Direct Determination of Drug Content in Semisolid Formulations Using Step-Scan FT-IR Photoacoustic Spectroscopy

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INTRODUCTION

In pharmaceutical practice the drug content in semisolid formulations is measured by using analytical standard methods such as HPLC, GC or HPTLC. For this purpose, the drug has to be separated from the complex semisolid vehicle system using liquid/liquid or liquid/solid phase extraction. This is followed by specific analytical assays in order to quantify the drug content. Obviously, such a procedure is laborious, time consuming and in many cases has low reproducibility.

To our knowledge, there are no literature reports of effective methods for the direct determination of drug content in semisolid formulations such as ointments and creams. In the last decade, Fourier transform infrared (FT-IR) spectroscopy combined with very sensitive photoacoustic (PA) detection has gained acceptance as a valuable qualitative as well as quantitative technique for the analysis of a large variety of materials (1–3). Photoacoustic spectroscopy (PAS) is employed in situations where a sample is deemed unsuitable or too valuable to grind or modify for traditional IR sampling techniques. It is very helpful that in the PA mode of operation no sample preparation is required. Until now, there are only a few pharmaceutical applications using PAS. For example, FT-IR PAS was successfully used to quantify a three component solid mixture (aspirin tablet) with concentrations varying from 0 to 60% (4). Modern developments with the application of digital signal processing (DSP) electronics to step-scan FT-IR PAS appear to offer special advantages (5): i) spectral depth profiling and ii) detection of weakly absorbing components in the presence of a strongly absorbing matrix. The last application has prompted us to study the possibilities and limitations of the step-scan FT-IR PAS with DSP demodulation for measuring drugs in the relevant concentration ranges.

We wish to report the determination of drug content in semisolid formulations using step-scan FT-IR PAS. Dithranol (DT) and brivudin (BV) were the drugs examined with Vaseline® the semisolid vehicle system. The results obtained are compared with those of standard analytical methods such as HPLC for DT and capillary zone electrophoresis (CZE) for BV.

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EXPERIMENTAL

Materials

Brivudin and dithranol were obtained from Berlin Chemie (Berlin, Germany). The samples were prepared as weight percent mixtures of Vaseline® with BV and DT, respectively.

Capillary Zone Electrophoresis

A Hewlett Packard ^{3D}CE (Waldbronn, Germany) system fitted with a 600 (515) × 0.005 mm (extended lightpath) fused silicia capillary (Hewlett Packard) and an on-column diode array detector (190···600 nm) was used. The capillary was preconditioned for 10 min with 1.0 M NaOH before the first run and then for 3 min with 0.1 M NaOH and 3 min with run buffer prior to each following run. The separation conditions were: −30 kV voltage (detection end), 200 mbar*sec pressure injection, 20°C capillary temperature. The detection was done on the cathodic site at 250 nm. All solutions and samples were filtered through a membrane filter of 0.2 μm pore size and degassed by ultrasound before running. Borate buffer solutions, pH 10.0 (20 mM), were prepared using analytical grade sodium borate (Fluka, Buchs, Switzerland).

BV was separated from Vaseline® by shaking 10 mg of the ointment for 1 h with a mixture consisting of 2.0 ml of petroleum ether, 1.5 borate buffer of pH 10.0 and 1.5 ml of ethanol. After shaking the aqueous phase was separated, filtered and diluted with borate buffer pH 10.0 (1:10) for the CZE measurement.

HPLC Method

A Kontron Instruments HPLC apparatus (Neufahrn, Germany) equipped with a pump P235, a Kontron Diode Array 440 detector, and a SA 360 autosampler was used for determining the DT content in the ointment. The following conditions were applied: flow rate 1.0 ml/min; eluent: acetonitrile, water, acetic acid (70(V): 29.5(V): 0.5(V); column: Nucleosil RP-18 (5 μm), 100 × 4 mm (Knauer, Berlin, Germany); detection wavelength 252 nm. For the analysis 10 mg of the ointment were shaken with 1 ml $CHCl_3$ for 1 h. Then 200 μl of the sample were diluted with 2 ml of methanol, filtered and injected into the HPLC device.

