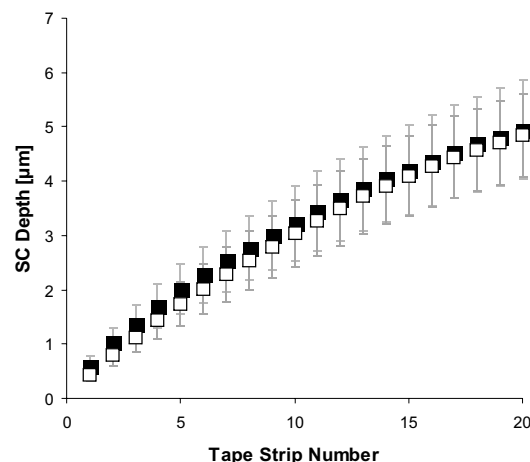
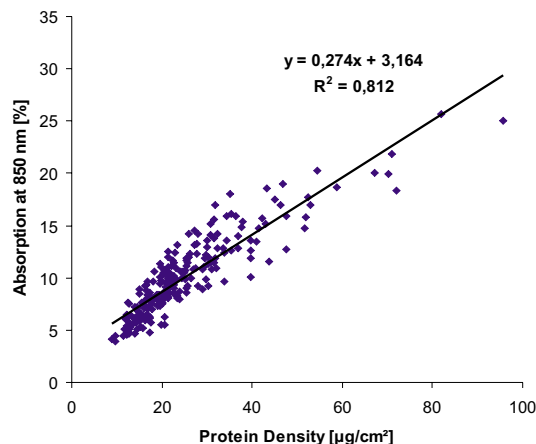


Infrared densitometry (IR-D) has recently been proposed for analysis of stratum corneum (SC) proteins on adhesive films obtained during tape stripping on human volunteers in vivo [1] and in vitro [2]. The mass of corneocytes on the tapes was shown to correlate linearly to the optical pseudo-absorption of the proteins as determined at 850 nm. However, this method has not yet been validated for the quantification of porcine SC proteins. Since porcine ear skin is a highly suitable in vitro model for tape stripping, there is a need for rapid and accurate quantification of the protein content removed with individual tapes.

Consecutive tape stripping of 20 adhesive films was performed on porcine ear skin. Two different brands of adhesive film, namely Corneofix® and D-Squame® tapes, were investigated in respectively 12 individual experiments. The optical pseudo-absorption of the corneocytes fixed to the individual tapes was determined with the SquameScan®850A (Heiland electronic GmbH, Wetzlar, Germany). The SC proteins were subsequently extracted from the tapes with 1M NaOH, neutralised with 1M HCl and their amount was determined with the Micro BCA® protein assay using a microplate reader (Tecan infinite®200, Tecan Ltd., Maennedorf, Switzerland). Upon addition of the reagent, the formation of a chelate complex of bicinchoninic acid (BCA) and cuprous ions led to a coloured product which was analysed at 550 nm. The quantification of the protein content was performed using bovine γ -globulin as an external protein standard.

A linear regression analysis was conducted to correlate the pseudo-absorption of porcine SC corneocytes to the protein content as determined after extraction of tape strips and subsequent protein analysis with the MicroBCA® protein assay.

The correlation between pseudo-absorption and protein content of the tapes was found to be highly linear, especially in the case of Corneofix® (Fig.1). This shows that IR-D is suitable for the quantification of not only human, but also porcine SC proteins despite the different pattern of protein removal. A proportionality factor between protein amount and pseudo-absorption was calculated after linear regression analysis of unbiased samples with homogeneous protein coverage. This factor $k=0.41$ can be employed to conveniently calculate the protein mass removed with each tape. The accuracy of the obtained results was confirmed by correlating the thickness of the removed SC layers as determined by the two employed methods against the respective tape strip number (Fig. 2). Almost identical mean values around $4.90 \mu\text{m}$ were obtained. In addition, separate studies in which the whole SC was removed with 80 to 130 adhesive tapes revealed a mean SC thickness of $7.96 \pm 3.25 \mu\text{m}$, which is in good agreement with previously reported data.



Overall, Corneofix® tapes with lower adhesive power were found to be more suitable for optical analysis of porcine SC proteins via IR-densitometry due to their more homogeneous protein coverage (Fig.3). If the tape stripping procedure is performed correctly and intact porcine ear skin is used, reasonably accurate results can be expected.