In vivo evaluation of drug delivery after ultrasound application: A new use for the photoacoustic technique

P.R. Barja^{1,*}, D. Acosta-Avalos¹, P.C.B. Rompe¹, F.H. dos Anjos¹,
F.R. Marciano¹ and M.D. da Silva²

¹Instituto de Pesquisa & Desenvolvimento (IP&D), Universidade do Vale do Paraíba (UNIVAP), Av. Shishima Hifumi 2911, São José dos Campos 12244-000, SP, Brazil

²Instituto Nacional de Pesquisas Espaciais (INPE), São José dos Campos, SP, Brazil

Abstract. Ultrasound application is a therapeutical resource widely employed in physiotherapy. One of its applications is the phonophoresis, a technique in which the ultrasound radiation is utilized to deliver drugs through the skin to soft tissues. The proposal of our study was to employ the Photoacoustic Technique to evaluate the efficacy of such treatment, analyzing if phonophoresis could enhance drug delivery through skin when compared to the more traditional method of manual massage. The configuration of the system employed was such that it was possible to perform *in vivo* measurements, which is a pre-requisite for this kind of study. The changes observed in the photoacoustic signal amplitude after each form of drug application were attributed to changes in the thermal effusivity of the system, due to penetration of the drug. The technique was able to detect differences in drug delivery between the specified physiotherapy treatments, indicating that phonophoresis enhances drug absorption by tissue.

1. INTRODUCTION

Ultrasound (US) is a therapeutical resource widely employed in physiotherapy. One employment of the US therapy is the so-called phonophoresis, a technique in which the therapeutical properties of this US are utilized to help drug delivery through the skin to soft tissues. While the US technique has been widely employed with this purpose, its actual effectiveness is still not fully understood. physiotherapists have been interested in finding a way to perform a quantitative analysis of the penetration of a determined drug in the human skin after US application. However, it is difficult to extrapolate conclusions obtained through *in vitro* measurements to the *in vivo* problem. In other words, the quantitative analysis must be performed through *in vivo* measurements. In the present work, we propose the use of the Photoacoustic (PA) Technique in performing this kind of *in vivo* evaluation.

The PA technique has already been employed in the characterization of human skin and in pharmacokinetics studies of topically applied drugs [1-3]. As the PA signal depends on thermal and optical properties of the sample, structural changes in the system under analysis (skin plus drug) account for signal variations in time. The present study utilized the diclofenac resinate (Cataflan®) as the topically applied drug. We compare different forms of application of this drugs into human skin: a) manual massage (no US application); and b) phonophoresis (US in continuous mode).

* e-mail: barja@univap.br

aluminum foil. The light incidence was the front one, impinging first on the glass window. The aluminum foil absorbed the light generating the PA signal. As the signal depends on the thermal properties of the material over the aluminum foil, changing the "base" the amplitude and phase of the signal changes. The photoacoustic signal was monitored by 25 minutes. First, the signal of the aluminum foil plus about 10 ml of methylene blue (diluted at physiological serum at 1:1 proportion) on its top was measured by 5 minutes. At the minute 5 the solution of methylene blue was irradiated with red light (640 nm, 10 mW/cm²) by 1 minute. The radiation source used was a LED. As a control, the PA signal of methylene blue was monitored without irradiation. For each, control and irradiated, 24 measurements were done and a medium graph was calculated using the software Microcal - Origin.

Figure 1. PA cell and measurement setup.

3. RESULTS AND DISCUSSION

Figure 2 shows the mean curve (control and irradiated) of 24 different curves. As can be seen, after irradiation the PA signal increases, meaning that less heat is transmitted to the methylene blue drop. The immediate interpretation is that methylene blue changes its thermal properties after irradiation with red light. But a continuous increase does not correspond with changes photoinduced by 1 minute irradiation. A closer look at figure 2 reveals that the methylene blue irradiated curve presents undulations with about 5 min of periodicity. Two possible explanations could help to understand this continuous increase in the PA signal. The first one has to be with the amphiphilicity of the molecule. In PDT, amphiphilic photosensitizers are better because they are generally more photodynamically active than symmetrical hydrophobic or hydrophilic molecules. Amphiphilicity describes molecules that are partially hydrophobic and hydrophilic at different positions so that each region interacts with the solvent independently. It is also important because it can affect the degree to which a compound aggregates [5]. Perhaps, during irradiation the methylene blue molecules change from hydrophilic to amphiphilic and after irradiation this process continues, as a chain reaction. The other explanation has to be with the reaction capacity of the methylene blue molecule. It is known that this molecule reacts easily in the presence of electron donors. As the methylene blue drop is on a metallic foil, that behaves as an electron donor, the methylene blue could be oxidizing the aluminum surface after irradiation. In this case, the surface oxidation would correspond with a photoprocess. Experiments must be done with electric isolant materials as base, to verify this hypothesis. The 5 minutes periodicity observed in the graph does not have explanation. This observed behaviour must be studied in the future in our laboratory.

Figure 2. Photoacoustic signal as function of time. Continuous line: methylene blue no irradiated. Black triangles: methylene blue irradiated at the minute 5 by 1 minute. Data shown are the mean of 24 different measurements.

4. CONCLUSION

The study of the photoproperties of molecules used in PDT can be done with photoacoustic techniques. In this report it was observed that the thermal effusivity of irradiated methylene blue decrease in the time. This behaviour is under study to understand if it is due to the interaction between the molecule of methylene blue and the aluminium foil, because this dye reacts easily with electron donors.

References

- [1] Vargas, H., Miranda, I., C. M. *Phys. Rep.* **161**: 43 (1988)
- [2] Rosencwaig, A., Gersho, A. *J. Appl. Phys.* **47**: 64 (1976)
- [3] Yunus, W. M. M., Sheng, C. K., Yunus, W. M. Z. W. *J. Nonlin. Opt. Phys. Mater.* **1**: 91 (2003)
- [4] George, N. A., Aneeshkumar, B., Radhakrishnan P., Vallabhan, C. P. G., *J. Phys. D: Appl. Phys.* **32**: 1745 (1999)
- [5] Macdonald, I. J., Dougherty, T. J. *J. Porphyrins Phthalocyanines* **5**: 105 (2001)