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A new FTIR-ATR cell for drug diffusion studies†

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The drug diffusion of most compounds, particularly hydrophilic molecules through the skin is limited by the permeation of the outermost cell layers of the epidermis, the *stratum corneum* (SC). For this reason it is of interest to characterize drug diffusion processes through this skin layer. A new FTIR-ATR cell was developed for non-invasive real time measurements of drug diffusion. The diffusion of water through an artificial polyethyleneglycol–polydimethylsiloxane membrane was studied. Additionally the diffusion of urea in human SC was analyzed. Based on a mathematical model the diffusion coefficients were derived. We could reveal that this cell associates the advantages of the Franz diffusion cell and the FTIR-ATR spectroscopy as a new powerful method for determining drug diffusion through biological membranes.

1. Introduction

The efficiency of topically applied drugs is often limited by their poor penetration through the *stratum corneum* (SC), the outermost layer of the skin. Therefore, it is of great importance to describe their diffusion through this outermost layer of the skin. This is frequently characterized in terms of the diffusion coefficient. Fourier transform infrared (FTIR) spectroscopy with an attenuated total reflection (ATR) unit is a well establish method to monitor the penetration of the lipophilic compound cyanophenol through the SC.[1–4] The penetration of the lipophilic model compound undecanitrile into human SC was also studied by Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS).[5] The diffusion coefficient of the small hydrophilic compound urea through the SC is unknown, even though it is an important substance for many dermatological and cosmetic applications. Although Ackermann *et al.* studied the permeation behaviour of urea on excised mice skin about 20 years ago using a diffusion cell and radio labelled ^{14}C-urea, a well-accepted method to measure the urea diffusion through SC is still lacking.[6] As explored in this paper, FTIR-ATR spectroscopy seems to be a very promising choice.

The most important advantage of the ATR technique is that it is non-destructive and provides online data of drug penetration. Typically, an appropriate artificial or natural membrane acting as an acceptor is sandwiched between an impermeable ATR crystal and a reservoir of penetrant, the donor. Drug diffuses through the membrane, initially devoid of diffusant, until it reaches the impermeable ATR crystal. Due to the diffusion through the membrane there will be a build up of penetrant concentration at the interface between membrane and crystal, which can be monitored online by the appearance and increase of drug specific IR bands as a function of time. Because the typical range of sampling depth is 1 to 2 μm, information obtained from an ATR spectrum pertains only the immediate layer in contact with the crystal. Therefore, an intimate constant optical contact during the diffusion experiment between the membrane and the ATR crystal is crucial, but often difficult to guarantee.

However, because of the rough surface of SC, the optical contact to the ATR-crystal is reduced. When SC serves as a membrane, there is an additional decrease of optical contact between crystal and SC in the cause of diffusion from aqueous drug solutions. The reason is the formation of a thin water layer between the crystal and the SC. Therefore, the common ATR penetration set-up is not the best one for diffusion experiments on the SC from aqueous drug solutions.

In the research described here, the characterization of diffusion processes with a new developed FTIR-ATR diffusion cell is represented. The opportunities of the cell for determining diffusion coefficients are described on two systems—water and urea in an artificial silicone membrane and urea in human SC. For the processing of the measured data a mathematical diffusion model is used to estimate the diffusion coefficient.

2. Materials and methods

2.1 Materials

The polydimethylsiloxane (PDMS) was supplied by Silikonwerke Nünchritz (Nünchritz, Germany). Polypropylene glycol 2000 (PPG) was obtained from Baker (Deventer, Netherlands). Trypsin (Type II), sodium bicarbonate and polyethylene glycol 400 (PEG) were obtained from Sigma (Deisenhofen, Germany).

