Penetration Studies of Clotrimazole from Semisolid Formulation Using Step-Scan FT-IR Photoacoustic Spectroscopy

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Purpose. The aim of this study was to elucidate the potential use of the step-scan FT-IR photoacoustic spectroscopy (PAS) for the non-destructive determination of drug penetration into membranes.
Methods. The penetration of clotrimazole from a 10% (w/w) suspension in Vaseline® into a dodecanol-collodion acceptor membrane was studied by three methods: the step-scan FT-IR PAS with a phase modulation, a multilayer membrane system, and a modified libration model. Based on Fick's second law, the diffusion coefficient of the drug in the membrane was derived by numerical fitting of the experimental data.
Results. The three methods applied provided almost the same diffusion coefficient $D = 2.2\ 10^{-9}$ cm²/s for clotrimazole in the membrane. Because of the non-destructive mode of operation, the accuracy of results obtained by FT-IR PAS is much better than that attainable by other two methods.
Conclusions. Step-scan FT-IR photoacoustic spectroscopy in conjunction with a phase modulation is useful to determine the penetration of drug through membranes. The fact that samples can be investigated without elaborate preparation is an advantage of this spectroscopic technique.

KEY WORDS: penetration; step-scan FT-IR photoacoustic spectroscopy; multilayer membrane; diffusion coefficient; clotrimazole.

INTRODUCTION

Studying the mechanism of drug transport through human skin is of considerable interest for understanding the barrier function of the stratum corneum as well as for many medical and cosmetic applications. For this purpose, a variety of spectroscopic methods among which photoacoustics, fluorescence and remittance spectroscopy in the UV range have been applied (1,2). Presently, Fourier transform infrared (FT-IR) spectroscopy with the attenuated total reflection (ATR), has emerged as one of the standard techniques used to investigate penetration kinetics (3–5).

Recent developments in step-scan FT-IR photoacoustic spectroscopy (PAS) appear to offer new possibilities. In particular, PAS was employed with samples that are either deemed unsuitable or too valuable to grind for analysis by traditional IR sampling techniques. Although samples can be readily analyzed without further preparation, FT-IR PAS has not been extensively utilized so far. Modern spectrometers operating with digital signal processing (DSP) electronics to step-scan FT-IR PAS offer additional advantages, such as possibility for spectral depth profiling and the detection of weakly absorbing compounds in strongly absorbing matrices.

As to the latter, we have recently reported on the quantitative drug analysis in semisolid formulations using step-scan FT-IR PAS (6). In the work described here, step-scan FT-IR PAS was applied to investigate the penetration of drugs from semisolid vaseline formulations into artificial membranes; clotrimazole (CT) was chosen as a model drug. These results of the studies are compared to those obtained from a multilayer membrane system and a modified liberation model. A mathematical model based on the Fick's second law is applied to quantify the process of drug diffusion into the model membrane system.

EXPERIMENTAL

Materials

Clotrimazole and chloroform (HPLC grade) were supplied by Sigma-Aldrich Chemie, Deisenhofen (Germany). Collidion solution (4% w/w in ether/ethanol) was purchased from Caelo, Hilden (Germany). 1-dodecanol was obtained from Merck-Schuchardt, Hohenrunn (Germany).

The ointments were prepared as a 10% (w/w) suspension in Vaseline®.

Preparation of the Membranes

The preparation technique of the model membranes has been described previously (7). Four grams of 1-dodecanol were dissolved in 96 g ether/ethanol (85:15, w/w) and a 100 g collodion solution added. This mixture was placed on a membrane preparation apparatus (8) that provides membranes of uniform thickness and 1-dodecanol content. The dodecanol-collodion (DDC) membrane was cut into disks after solvent evaporation.

