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^{19}F-NMR for drug quantification in tape stripping experiments

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Introduction

Tape stripping is a well-known method to characterise the penetration of a drug into the stratum corneum. While the amount of corneocytes on the tape strips can easily be quantified by NIR or UV/Vis [1], the amount of active drug is usually determined by HPLC. In the present study, we employed 19F-NMR experiments for a quantitative analysis of flufenamic acid.

Experimental methods

Formulations

Microemulsions based on natural surfactants were developed. The formulations contained lecithin, sucrose laurate, alkylpolyglycoside or a mixture thereof as surfactant. Isopropanol served as co-surfactant. Moreover, the formulations contained water and neutral oil. Flufenamic acid served as model drug. 3% of the active substance was incorporated into the microemulsions.

Tape stripping procedure

Tape stripping experiments were executed on full-thickness porcine ear skin. Hair was carefully removed by clipping. Subsequently, 5 mg cm^{-2} of the formulation was applied. After a residence time of one hour, twenty tape strips were torn off at a defined spot and analysed for their content of drug and corneocytes. The quantification of the corneocytes was performed by NIR, while the drug content was analysed by NMR and HPLC after extraction with deuterated methanol.

NMR analysis

NMR experiments were performed on a BrukerAvance DRX 600 NMR spectrometer (BrukerBioSpin GmbH, Germany) operating at 564.69 MHz for ^{19}F as described in Schwarz et al., 2011 [2]. A 5 mm quadruple observe probe equipped with z-axis gradient coil was used. All measurements were done in deuterated methanol at a temperature of 298 K. Typical acquisition parameters chosen are: 15 ppm spectral width, 32000 data points, 90° excitation pulse, 2 s acquisition time, 1000 scans. The spectra were referenced externally (^{19}F: CCl_3F δ = 0 ppm). The processing and the analysis of the NMR spectra were done within the Topspin Software, Version 3.0 (BrukerBioSpinGmbH). For the processing the amount of data points was doubled by zero filling and an exponential window function with a line broadening factor of 3 was applied. To improve the accuracy of the integration the signals were deconvoluted by fitting a Lorentzian line shape.

In order to obtain the absolute concentrations the intensities from the integration were referenced to a calibratrion series, which was measured in the range from 0.5 to 1000 µg ml^{-1}.

Results

It was possible to determine the amount of flufenamic acid by ^{19}F-NMR (Figure 1). Moreover, the obtained results showed a highly linear correlation (R2=0.988) with the drug amounts determined by HPLC (Figure 2). Therfore, it could be shown that ^{19}F-NMR is highly suitable for the quantitative analysis of fluorinated drugs in tape stripping experiments.

Figure 1: Amount of flufenamic acid on tape strips quantified by ^{19}F-NMR

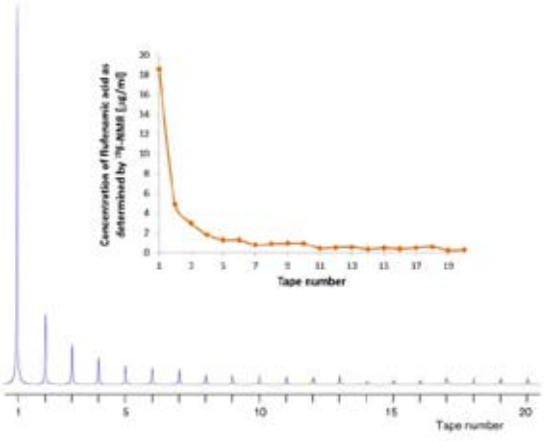
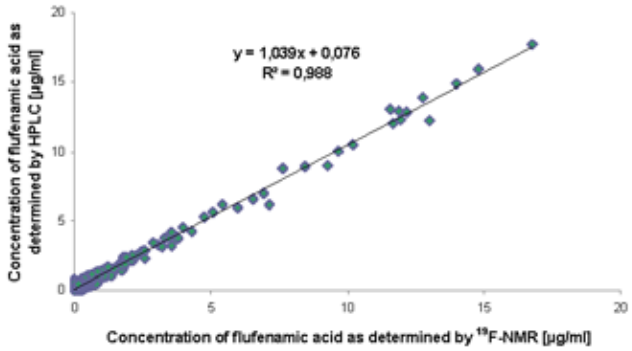


Figure 2: Linear correlation of the amounts of flufenamic acid on tape strips as determined by by ^{19}F-NMR and HPLC



References

[1] Schwarz, J.C., Klang, V., Hoppel, M., Valenta, C., 2012. Corneocyte quantification by NIR densitometry and UV/Vis spectroscopy for human and porcine skin and the role of skin cleaning procedures. Skin Pharmacol. Physiol. (in press).

[2] Schwarz, J.C., Kählig, H., Matsko, N.B., Kratzel, M., Husa, M., Valenta, C., 2011. Decrease of liposomal size and retarding effect on fluconazole skin permeation by lysine derivatives. J. Pharm. Sci. 100, 2911-2919.