Artificial polyethyleneglycol–polydimethylsiloxane (PEG-PDMS) membranes were produced by evaporating 4.9 ml of the membrane solution in a glass ring with a diameter of 6.8 cm on a Teflon plate over 12 h. For generating this solution, 4.41 ml of 10% (m/v) PDMS dissolved in ether was mixed with 0.49 ml of 10% (m/v) PEG dissolved in ether/ethanol (85:15, v/v). The

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cross linking of the silicone was catalyzed by adding dibutyltin dilaurate to the membrane solution as described by Richter *et al.*[7]

The drug urea was purchased from Merck (Darmstadt, Germany). Distilled water was utilized as acceptor for diffusion experiment with the SC as well as for producing the urea solutions used as donor. For the membrane experiments and for the SC experiments aqueous urea solutions 20% (m/m) and 10% (m/m), respectively were applied.

The skin from 33 year old donor was freshly taken from plastic surgery (mamma reduction) and the fat and parts of the dermis were separated by mechanical preparation. This tissue sample was purchased from the German Institute for Cell and Tissue Replacement (Berlin, Germany). The skin was stored at −82 °C. SC was freshly prepared by enzymatic dissection.[8] The skin sample was spread, SC side up, on filter paper soaked with 0.001% trypsin in 0.5% sodium bicarbonate. After 14 h of incubation at 37 °C, the SC was peeled away from underlying layer. This sample was washed in distilled water and incubated for several hours at 90% relative humidity and 20 °C.

2.2 FTIR-ATR spectroscopic studies

The IR spectra were acquired by using a Bruker spectrometer IFS 28 (Bruker Optics, Ettlingen, Germany) equipped with a Thermo Spectra-Tech HATR attachment (Shelton, CT, USA). The sampling compartment is a Fresnel ATR accessory that uses a ZnSe crystal with an angle of incidence of 45° in a horizontal orientation. The diameter of the top of the crystal is 20 mm. Each IR spectrum was collected at about 22 °C with 32 scans and a resolution of 2 cm^{-1} using the Norton–Beer medium apodization.

2.2.1 FTIR-ATR diffusion cell. The new developed FTIR-ATR diffusion cell combines the advantages of the ATR-method with the Franz-type diffusion cell. The scheme of the cell is shown in Fig. 1.

The compartments for acceptor and donor are made of stainless steel. Both are one-sided sealed with nitrilebutyl rubber O-rings (thickness 1 mm) to the crystal and to the membrane. The diffusion area of the membrane is 0.385 cm^2. The acceptor volume was 66.5 µl and the donor volume 2.66 ml. The acceptor covers only a part of the crystal and the IR beam detects the whole crystal. Therefore, any leakage of the cell would cause a noticeable change in the IR-spectrum of the acceptor. It could be proved that the cell was tightly fixed.

In case of water diffusion experiments through the PEG-PDMS membrane, PPG 2000 acted as acceptor solution. For the diffusion experiment with the SC, distilled water was filled in the acceptor compartment. The membrane was laid on the surface of the acceptor solution.

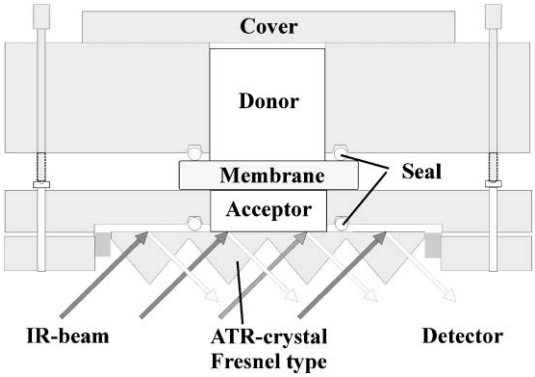

Fig. 1 Schematic representation of the FTIR-ATR diffusion cell.

Water absorbs strongly near 1640 cm^{-1} and 3300 cm^{-1} as a result of O–H bending and stretching vibrations, respectively. The spectrum of PPG 2000 is also characterized by an O–H stretching vibration near 3300 cm^{-1}. Consequently, the diffusion of water was monitored by its characteristic absorbance at 1640 cm^{-1}. Water is fast evaporating from the uncovered PPG 2000 water mixture. Therefore, for the evaluation of the water diffusion a calibration is not realizable and monitoring of the integral absorbance in the spectral region from 1564 to 1723 cm^{-1} with a straight baseline method provides secure results.

