Determination of drug content in semisolid formulations by non-invasive spectroscopic methods: FTIR - ATR, - PAS, - Raman and PDS

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Abstract. This study elucidates the potential use of photothermal deflection spectroscopy (PDS), FTIR photoacoustic (FTIR-PAS), FT Raman, and FTIR-attenuated total reflection (FTIR-ATR) spectroscopy as analytical tools for investigating the drug content in semisolid formulations. Regarding the analytical parameters, this study demonstrates the photothermal beam deflection to be definitely comparable to well established spectroscopic methods for this purpose. The correlation coefficients range from 0.990 to 0.999. Likewise, repeatability and limit of detection are comparable.

1. Introduction

The state of the art in pharmaceutical practice to determine the drug content in semisolid formulations comprises analytical standard methods with the disadvantage of time consuming and laborious processes for separating the drug from the complex vehicle system. To overcome these obstacles, diverse spectroscopic methods have been applied to evaluate the drug content in semisolid and solid formulation [1]. Notably, the FTIR attenuated total reflection (FTIR-ATR) spectroscopy and the FTIR photoacoustic spectroscopy (FTIR-PAS) turned out to be suitable methods for the characterization of the drug penetration [2, 3]. Neubert et al. evidenced the step-scan photoacoustic spectroscopy with phase modulation is a useful analytical tool for the drug analysis [4]. FT Raman spectroscopy widely has been used to characterize pharmaceuticals. This method seems to be a good complement to IR spectroscopy for lipophilic molecules with many non-polar functional groups. The photothermal spectroscopy comprises various methods differing in the modus they apply for excitation and detection. In the past years, inexpensive and non-destructive variants of photothermal spectroscopy have been successfully developed [5-7]. The photothermal deflection spectroscopy (PDS) has proved to be a robust but also sensitive option for investigating almost any species of solid samples.

The objective of this article is to compare the potential use of PDS with the well known methods: FTIR-PAS, FT-Raman and FTIR-ATR were applied in pharmaceutical research exemplifying the direct determination of ketoconazole and methoxsalen suspended in white soft paraffin. The comparison procedure is based on validation parameters like limit of detection, linearity and specificity, which allow for a comparison of PDS with spectroscopic techniques and classification among the optical methods.

2. Experiments

2.1. \textit{FTIR-ATR / -PAS spectroscopy}

The IR spectra were acquired by using a Bruker spectrometer IFS 28 (Bruker Optics, Ettlingen, Germany) equipped with a Thermo Spectra-Tech horizontal ATR attachment. The sampling compartment is a Fresnel ATR accessory (Shelton, CT, USA) that uses a ZnSe crystal with an angle of
incidence of 45° in a horizontal orientation. For measurement each suspension was dispersed directly on the ZnSe crystal. The FTIR-PAS experiments were carried out with a MTEC 200 photoacoustic cell (MTEC, Ames, IA, USA). The topical formulation was loaded in a brass cup (5 mm diameter; 0.5 mm depth) that fits into the sample holder of the PAS cell. For the step-scan (ssc) experiment, the phase modulation technique was applied with five modulation frequencies in the range 16-54 Hz, and a modulation amplitude of 2\lambda_{\text{HeNe}} (1.266 µm). The demodulation of the photoacoustic signal with the modulated IR beam as a reference was achieved by using the “inphase” (I) and in “quadrature” (Q) components. The magnitude spectrum, M = (I^2 + Q^2)^{1/2} has been calculated.

2.2. Raman spectroscopy
FT Raman scattering at 180° to the incident beam was recorded by means of a Bruker FT Raman spectrometer RFS 100/S (Bruker Optics, Ettlingen, Germany). The excitation source was a diode pumped Nd:YAG laser at an operating wavelength of 1064 nm. Typical spectra were acquired with 200 scans and a laser power of 300 mW at the sample location. The data processing and evaluation of the spectra (FTIR-ATR, FTIR-PAS and FT-Raman) were carried out using the Bruker OPUS software.