Multilayer Membrane System

The multilayer membrane system (MLMS) schematically outlined in Fig. 1, has been used as a penetration model by various authors (8–10). Each acceptor system contained one DDC membrane (diameter 4 cm, thickness 18 μm). The topical formulation (ca. 10 mg) was applied to an exposed membrane area (4 cm²). Usually, five cells were fitted together and placed in a chamber maintained at 22°C during the experiment. The experiment was performed at room temperature in order to ensure conditions comparable to those used in photoacoustic measurements. At selected time intervals, one penetration cell of the model system was removed from the thermostated chamber; the remained quantity of ointment was then carefully wiped away from the membranes. In order to determine the extent of drug penetration, each membrane was extracted by shaking with 5 ml chloroform for 20 min. The UV absorption at 260 nm recorded with a Shimadzu UV-spectrometer UV-1202 was used as a measure of the diffused amount of CT. For that purpose the calibration was carried out with CT standard solutions in the concentration range of 0–400 μg/ml.

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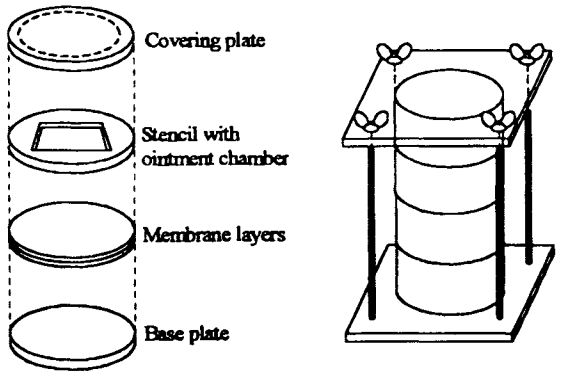

Fig. 1. Multilayer membrane system (MLMS).

Modified Liberation Model

The modified liberation model (MLiM) set-up (11) made of acrylic glass and schematically shown in Fig. 2, consists of two chambers separated by DDC membranes (17–20 μm thick). The chamber (2.0 × 2.0 × 1.25 cm) in the middle contained the donor solution, i.e. 5 ml clotrimazole solution (100 mg/l) in a mixture of dodecanol and ethanol (1:1; v/v). The membranes and the chambers were sealed using silicone paste and then bolted together.

Using a holder arm and a tripod, the model thus prepared was hung in a 150 ml glass beaker filled with 100 ml dodecanol-ethanol-mixture (1:1; v/v), i.e. the acceptor solution. The acceptor solution was magnetically stirred in the course of the experiment

The drug diffused from the donor solution through the membrane into the acceptor. At appropriated time intervals, 1 ml sample of the acceptor solution was taken and the reduced volume compensated by adding of 1 ml of fresh acceptor. When required, the samples were diluted with ethanol and the UV absorption measured at 260 nm. The CT amount was determined by calibration using standard solutions the concentration of which ranged between 0 and 500 μg/ml; this procedure was repeated until the equilibrium was reached.

Photoacoustic Spectroscopy

The photoacoustic experiments were carried out on a Bruker FT-IR spectrometer IFS 28 equipped with a MTEC Photoacoustics model 200 photoacoustic cell. 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. The DDC membrane was placed on the ointment surface without any air bubbles.

The step-scan experiments were conducted by applying the phase modulation technique with a modulation frequency of 91 Hz and a modulation amplitude of 2 λ_{HeNe} (1.25 μm). In this set up the moving mirror of the interferometer oscillated sinusoidally about the stopping position with the selected modulation frequency and modulation amplitude. This has enabled the sinusoidal phase modulation of the radiation. The demodulation of the PA signal with the modulated IR beam as a reference was achieved by the acquisition processor; this resulted in the "in-phase" (I) and "in-quadrature" (Q) components. Using the two signal components I and Q, the magnitude spectrum $M = (I^2 + Q^2)^{1/2}$ has been calculated. All PA spectra were acquired at a resolution of 12 cm^{-1} using a strong Norton-Beer apodization and Mertz phase correction. A delay time of 150 ms was chosen for stabilizing the step-scan position. Using 10 or 20 coadditions the measuring time per spectrum amounted to 670 s or 1340 s, respectively. Prior to the experiment the PA cell was helium purged for 30 s. All spectra were normalized to that of a carbon black sample.