A calibration set-up of 38 aqueous samples containing urea in the concentration range from 0 to 10 wt% was used for quantifying the urea uptake in the acceptor due to the urea diffusion through the skin. The urea content derived from the IR-spectra in the course of diffusion was determined by using the multivariate analysis Quant2 of the OPUS software. The spectral region from 1269 to 1581 cm^{-1} was utilized for the quantification of the drug by cross validation. The characteristic parameters of this procedure were the root mean square error of cross validation (RMSECV) of 0.0324 and the correlation coefficient (R^2) of 99.99.

3. Mathematical model

In order to derive the diffusion coefficient of urea in the SC from the experimental data, it is necessary to assume an appropriate model based on Fick's second law of diffusion eqn. (1.1) as shown in Fig. 2.

$$\frac{\partial u}{\partial t} = D\frac{\partial^2 u}{\partial x^2};\ 0 \le x \le L \text{ for } t \ge 0 \qquad (1.1)$$

where L is the thickness of the membrane, t is the time and D is the effective diffusion coefficient.

The boundary conditions are:

$$u(t,\ x\ =\ 0)\ =\ u_{\mathrm{D}}\ =\ \text{constant} \qquad (1.2)$$

$$u(t,\ x\ =\ L)\ =\ v(t) \qquad (1.3)$$

where $u(t, x)$ is the drug concentration within the membrane, u_{D} is the drug concentration in the donor, and $v(t)$ is the drug concentration in the acceptor

The initial conditions are:

$$u(t\ =\ 0,\ x)\ =\ 0 \qquad (1.4)$$

$$v(t\ =\ 0)\ =\ 0 \qquad (1.5)$$

Using the symmetry of the arrangement and the fact that the diffusion of drug in acceptor is much faster than the diffusion within the membrane, one obtains a linear one-dimensional equation with a dynamic boundary condition at the membrane–acceptor contact. It is this dynamic boundary condition in which the model departs from the standard arrangement. However, it still possesses a Fourier series solution as it has been derived in:[9]

$$u(t,x) = u_{\mathrm{D}}\left(1 - \sum_{n=0}^{\infty} c_n \exp\left(-\lambda_n^2 Dt/L^2\right)\sin\left(\lambda_n x/L\right)\right) \qquad (1.6)$$

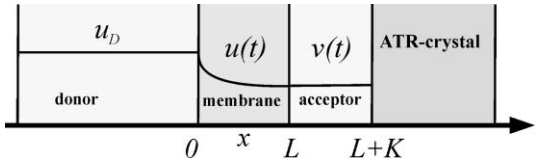

Fig. 2 Sketch and assumption of the mathematical model.

with eigenvalues $n\pi \leqslant \lambda_n \leqslant (n + 1/2)\pi$ defined by

$$\lambda_n K/L \sin\lambda_n - \cos\lambda_n = 0$$

and

$$c_n = 2/(\lambda_n(1 + K/L\sin^2\lambda_n))$$

where K is the height of the acceptor compartment.

To estimate the diffusion coefficients, *i.e.* to fit the measured data with this series [eqn. (1.6)] the MATHEMATICA system (Wolfram Research, Champaign, IL, USA) was utilized.