2.3. Photothermal spectroscopy
The PDS experiments were performed with a photothermal device (Monobloc Phototherm Dr. Petry, Saarbruecken, Germany) with beam deflection detection of the oscillating surface temperature. A tuneable CO_2 laser (Access Laser, Model LASY 3S, Marysville, WA, USA) at a wavelength of 10.225 µm and an intensity range from 5 to 100 mW, was applied as the pumping source. The incident heating beam was modulated by a mechanical chopper (New Focus, San Jose, CA, USA). A HeNe laser (wavelength \(\lambda = 633\) nm, 1 mW; JDS uniphase, CA, USA) was used as probe beam source. The probe beam deflection signal (normal component) was detected by a four-quadrant position sensitive detector and processed by means of a lock-in amplifier. For scanning purpose, an XY-translation stage (Limes 90, OWIS, Staufen, Germany) was used, which has allowed to move the sample with a step accuracy of 0.5 or 1 µm. Pure white soft paraffin and white soft paraffin drug formulations of ketokonazole or methoxsalen were filled into cavities (0.25x0.5x1 mm³) positioned on a sample holder. All samples were measured at 5 modulation frequencies (16, 20, 24, 28 and 32 Hz).

2.4. Materials
Ketoconazole (KE) and methoxsalen (ME) were supplied by Sigma-Aldrich Chemie (Deisenhofen, Germany). White soft paraffin, dodecanol and collodion were purchased from Caesar & Loretz GmbH (Hilden, Germany). The samples were prepared as weight percent mixtures (2%, 4%, 5% and 8%) of white soft paraffin with KE and ME. A lipophilic skin model membrane composed of dodecanol and collodion (DDC membrane) was used for monitoring methoxsalen penetration.

3. Results and discussion
The drug content in both semisolid concentration series ranged from 2 % to 8 % (% w/w). The measurements were repeatedly performed (n ≥ 5) by means of all methods. Linearity over the investigated range was provided by all techniques considering the correlation coefficients of the linear regression of the calibration curves (R^2 > 0.99 of all calibration curves). Previously it was ascertained if FTIR-PAS ssc (step scan) or rsc (rapid scan) mode was suited best for this purpose. In the case of measuring the drug penetration into membranes the ssc mode was applied frequently, since this option allows for investigating deeper sample regions (figure 1 B) and even depth resolved characterization is possible. However, the rsc mode is favored when short measurement times are required. A comparison of both FTIR-PAS options concerning quantitative determination of model formulations produced better detection limits and repeatability values for the ssc mode and turned the balance in support of the ssc technique for the study.
Following, when FTIR-PAS is written the step scan method is intended. The specificity of the analytical procedures using the spectroscopic methods of FTIR-PAS, FTIR-ATR and FT-Raman was provided, since the integration of specific drug bands and spectral regions was used for quantification of the drug amount (table 1).

As the PD signal is the result of a monochromatic excitation the wavelength of the excitation beam was adapted to $978 \text{ cm}^{-1}$ at which ketoconazole and methoxsalen exhibit local absorption maxima. This procedure allowed for a good correlation of drug amount with PD signal intensity.

The relative standard deviation of the performed measurements was between $1.7 \%$ and $5.9 \%$ (table 2). These outcomes indicate an appropriate repeatability of the investigations. However, the standard deviation of the ATR measurements is higher then $5 \%$ for both series. In contrast FTIR-ATR offers a high sensitivity. Comparing FTIR-ATR and PDS, the disadvantage of ATR technique, which allows analysing only very thin surface layers, becomes apparent given by a high standard deviation. The generation of the signal is merely proceeding in a $2 \mu m$ layer of the specimen facing, so that particles positioned outside of this thin plane contribute to the signal less. The values obtained by means of FT-Raman spectrometer exhibit a low standard deviation. The $1 \text{ mm}$ laser diameter of the FT-Raman gives average spectra from the sample surface resulting in a good repeatability. The best limit of detection for the KE formulation arose from the FT-Raman method which is $6$ times lower then approachable by means of the PDS technique (table 2). Contrary results were obtained for the ME concentration series, where the FTIR-ATR technique exhibits best detection limit closely followed by PDS and FT-Raman. When comparing PDS and Raman method, it becomes evident that parameters like limit of detection and repeatability definitely depend on the physicochemical properties of the substance.

**Table 1.** Spectroscopic techniques with corresponding spectral region applied for quantitative determination of drug content in semisolid formulations. In the case of the PDS measurement the wave number of excitation beam is presented.

| Method  | Methoxsalen [cm$^{-1}$] | Ketoconazole [cm$^{-1}$] |
|---------|-------------------------|--------------------------|
| FTIR-PAS| 1560 - 1840             | 1540 - 1740              |
| FTIR-ATR| 1530 - 1750             | 1560 - 1660              |
| FT-Raman| 1660 - 1760             | 1530 - 1660              |
| PDS     | 978                     | 978                      |

Figure 1.

