Photoacoustic study of the penetration kinetics of nimesulid into human skin

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Abstract. The photoacoustic (PA) effect is observed when modulated (or pulsed) light is absorbed by a sample inside a closed chamber and converted in heat, generating acoustic waves; PA measurements have been employed to evaluate transdermal penetration of topically applied drugs. Phonophoresis is the utilization of ultrasonic (US) energy to enhance absorption of drugs across the epidermal barrier, and its usefulness has been shown by PA measurements. The aim of the present work was to determine the characteristic absorption times of the anti-inflammatory Nimesulid (gel) in human skin, with and without help of therapeutic phonophoresis. After local cleaning, measurements were performed in the forearm of each volunteer before Nimesulid application and for different times after application through massage with the US equipment head; the protocol was repeated for the opposite forearm, but without US emission. Curves of the PA signal level as a function of time were adjusted by a Boltzmann equation, leading to the determination of the characteristic absorption time (about 12 minutes). No significant gain was observed in Nimesulid absorption with the utilization of US radiation, indicating that topic application of Nimesulid does not require the use of phonophoresis, due to the natural fast penetration of the Nimesulid gel.

1. Introduction
The photoacoustic (PA) effect is observed when modulated (or pulsed) light is absorbed by a sample inside a closed chamber and converted in heat, thus generating acoustic waves. The PA technique has been increasingly employed in different biological and biomedical studies, allowing in vivo skin measurements not easily performed with other techniques. The penetration of substances topically applied to skin can be evaluated through PA measurements taken as a function of time [1, 2].

The diffusion rate of a drug into skin depends on the physical-chemical characteristics of both the active principle and the vehicle of the formulation. It also depends on skin factors, such as: thickness, water content, condition and age. Drug concentration is a secondary factor [3]. In terms of solubility, the diffusion rate of a determined molecule is inversely proportional to its molecular weight [4].

Phonophoresis can be defined as the utilization of ultrasound (US) waves to enhance absorption of drugs across the epidermal barrier. The utilization of topically applied anti-inflammatories is frequently adopted in the control of acute pain caused by muscular contracture, being normally employed with association to other therapeutic methods, as application of ultrasound (US) radiation. Recently, Barja et al reported in vivo PA measurements performed to evaluate transdermal delivery with and without phonophoresis (continuous mode) and showed that, for the specific formulation analyzed (diclofenac resinate), US therapy enhanced drug delivery through skin [5].
The objective of the present study was to determine the characteristic absorption times of the anti-inflammatory Nimesulid (gel formulation) in human skin, with and without help of therapeutic phonophoresis, in order to determine if this application method presents advantages when compared to the more traditional method of manual massage.

2. Materials and Methods

2.1. Sample
Measurements were performed in 16 women between 20 and 30 years-old. Volunteers should not present neither allergy to the drug employed, nor injury or metallic implant in the region of the skin under analysis.

2.2. Experimental Setup
PA measurements employed a tungsten lamp (24V, 250W) as light source, modulated at 17Hz by a mechanical chopper (Stanford Research Systems, mod.SR540). Light was directed to a double-faced PA cell closed by a glass window in one side and with a 65µm-thick aluminum foil (thus in the thermally thin regime for the modulation frequency employed) closing the opposite side. The chopper and the electret microphone of the cell were connected to a lock-in amplifier (Stanford Research Systems, mod.SR530) used to capture the PA signal, which was sent to a microcomputer that controlled the data acquisition process.

2.3. Formulation
The formulation analyzed is commercially available in Brazil as Scaflam® Gel, being indicated to the local treatment of inflammations and pain. Clinically tested, its active principle is the Nimesulid at the concentration of 3% (30mg for each 1g of product). The vehicle is a water-based gel with excipients: diethylene glycol monoethyl ether, disodium edetate, caprylcaproyl macrogol glycerides, carbomers, methyl-parahydroxybenzoate, propyl-parahydroxybenzoate, triethanolamine and purified water.

2.4. Phonophoresis
The equipment utilized for phonophoresis was the Sonopulse Compact 1.0MHZ (Ibramed, Brazil), at the intensity of 1W/cm² and US frequency of 1MHz (continuous mode). The application period adopted was three minutes (for an area of 9cm²).

2.5. Topical application protocol
The skin region to be evaluated (forearm) was previously cleaned with water and soap. Topical application of the formulation employed a disposable plastic syringe (0.2mL for each application). After local cleaning, measurements were taken for each volunteer.

2.6. Photoacoustic measurements
The first series of measurements were performed in the forearm before Nimesulid application and for different times after application through massage with the US equipment head, but without US emission. For each measurement, the volunteer gently pressed the skin against the Al foil on the PA cell and the PA signal was registered at a rate of two points per second, up to a total of 80 points (40s). Preliminary measurements helped to define a regular interval between successive measurements, established in five minutes; the first measurement was performed immediately after asepsis, and the last measurement began exactly 15 minutes after Nimesulid application. Measurements performed in the opposite forearm followed the same protocol, but with the US equipment turned on.