Photoacoustic Spectroscopy

The spectra were collected on a Bruker FT-IR spectrometer IFS 28 (Karlsruhe, Germany) equipped with a MTEC Photoacoustics model 200 photoacoustic cell (Ames, Iowa, USA). The ointment was placed in a brass cup (5 mm diameter and 0.5 mm depth) which fits into the PAS sample holder. Care was taken to prepare a flat sample surface. Using the Bruker OPUS software package the step-scan experiments were conducted by applying a sinusoidal phase modulation technique with a modulation frequency of 25 Hz and a modulation amplitude of $2\lambda_{HeNe}$ (1.25 μm) and 10 coadditions. The demodulation of the PA signal with reference to the IR beam modulation was provided by the acquisition processor and resulted in the "in-phase" (I) and "in-quadrature" (Q) components. The PA spectra of the ointment samples were acquired at a resolution of 12

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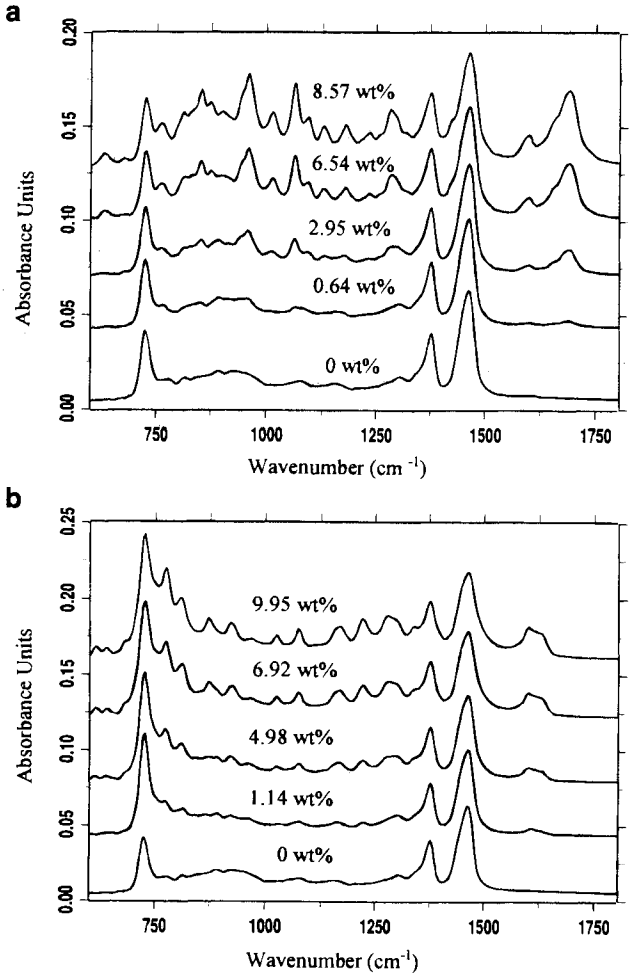

Fig. 1. Step-scan FT-IR PA magnitude spectra of a) mixtures Vaseline®/brivudin and b) mixtures Vaseline®/dithranol. The various drug content is indicated.

cm^{-1} using a strong Beer-Norton apodization function and Mertz phase correction. A delay time of 150 ms was chosen for stabilizing the step-scan position. Thus, the total scan time amounted to 1284 seconds. The PA cell was purged with helium prior to measuring each sample. From the two signal components I and Q the magnitude spectrum $M = (I^2 + Q^2)^{12}$ was calculated. All spectra were normalized by ratioing the sample spectrum with a carbon black spectrum.

RESULTS AND DISCUSSION

The normalized PA magnitude spectra in the range between 600 and 1800 cm^{-1} for mixtures of Vaseline® with various concentration of BV and DT are presented in Figure 1a) and 1b), respectively. The spectra exhibit an excellent signal to noise ratio and bands belonging to the drug component are clearly observable even at the lowest amount of drug (about 0.5 wt %). In comparison with the conservative rapid-scan PAS technique we found an increase in the signal to noise ratio by more than an order of magnitude (equal measuring time supposed).

For the quantitative analysis we used the well separated bands between 1545 and 1770 cm^{-1} for BV and between 1560

Table 1. Determination of Brivudin in Vaseline® Formulation Using CZE and FT-IR PAS

BV content [%] according declaration	BV content [%] determined using		
	CZE		FT-IR PAS
	Mean	S.D. rel [%]	mean
0.5	0.64	8.5	0.51
1.0	1.22	14.4	1.29
2.0	2.95	12.1	3.07
6.0	6.54	17.3	6.48
8.0	8.57	3.0	8.57
10.0	9.89	11.1	9.98
	detection limit: 70.0 [μg/ml]		