Mathematical Analysis

The transport of drug from the suspension (donor) to the membrane (acceptor) occurs sequentially, i.e.: (i) the solid drug particles dissolve in the liquid phase of Vaseline®, (ii) dissolved drug molecules diffuse in the donor, (iii) the drug migrates from the donor to the acceptor, and finally (iv) the drug diffuses in the acceptor. For the suspension studied here, the first step is not essential, because of the high drug content in the formulation. Therefore, we suppose that the saturation concentration of drug in the liquid phase of the Vaseline® ($c_{d\,sat}$) does exist during the entire penetration process. This implies that the diffusion of drug in the donor system is negligible. The partition coefficient k accounts for the transfer of the drug from the donor to the acceptor. The drug diffusion in the acceptor obeys Fick's second law for the one-dimensional case, i.e.:

$$\frac{\partial c_a}{\partial t} = D\frac{\partial^2 c_a}{\partial x^2}, \qquad (1)$$

where c_a is the drug concentration in the acceptor and D is the diffusion coefficient. The equation (1) was solved for the

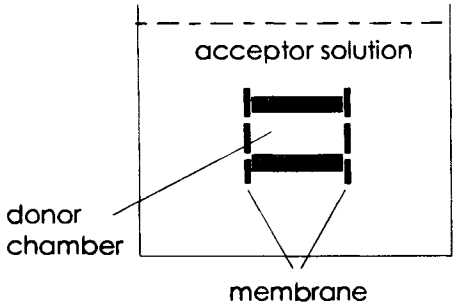

Fig. 2. Modified liberation model (MLiM).

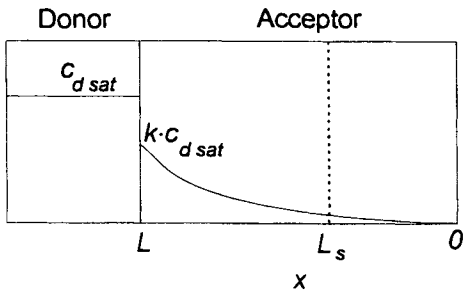

Fig. 3. Concentration profile of the drug in the donor and acceptor following the application of the formulation. L is the membrane thickness, L_S is the photoacoustic sampling depth, k is the partition coefficient and $c_{d\,sa}$ stands for the saturation drug concentration in the liquid phase of Vaseline®.

following initial and boundary conditions:

$$\text{at } t = 0: \quad c_a = 0 \quad \text{for } 0 < x < L, \tag{2}$$

$$\text{at } t > 0 \quad c_a (x = L) = kc_{d\,sat} (= c_{a\,sat}), \tag{3}$$

$$\text{and} \quad \frac{\partial c_a}{\partial x} = 0 \quad \text{for } x = 0, \tag{4}$$

where L is the thickness of the membrane (see also Fig. 3) and $c_{a\,sat}$ is the saturation concentration of the drug in the acceptor membrane. The boundary condition (3) indicates that the drug concentration on the left edge of the membrane remains constant during the entire penetration process. The condition (4) for zero flow implies that no drug can traverse the right membrane boundary.

In the case of the multilayer membrane system, the experiment provides the total mass of the drug in the membrane

$$M_{MLMS} = \int_0^L c_a \, dx, \tag{5}$$

whereas, in the case of the PAS experiment, the mass detected is given by the sampling depth of the IR beam (L_S)

$$M_{PAS} = \int_0^{L^s} c_a \, dx \tag{6}$$

Based on this model and the experimental data obtained, one has determined the diffusion coefficient in the acceptor. The numerical calculations were carried out using the nonlinear least-square data fitting by Gauss-Newton method of the software package MATLAB (The Mathworks Inc., Natick, Mass. USA).