4. Results and discussion

4.1 Water diffusion through membrane

FTIR-ATR spectra in the spectral range between 1200 and 1800 cm^{-1} for the aqueous urea solution/PEG-PDMS membrane/PPG 2000 system at various times t of the diffusion experiment are represented in Fig. 3. At the beginning of the diffusion experiment, the IR spectrum of pure acceptor solution (PPG 2000) was observed. The increase in absorbance of the IR band belonging to water is evident in the course of the diffusion experiment. The detection of urea was not possible within the limits of the experiment. Fitting the spectroscopic data by the mathematical model allows calculation of the diffusion coefficient of water in the membrane. The normalized absorbance $I(t)/I$ (t = 900 min) *versus* time is shown in Fig. 4. In the case of water diffusion, the diffusion coefficient derived in this way amounts to $(6.72 \pm 0.26) \times 10^{-8}$ cm^2 s^{-1}. The course of the experimental data observed is close to the fitting curve, reflecting the agreement of experimental data with the mathematical model describing the diffusion process. Therefore, the determination of diffusion coefficients with the FTIR-ATR diffusion cell can be considered as reliable.

4.2 Urea diffusion through stratum corneum

Fig. 5 shows the FTIR-ATR spectra for the aqueous urea solution/SC/water system at various times t of the diffusion experiment in the spectral range between 1000 and 1900 cm^{-1}.

As has been noted already, at the beginning of the diffusion experiment, the IR spectrum of pure acceptor (water) was observed. Then the absorbance of IR bands belonging to urea increases in the time course of the diffusion experiment.

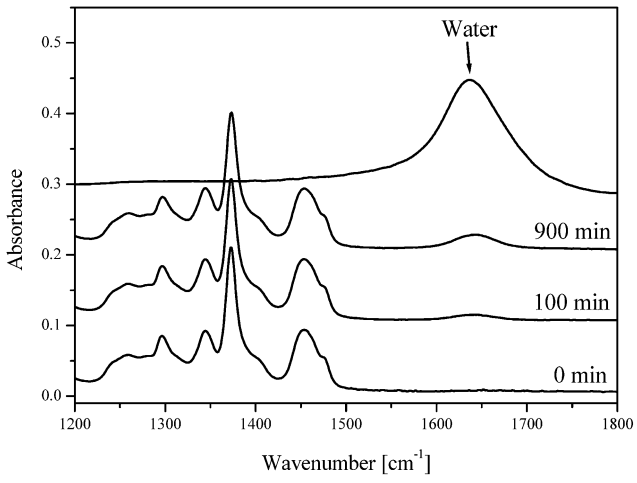

Fig. 3 FTIR-ATR spectra of the acceptor (PPG)/water system recorded at various times. The arrow indicates water band. For comparison, the FTIR-ATR spectrum of pure water is also represented on the top.

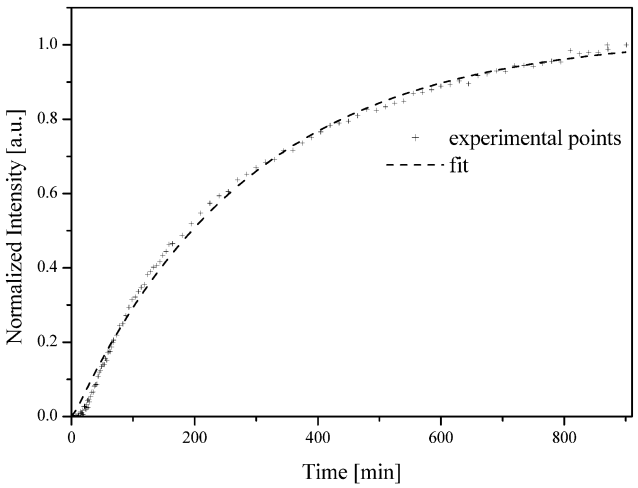

Fig. 4 Integral absorbance of the water band between 1564 and 1723 cm^{-1} in the acceptor *versus* time. The fitting curve with $D = 6.72 \times 10^{-8}$ cm^2 s^{-1} was obtained using the eqn. (1.6).

