Development of a photothermal double beam laser scanning system in biopharmaceutical applications

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Abstract. Photothermal beam deflection (PDS) has been applied to obtain information regarding the penetration of methylorange (MO) and ditranol (DI) into artificial membranes. The measurable depth range is 56 µm. Photothermal beam deflection allows on the one hand depth resolved investigations by the use of a frequency modulation of the excitation beam to reach deeper regions even in opaque sample, and on the other hand lateral imaging. To explore the potential use of a novel photothermal double beam laser scanning system, measurements in drug delivery analysis have been used for depth profiling and imaging into an artificial membrane, which represents stratum corneum or bovine hoof, appropriately.

1. Introduction
The human skin is an extraordinarily complex organ that consists of several layers which complete a multiplicity of functions. On the one hand the skin protects the organism from exhaustive water- and heat loss. On the other hand the barrier functions against penetration and permeation of microorganisms and toxics into the organism is one of the main duties in particular of the outermost layers of the skin, the horny layer or the stratum corneum (SC). The SC is a heterogeneous membrane which is composed of keratin-rich corneocytes embedded in a lipid matrix. The so called “brick and mortar” model builds an effective barrier that restrain not just toxics and microorganisms but also pharmaceutical agents from penetration into the skin. The improvement of pharmaceutical formulations concerning drug delivery and skin penetration is a main challenge for the dermatopharmacy [1, 2, 3]. For characterising the complex process of drug penetration and permeation and in order to get consistent with high requirements we developed a photothermal double beam laser scanning system. We present a novel photothermal double beam laser scanning system and its applications to study the migration processes in tissues by exemplifying several applications in pharmaceutical science.
2. Photothermal scanner device
The method used is the photothermal beam deflection spectroscopy (PDS). A sample is heated by an incident laser beam. The resulting gradient of index of refraction in the gas phase adjacent to the sample surface is probed by beam deflection [4]. Both, excitation beam and probe beam are guided simultaneously over the sample surface as the position of the probe and excitation beam is constant to one another during the imaging process. A lateral diode which is used to measure the probe beam deflection is synchronously aligned with the probe beam. Figure 1 depicts the novel recently patented [5] Photothermal double beam laser scanning system:

For the visible absorption range a diode pumped frequency doubled 100 mW Nd:YAG laser (wavelength: 532 nm, B&WTEK, Newark, DE, USA), modulated by a mechanical chopper (New Focus, San Jose, CA, USA Model NFO-3501), was used as excitation source. A He-Ne laser (wavelength \( \lambda = 633 \text{ nm} \), 1 mW; JDS uniphase Model 1101P, Montea, CA, USA) served as probe beam source. In order to sync both laser beams they superposed by a dichroitic mirror 1, and after passing through two scanner devices (GSI Lumonics, Munich, Germany), they were separated by a second dichroitic mirror 2. The probe beam deflection signal (normal and transverse components) was detected by a lateral position detector (PSD) and processed by means of a lock-in amplifier.

![Figure 1. Photothermal double beam laser scanning system.](image-url)
The probe-beam deflection angle is given, in vector notation, by the line integral eq.1 [6] Where, P is the probe beam path, n is the gas index of refraction $T_g$ is the periodic temperature distribution in the air, and $dl$ is an incremental distance along pathway P.

$$\varphi = \int_{P} \frac{dn}{ndT} \nabla T_g \times dl$$  \hspace{1cm} (1)

$$\varphi_n = \frac{dn}{ndT} \int_{0}^{\infty} T_g(\lambda)b \exp(-b z) \cos(\lambda y) d\lambda$$  \hspace{1cm} (2)

$$\varphi_t = \frac{dn}{ndT} \int_{0}^{\infty} T_g(\lambda) \exp(-b z) \sin(\lambda y) d\lambda$$  \hspace{1cm} (3)

Where $\varphi_n$ (eq.2) is a deflection moving the probe beam normal to the sample surface, and $\varphi_t$ (eq.3) is a deflection in a plane parallel to the sample surface, and $\overline{T_g(\lambda)}$ is the Hankel spectrum of the surface temperature.

4. Experiments and results

4.1. PDS-Depth profiling

As first experiments, single point measurements of an artificial membrane (consisting of 3 layers each about 25 µm thickness) have been performed. In order to investigate the penetration of a model drug into a hydrophilic acceptor membrane depth resolved the frequency regime at 10 Hz, 20 Hz, 30 Hz, 100 Hz was applied. The artificial membrane (glycerol collodion) was placed above a formulation (methylorange (MO) 10% in white soft paraffin) (Fig.2). The up-grow of the PDS signal by increasing penetration time arising from the increasing MO amount is evident in four depth ranges (Fig. 3). After an exposure time of about 700 minutes the drug has penetrated a path length of almost 60 µm through the membrane and reaches the topmost layer at 17 µm [100 Hz].

![Figure 2. Scheme of the depth profiling.](image-url)
Assuming a thermal diffusivity of the DDC-membrane $\alpha = 9.9 \times 10^{-4} \text{[cm}^2 \text{s}^{-1}]$ (organic material) [7] the thermal diffusion length can be calculated by means of eq. 4. One of the main advantages of the PDS method is the possibility of varying the sampling depth

$$\mu = \left( \frac{\kappa}{\pi f \rho c} \right)^{1/2}$$

where $\kappa$ is the thermal conductivity, $c$ is the heat capacity, and $f$ is the light modulation frequency. While this formulation of $\mu$ applies strictly to homogeneous sample, understanding the concept of the thermal sampling depth for such samples is essential before expanding the analysis to depth profiling of heterogeneous materials.

4.2. PDS Imaging

The experimental arrangement applied for the imaging experiment is presented in figure 4 c. A Nd:YAG laser (532 nm) was used to excite the sample. The pure DDC (dodecanol/collodion) membrane is a lipophil acceptor system and does not absorb the irradiating laser. However, the model substance ditranol shows light absorption at this wavelength, which allows the selective excitation and detection of the substance. Photothermal images of the penetration process of the drug within an artificial membrane are presented in figure 4. The modulation frequency of the excitation beam was 18 Hz. On the subject of the membrane thickness (28 µm), the PDS-signal is a mean value, which represents the cumulative signal of the whole membrane thickness.

The image was recorded after 25 hours of penetration time and exhibits a signal gradient by increasing penetration way. Since we relate the PDS-signal to the concentration of ditranol the gradient correlates with the concentration profile of the drug in the membrane. The results show highest PDS signals arising near the boundary of the reservoir after 25 hours. The blank test with pure white soft paraffin prove the increasing signal is due to accumulating DI, since no PDS-signal generation more than the background noise was detectable. This results obtained with the scanner system achieve the requirements in non invasive in vivo experiments and are comparable with reference analytical optical methods for measurements of skin penetration [8, 9].

**Figure 3.** Frequency [Hz] dependent PDS signal versus time.

![Figure 3](image-url)
3. Conclusions
The potential use of a novel photothermal double beam laser scanning system was demonstrated exemplifying measurements in drug delivery analysis. By modulating the excitation beam it was possible to measure various depth ranges of the sample. Hence the process of diffusion of the drug could be followed in 4 depth regions of the acceptor membrane. Furthermore the new scanner system allows for imaging the sample surface and even deeper layers without moving the sample.

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