RESULTS AND DISCUSSION

Typical examples of normalized photoacoustic magnitude spectra obtained for the membrane/Vaseline®/CT system during the penetration experiment are presented in Fig. 4. For a 31 μm thick membrane, only the spectrum of the pure membrane was observed at the beginning of the experiment. It can be seen that characteristic IR bands of the drugs appear in the course of penetration. The increasing intensity of the bands in the range 710–800 cm^{-1} and 1190–1240 cm^{-1} as well as of the shoulder at 1500 cm^{-1} (Fig. 4a) clearly indicates the penetration of CT into the membrane. It has been proved that IR bands in these spectral regions are caused by the drug; for pure CT strong bands appear at 762 cm^{-1}, 1212 cm^{-1}, and 1493 cm^{-1} (spectrum not shown).

Repeating the experiment with a thinner (17 μm) membrane, one obtains spectra showing CT bands at the onset of the measurement. This observation suggests sampling depth

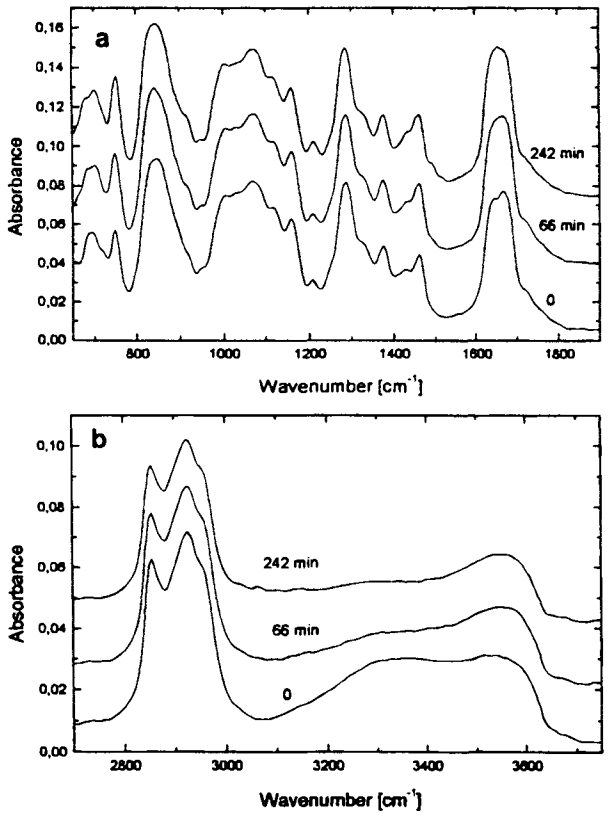

Fig. 4. Step-scan FT-IR photoacoustic spectra in the spectral ranges (a) 650–1900 cm^{-1}, and (b) 2700–3750 cm^{-1} for the membrane/clotrimazole/Vaseline® system recorded at various times of the penetration experiments. From bottom to top t = 0; t = 66 minutes; t = 242 minutes. Thickness of the membrane 31 μm.