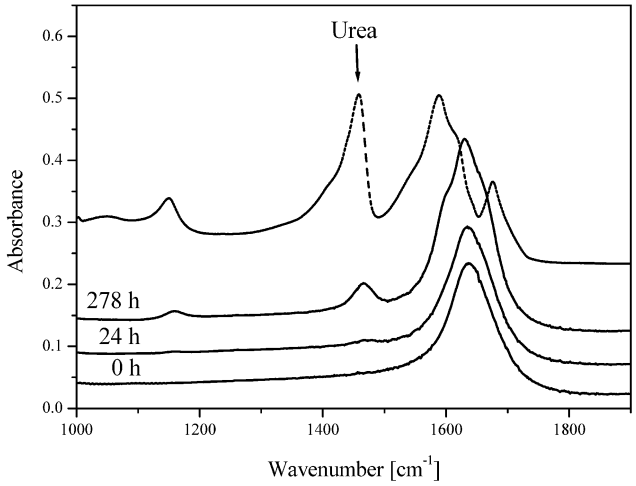

Fig. 5 FTIR-ATR spectra of the acceptor (water)/urea system recorded at different times. The arrow indicates the urea band.

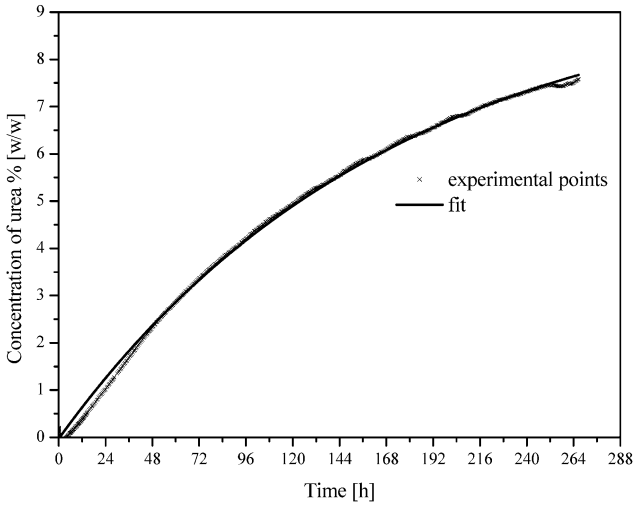

Fig. 6 Urea mass in % (m/m) in the acceptor *versus* time for the system aqueous urea solution/SC/water. The fitting curve with $D = 6.68 \times 10^{-10}$ cm^2 s^{-1} was obtained using the eqn. (1.6).

The time dependence of the urea amount in the acceptor as represented in Fig. 6 was quantified by multivariate calibration described. The low RMSECV (0.0324) and high R^2 (99.99) values prove that the chemometric method is suitable for this quantification.

Taking the spectroscopic results as input data to the mathematical model, the diffusion coefficient of urea in the SC has been determined. The diffusion coefficient of $(6.68 \pm 0.39) \times 10^{-10}$ cm^2 s^{-1} shows that the diffusion of the small hydrophilic substance urea through SC is a slow process. Obviously this parameter is rather small, indicating that the intact SC represents a strong barrier for such small hydrophilic molecules.

5. Conclusions

The ATR-diffusion cell combines the properties of the Franz diffusion cell with the advantages of the ATR-technique such as in process determination of drug concentration with time. This combination offers new possibilities for drug permeation studies.

The advantages of the cell in comparison to the common ATR penetration set-up are as follows:

(i) the acceptor and donor are well defined,

(ii) the membrane is not in contact with the crystal: the optical contact between the liquid acceptor and the ATR crystal is optimal,

(iii) the SC is always fully hydrated,

(iv) the physiological conditions can be better simulated in comparison with an arrangement in which SC is directly placed on the impermeable crystal.

The mathematical model in conjunction with spectroscopic data offers the possibility to determine the diffusion coefficient. In this way, the diffusion coefficient of urea in the SC was determined. All diffusion experiments carried out with a Franz cell can also be performed with the new FTIR-ATR diffusion cell. This method also allows determining diffusion coefficients of hydrophobic molecules by using lipophilic acceptor solution.

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