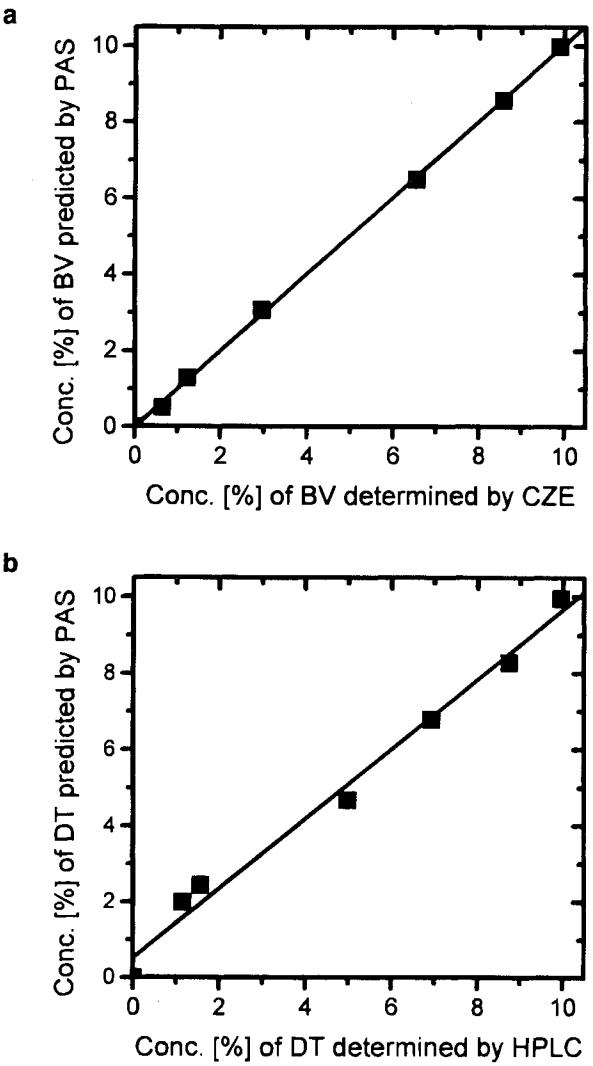

Fig. 2. Drug concentration predicted by PAS versus actual drug content a) mixtures Vaseline®/brivudin; brivudin content determined by CZE b) mixtures Vaseline®/dithranol; dithranol content determined by HPLC

Table 2. Determination of Dithranol in Vaseline® Formulation Using HPLC and FT-IR PAS

DT content [%] according declaration	DT content [%] determined using		
	HPLC		FT-IR PAS
	Mean	S.D. rel [%]	mean
1.0	1.14	3.7	1.99
2.0	1.55	17.0	2.44
4.0	4.98	9.9	4.67
6.0	6.92	4.5	6.79
8.0	8.74	6.1	8.28
10.0	9.95	7.2	9.95
	detection limit: 131.7 [μg/ml]		

and 1670 cm^{-1} for DT. The integrated intensities of these bands were determined and ratioed to the integrated intensity of the Vaseline® band at 1462 cm^{-1}, whereby in the case of Vaseline® half of the band ($\upsilon > 1462$ cm^{-1}) only was considered.

The results obtained by PAS and analytical standard methods for Vaseline®/BV and Vaseline®/DT are listed in Table 1 and 2, respectively. It appears that the reproducibility of the standard methods varies between 3 and 17%, whereas, the instrumental error of the step-scan FT-IR PA spectrometer amounts to less than 2%. For calibrating the photoacoustic spectra we used the sample with a drug concentration of 8.57% for Vaseline®/BV and 9.95% for Vaseline®/DT. The drug content, derived in this manner from the PAS data, versus the actual content, obtained by CZE and HPLC, respectively, is plotted in Figure 2. These plots clearly demonstrate that Beer's law is fulfilled. The results show excellent correlation coefficients: 0.9998 for Vaseline®/BV and 0.994 for vaseline/DT. As can be seen in Figure 2b, the deviations of the data points from the linear regession curve, particularly in the range of lower content, are larger for DT than for BV. In the case of Vaseline®/DT, the standard deviation amounts to 0.451 being five time greater than that for Vaseline®/BV. The HPLC detection limit for DT is also relatively high (131.7 μg/ml). This greater uncertainty for determining DT is caused by the HPLC method.

In conclusion, it appears that step-scan FT-IR PAS in conjunction with a phase modulation technique can be profitably employed in the drug analysis of semisolid formulations. It is possible to determine precisely drugs down to concentration of 0.5 wt%. The minimal sample preparation required is a big advantage of the technique.

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