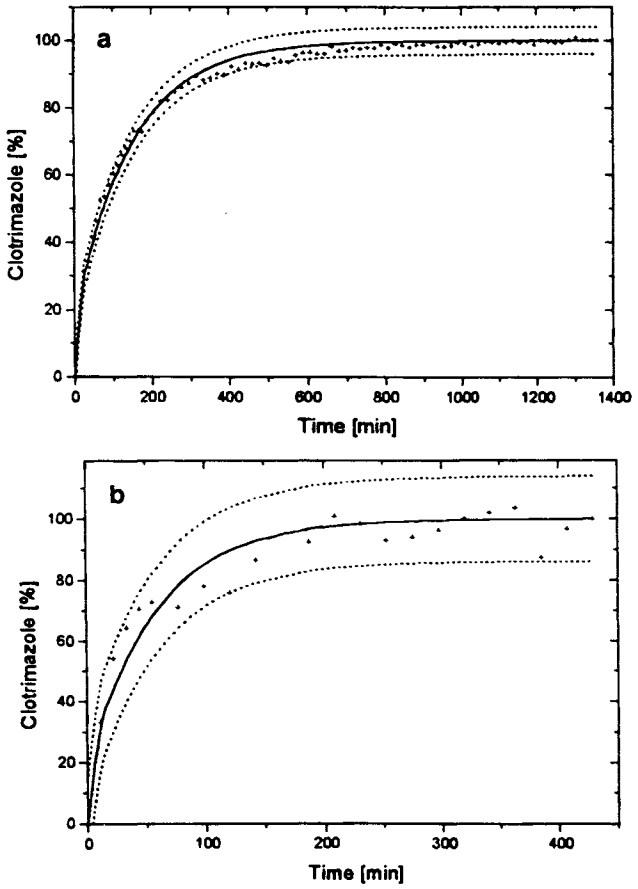

Fig. 5. The content of clotrimazole in DDC membrane (normalized to 100%) plotted versus the penetration time as calculated from (a) FT-IR PAS, and (b) MLMS experiments. (+) experimental data; (—) calculated; (•••) error bounds of the fitted curve.

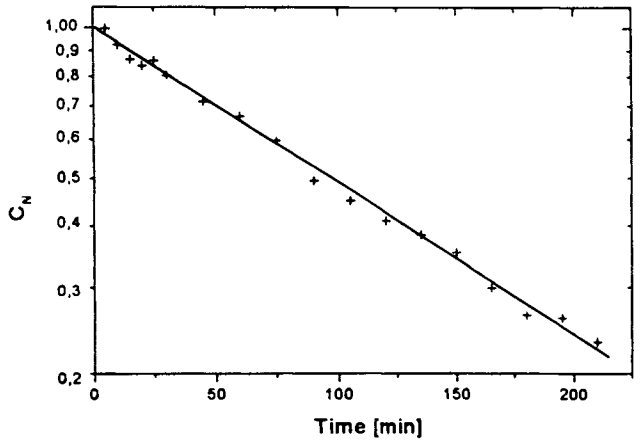

Fig. 6. Semilogarithmic plot of $C_N = [c_{DC}(t) - c_{DC}(t \rightarrow \infty)]/[c_{DC}(t = 0) - c_{DC}(t \rightarrow \infty)]$ versus time; $c_{DC}(t)$ is the concentration of clotrimazole in the donor chamber at the time t.

within the membrane being 15 μm. Another point of interest is that changes in the spectral range 3200–3500 cm^{-1} (Fig. 4b) reflect alterations in the hydrogen bond system of the membrane caused by the drug penetration.

For the quantitative analysis, we have applied the partial least-square program 'quant 2' of the Bruker OPUS software package taking into account the spectral ranges mentioned above. The spectra obtained from the membrane without drug as well as from a membrane contained the saturation amount of the drug, were taken for calibration. The content of CT thus obtained and normalized to 100%, the equilibrium value, is plotted in Fig. 5a versus time.

For comparison reasons, the results obtained with the multilayer membrane system are shown in Fig. 5b.

In the case of MLiM experiment, the diffusion coefficient was calculated from the drug concentration in the donor chamber using the following relationship (12)

$$\log \frac{c_{DC}(t) - c_{DC}(t \rightarrow \infty)}{c_{DC}(t = 0) - c_{DC}(t \rightarrow \infty)} = -\frac{D \cdot A \cdot k_s}{2.303 \cdot d \cdot V} \cdot t, \quad (7)$$

where $c_{DC}(t)$ is the drug concentration in the donor chamber at the time t, A is the area of the membrane, k_S is the membrane/donor partition coefficient, d is the membrane thickness, and V is the volume of the donor chamber. The semilogarithmic plot of $C_N = [c_{DC}(t) - c_{DC}(t \rightarrow \infty)]/[c_{DC}(t = 0) - c_{DC}(t \rightarrow \infty)]$ versus time is shown in Fig. 6.