FTIR-PAS measurement A) in step scan mode (54 Hz) of methoxsalen in semi solid formulation at various concentrations (% w/w). B) Penetration curves of methoxsalen from a semisolid formulation into an artificial DDC membrane measured in step scan and rapid scan mode. Thermal diffusivity $\alpha = 9.9 \times 10^{-4} \text{ cm}^2/\text{s}$ [8] was used to calculate the penetration depth $\mu$ of the measurements.
While ME has non-polar chemical groups and is an intensive Raman scattering substance, KE displays a higher specific extinction coefficient at the laser wavelength used here in PDS measurements. The correlation coefficients for calibration curves compared with PDS calibration range from 0.990 to 0.999 (table 3). These results confirm the comparability of the PDS technique with the other well-established methods. Since the PDS does not give any chemical information about the analyte, this technique does not provide such high selectivity as spectroscopic methods which produce a spectrum of the sample. Nevertheless, substance-specific thermal properties like thermal diffusivity or specific heat can be determined by means of this technique. An advantage of PDS is the capability to measure with a lateral resolution in the low micrometer range. Due to the spot size of the CO\textsubscript{2} laser used for the drug content determination, the lateral resolution of PDS was restricted to 50 µm. Depending on the optical system and excitation source, values less than 5 µm can be achieved. However, conventional FT-Raman spectrometers do not afford such resolution values with a minimum spot size of 100 µm. Furthermore, the diameter of the measurement point of FTIR-PAS extends to 7 mm and FTIR-ATR does not facilitate pointwise non-contact measurements at all. This characteristic gives PDS technique and spectroscopic methods coupled with microscopes the preference for many purposes. Finally, the comparison of PDS and its allied method FTIR-PAS show a good correlation. Concerning repeatability and limit of detection, both techniques show similar results. By increasing modulation frequency of excitation beam, the limit of detection and reproducibility worsen. Since ambient noise affects measurement results a lot, FTIR-PAS requires demanding measuring conditions. However, PDS is a quite robust method and is less sensitive for noise.

### 4. Conclusions

These results prove the proportionality of the PD signal to the concentration of two different drugs in semisolid pharmaceutical formulation and this study demonstrates PDS to be definitely comparable to well-established spectroscopic methods for this purpose. FTIR and FT-Raman methods are sensitive on different chemical structures, so that they complement each other. Concerning the limits of detection, PDS seems to be more independent from the chemical structure. However, the application of PDS with monochromatic excitation requires the adaption of the wavelength of excitation and absorption behaviour of the analyte. However, this photothermal technique does not provide the

### Table 2. Relative standard deviation and limits of detection of Methoxsalen and Ketoconazole. FTIR-PAS and PDS were performed at 16 Hz modulation frequency.

|                  | Methoxsalen | Detection limit [w/w %] |
|------------------|-------------|-------------------------|
|                  | Relative standard deviation [%] |                        |
| PDS              | 4.4         | 0.37                    |
| FTIR-PAS         | 3.0         | 0.29                    |
| ATR              | 10.3        | 0.27                    |
| FT-Raman         | 1.9         | 0.06                    |

|                  | Ketoconazol | Detection limit [w/w %] |
|------------------|-------------|-------------------------|
|                  | Relative standard deviation [%] |                        |
| PDS 16           | 2.8         | 0.19                    |
| FTIR-PAS         | 1.7         | 0.29                    |
| ATR              | 5.9         | 0.14                    |
| FT-Raman         | 1.9         | 0.06                    |

### Table 3. Correlation parameter R\textsuperscript{2} of calibration curves for Methoxsalen and Ketoconazole between PDS and the reference methods. FTIR-PAS and PDS were performed at 16 Hz modulation frequency.

|                  | Methoxsalen | PDS        |
|------------------|-------------|------------|
|                  | ATR         | 0.990      |
|                  | FT-Raman    | 0.995      |
|                  | FTIR-PAS    | 0.993      |

|                  | Ketoconazol | PDS        |
|------------------|-------------|------------|
|                  | ATR         | 0.993      |
|                  | FT-Raman    | 0.994      |
|                  | FTIR-PAS    | 0.999      |
chemical information. Referring to this, a linkage to another spectroscopic method, e.g. Raman microscopy, would be a reassessment. Furthermore, the PDS provides three dimensional sample characterization due to the combination of mapping mode and depth profiling [9]. In this manner the PDS can give information about homogeneity in a quite simple way. Due to the good robustness and flexibility PDS is a promising technique for the application in analytical pharmacy and biopharmacy.

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