2.7. Data analysis
Data was stored in the computer (software “Sin530r”) for posterior analysis. The software Microcal Origin 7.0® was utilized in the graphic analysis of the data. Statistical analysis (ANOVA or Student’s t
3. Results and discussion

Initially, an exponential fitting of the experimental data was attempted. However, after initial analysis, a much better data fitting for the PA amplitude was obtained with a sigmoidal curve (S-shaped curve), Boltzmann type (Origin 7.0): 

\[ PA(t) = \frac{A_1 - A_2}{1 + e^{\frac{t-t_0}{\Delta t}}} + A_2 \]  

(eq.1)

In (eq.1), the relevant parameters for our analysis are \( t_0 \) (typical absorption time, correspondent to an absorption of 50% in the skin layer investigated) and \( \Delta t \) (time interval such as 67% of the absorption process occurs between \( t_0-\Delta t \) and \( t_0+\Delta t \)). This function represents quite well the drug absorption process, presenting stabilization in the level \( A_2 \).

The better fitting obtained with the sigmoidal function, when compared to the exponential function, was confirmed by statistical analysis (Mann-Whitney test for the comparison between the chi-square values for each fitting expression), with \( p=0.03 \). Table I shows the results obtained with (eq.1) for each fitting parameter and each application method (massage × phonophoresis).

| Application method | \( t_0 \) (min) ± standard error | \( \Delta t \) (min) ± standard error |
|--------------------|----------------------------------|-----------------------------------|
| Massage            | 12.5 ± 1.2                      | 1.8 ± 0.4                         |
| Phonophoresis      | 11.7 ± 1.7                      | 1.7 ± 0.8                         |

Statistical analysis initially employed the paired Student’s \( t \) test; however, as the software INSTAT® pointed no significant data-pairing (\( p\geq0.10 \) for both relevant parameters), the analysis was repeated with the non-paired \( t \) test; the following \( p \) values were obtained: \( p=0.69 \) for \( t_0 \) and \( p=0.42 \) for \( \Delta t_0 \) (paired \( t \) tests); \( p=0.59 \) for \( t_0 \) and \( p=0.99 \) for \( \Delta t_0 \) (non-paired \( t \) tests). In this way, all the tests performed indicated absence of any significant difference between the two application methods employed. Therefore, the experiment shows that the transdermal penetration kinetics of the Nimesulid in the formulation evaluated is not enhanced by phonophoresis. For both application methods investigated, the characteristic absorption time for the skin layer under study is about 12 minutes, with a \( \Delta t \) of less than two minutes. A typical absorption curve (normalized for \( A_1=0 \) and \( A_2=100 \), with \( A_2 \) corresponding to the signal amplitude of the clean skin) is presented in Figure 1, that represents the penetration process of the drug into skin as a function of time, showing that approximately 90% of the absorption process is concluded in about 15 minutes.

**Figure 1.** Normalized typical curve (data + fitting) for the PA signal level as a function of time after Nimesulid application.
For the experimental conditions employed, the skin layer under study is about 40\(\mu\)m. As the stratum corneum (SC) is significantly thinner than that [7], in this work we are looking at drug penetration through SC into the underlying epidermis.

Now we must compare the results obtained in the present work with those reported for similar PA experiments, performed to evaluate the penetration \textit{in vivo} of other substances topically applied by massage or phonophoresis.

Gutiérrez-Juárez \textit{et al} (2002) studied the penetration of Nitrofurazone, Vaseline and Vaporub applied by massage in the forearm of volunteers, finding time constants between 15 and 60 minutes [6]. Working with Piroxicam, in a previous \textit{in vivo} experiment performed with essentially the same setup and method, our group obtained typical time constants of about 20 minutes for phonophoresis application and about 60 minutes for manual massage [5]. Still with the same experimental setup, employing \textit{in vivo} measurements and manual massage, the time constants verified for hydrating formulations were between 30 and 90 minutes [1], while the evaluation of the penetration kinetics of two gel formulations showed time constants of about 10 to 15 minutes [2].

Considering the reported results, the observation that the application method did not alter the penetration kinetics for the Nimesulid formulation here evaluated can be associated to the fact that the Nimesulid absorption kinetics is much faster than those presented for most of the products previously evaluated, with essentially the same experimental setup and method.

4. Conclusion and perspectives

In the present work, \textit{in vivo} PA measurements were employed to determine the effect of the topical application method (massage x phonophoresis) in transdermal drug delivery of a Nimesulid gel formulation. The results obtained show that: i) the Nimesulid gel presents fast transdermal penetration, with a typical constant of 12 minutes (for a skin layer of about 40\(\mu\)m); ii) the use of therapeutic US application does not enhance transdermal penetration for this specific formulation. This indicates that topical application of Nimesulid gel does not require the use of phonophoresis, due to the natural fast penetration of this formulation. However, it must be emphasized that different formulations may present different responses to phonophoresis.

Future studies in this field must employ additional measurements to evaluate the differences among topical drug application methods. For instance, PA measurements performed at different modulation frequencies could be correlated to different skin layer thicknesses, supplementing knowledge about transdermal penetration kinetics. In the specific field of phonophoresis, more studies must be carried out to investigate the influence of the application parameters of the US waves (intensity, mode, application period) in the absorption kinetics of topically applied products.

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