The diffusion coefficients D determined by the three methods are shown in Table 1. It is evident that the methods applied provide almost the same value of D. Obviously, the accuracy of the PAS experiment is superior to that of the other two methods. The variance between the experimental data and the calculated curve is one order smaller than in the case of the MLMS experiment. This is not surprising because the PAS as an on-line method is capable of providing much more data points than both other methods can do, reducing thereby substantially the errors due to the handling procedure.

Table 1. Diffusion Coefficient D of Clotrimazole in DDC Membrane Determined at Room Temperature by Using Various Methods

Method	D [10^{-9} cm^2s^{-1}]	Variance
PAS	2.21 ± 0.06	4.18
MLMS	1.87 ± 0.31	44.67
MLiM	2.73 ± 0.23	—

CONCLUSIONS

We have successfully demonstrated that a penetration of drug through membranes can be monitored by step-scan FT-IR photoacoustic spectroscopy in conjunction with a phase modulation technique. The fact that samples can be investigates with a minimum handling represents the most important advantage of this spectroscopic method.

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REFERENCES

1. B. Sennheim, K. Giese, K. Plamann, N. Harendt, and K. Köhnel. In vivo evaluation of the penetration of topically applied drug into human skin by spectroscopic methods. *Skin Pharmacol.* **6**:152–160 (1993).
2. E. Stahl and H. D. Breuer. Photoakustische Spektroskopie in der Pharmazie. *Arch. Pharm.* **318**:36–40 (1985).
3. H. M. Reinl, A. Hartinger, P. Dettmar, and T. M. Bayerl. Time resolved infrared ATR measurements of liposome transport kinetics in human keratinocyte cultures and skin reveals a drastic dependence on liposome size and phase state. *J. Invest. Dermatol.* **105**:291–295 (1995).
4. A. M. L. Tralhão, A. C. Watkinson, K. R. Brain, J. Hadgraft, and N. A. Armstrong. Use of ATR-FTIR spectroscopy to study the diffusion of ethanol through glycerogelatin films. *Pharm. Res.* **12**:572–575 (1995).
5. M. A. Pellet, A. C. Watkinson, J. Hadgraft, and K. R. Brain. Comparison of permeability data from traditional diffusion cell and ATR-FTIR spectroscopy. Part I. Synthetic membranes, Part II. Determination of diffusional pathlengths in synthetic membranes and human stratum corneum. *Int. J. Pharm.* **154**:205–215 and 217–227 (1997).
6. R. Neubert, B. Collin, and S. Wartewig. Direct determination of drug content in semisolid formulations using step-scan FT-IR photoacoustic spectroscopy. *Pharm. Res.* **14**:946–948 (1997).
7. R. Neubert, C. Bendas, W. Wohlrab, B. Gienau, and W. Fürst. A multilayer membrane system for modelling drug penetration in human skin. *Int. J. Pharm.* **75**:89–94 (1991).
8. S. Huth, R. Neubert, L. Boltze, and A. Büge. Experimental determination and mathematical modelling of propylene glycol transport from semisolid vehicles. *Chem. Pharm. Bull.* **44**:1263–1266 (1996).
9. R. Neubert, W. Wohlrab, and C. Bendas. Modelling of drug penetration into human skin using a multilayer membrane system. *Skin Pharmacol.* **8**:119–129 (1995).
10. M. T. Knorst, R. Neubert, and W. Wohlrab. Release of urea from semisolid formulations using a multilayer membrane system. *Drug Devel. Ind. Pharm.* **23**:259 (1997).
11. S. Pfeifer, P. Pflegel, and H.-H. Borchert. *Biopharmazie*, Verlag Ullstein Mosby GmbH, Berlin, 1995.
12. B. H. Lippold, Simulierung der gastro-intestinalen resorption von dissoziierenden Arzneistoffen mit einem Membranmodell, *Pharm. Ind.* **38**:208–215 (1976).