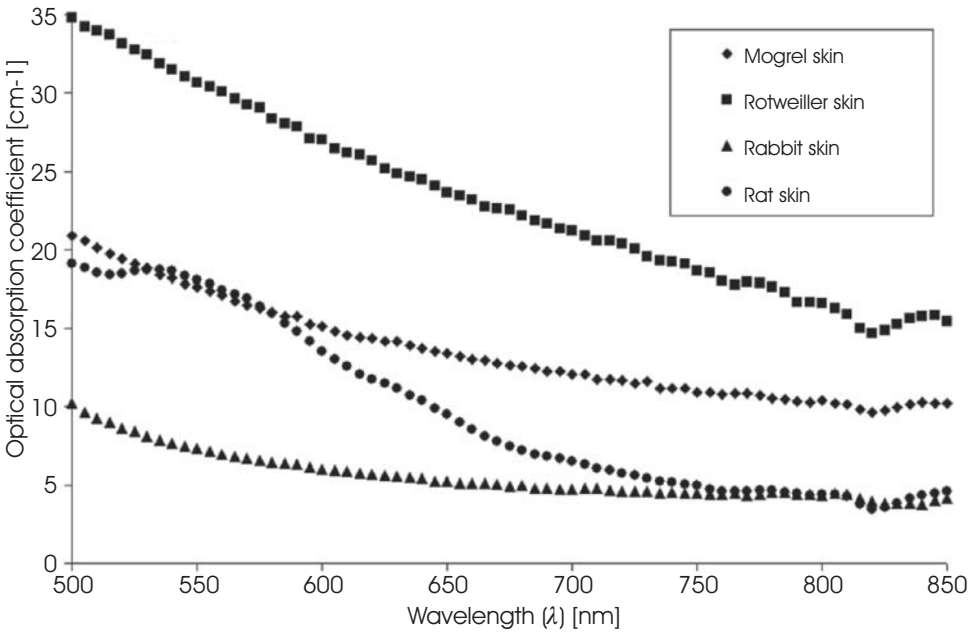


**Figure 2.** Optical absorption coefficient of each tissue.

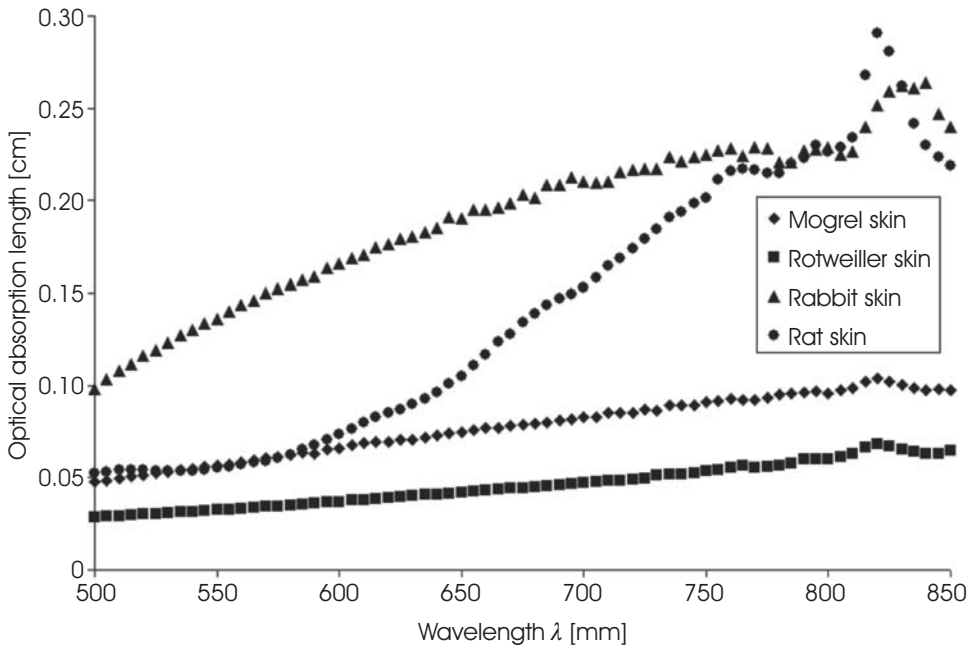


**Figure 3.** Optical absorption length reached for each tissue.

skin surface, and also in remotion surgery of tumours.[13]

## CONCLUSIONS

In this paper, the use of PAS in biomedical applications is shown, particularly to obtain the optical absorption coefficient of organic tissues. In this way, the correct wavelength can be obtained depending on the clinical application[14,15]. If the absorption coefficient is known for a particular tissue, then the ideal laser to be used in a medical application can be chosen. For example, if the aim is an application on skin, like scarring, it is possible to use some laser with a visible wavelength[16] (400 to 700 nm). However, if the application is on tissues with high water content to make cuts or reach deep zones of skin, then a good decision is to choose an infrared laser [17].

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