ATR-FTIR spectroscopic investigation of penetration enhancement by cineole and oleic acid

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Introduction

ATR-FTIR (Attenuated Total Reflectance FTIR) spectroscopy is used to study the effect of penetration enhancers on absorption across human skin and model membranes. One advantage of using this approach is that it allows the diffusion and partition coefficients of the drug in the membrane to be separated more easily than can be achieved with Franz diffusion cells. Mechanistic insight can therefore be gained into how the penetration enhancer exerts its effect which can facilitate and optimise formulation development. Furthermore, with the use of chemometric data analysis the diffusion of several components and their effect on the membrane can be followed. This study has used ATR-FTIR spectroscopy to investigate the effects of two penetration enhancers, cineole and oleic acid on the diffusion of a model permeant, cyanophenol across human epidermis

Materials and Methods

Diffusion experiments were performed using a Nicolet Avatar 360 fitted with an ATR multiple reflection accessory. Heat separated human epidermis was placed on the crystal such that the stratum corneum (SC) was in direct contact with the crystal. Saturated solutions of cyanophenol (CNP), in water or cineole or oleic acid (OA) which had been equilibrated with water were placed in a trough on top of the membrane and IR spectra were collected as a function of time. The experimental setup is shown in Figure 1. CNP solubility in the different solvents was determined by UV spectroscopy. Data analyses were performed using Scientist and Insight software programmes.

Experimental Setup

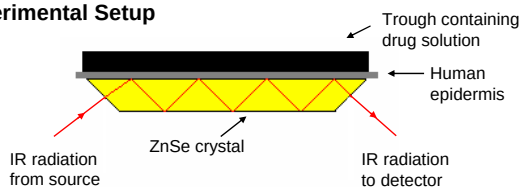


Figure 1 Illustration of the bulk diffusion ATR-FTIR setup to investigate diffusion across human epidermis.

Results and Discussion

CNP was selected as a model permeant because its CN stretching is in a spectrally silent skin region (Fig. 2) simplifying data analysis.

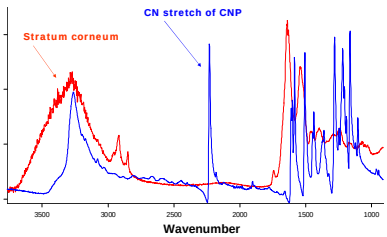
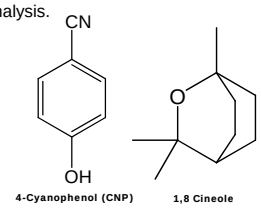


Figure 2. ATR spectra of CNP and mounted human epidermis (stratum corneum)



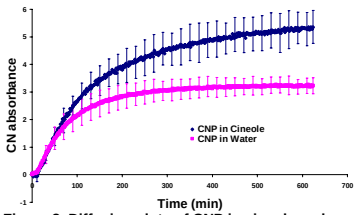
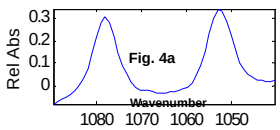


Figure 3. Diffusion plots of CNP in cineole and water across human epidermis

Fig. 3 shows that using cineole as a vehicle increases the plateau absorbance obtained, indicating that cineole increases the concentration of CNP in the stratum corneum.



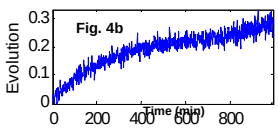


Figure 4a shows the extracted spectral profile of cineole in the 1090-1040cm^{-1} window obtained using *Insight*. Figure 4b shows the evolution of the extracted cineole spectral profile with time

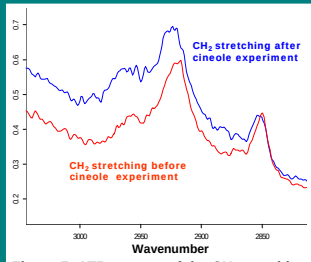


Figure 5. ATR spectra of the CH stretching region before and after a CNP in cineole experiment

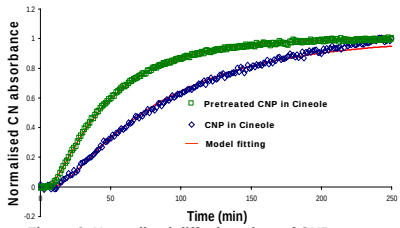
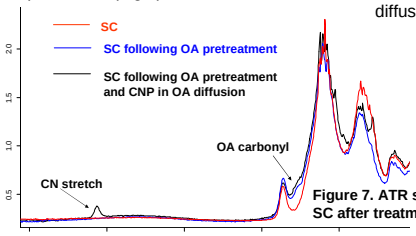


Figure 6. Normalised diffusion plots of CNP across human epidermis with model fitting

The CH stretching before and after the CNP in cineole experiment showed small shifts highlighting the possibility of cineole affecting stratum corneum lipid packing, perhaps increasing the diffusion coefficient of permeants (Fig 5).

To examine whether cineole lowers the diffusional resistance of the membrane, the epidermis was first pretreated for 24 hours on the ATR crystal prior to adding the CNP in cineole. The figure above shows that this does appear to be the case. Calculated pathlength normalised diffusion coefficients are given in Table 1.



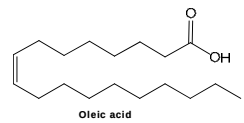
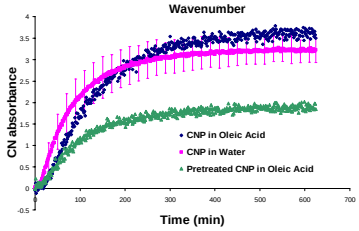


Figure 7. ATR spectra of the SC of human epidermis, the same SC after treatment with OA and after the CNP in OA experiment.



Initial experiments of CNP in oleic acid showed little enhancement above that in water (Fig 8). During pretreating experiments higher uptake of oleic acid was observed compared with non-pretreated experiments, which may be due to variability in the skin used (Fig. 7). For these experiments a lower CNP absorption plateau was observed. This suggests that CNP has a lower solubility in oleic acid than the SC lipids.

Figure 8. Diffusion plots of CNP in water and OA across human epidermis. Also diffusion of CNP in OA across epidermis pretreated with OA

	Solubility (mg/ml)	Plateau absorbance (AU)	Pathlength normalised diffusion coefficient (D/h^2) (x10^{-5} s^{-1})
CNP in water	17	3.2	10.6
CNP in Cineole	389	5.2	8.7
Pretreated CNP in Cineole	389	15.5	15.4
CNP in OA	35	3.6	7.1
Pretreated CNP in OA	35	1.9	9.3

Table 1. The solubility, plateau absorbances and diffusion coefficients obtained for CNP

Conclusions and Future Work

Typical Fickian diffusion profiles were obtained for CP in oleic acid and cineole and these data were modelled using *Scientist* to obtain pathlength normalised diffusion coefficients of CP across human epidermis. The cineole diffusion profile was extracted using *Insight*. Pretreating the epidermal membranes with cineole was observed to increase the diffusion of CNP across the membrane possibly through interaction with / extraction of the SC lipids. Oleic acid was not found to increase the diffusion coefficient of CNP across the membrane. Cineole was also found to increase the plateau absorbance of CNP indicating an increased concentration of CNP in the membrane. With pretreatment, oleic acid lowered the plateau absorbance of CNP. The data indicates that cineole should increase skin transport of CNP through increasing its concentration in the stratum corneum and by increasing its diffusion coefficient. No evidence was obtained for penetration enhancement by oleic acid. Further experiments are ongoing to verify these results and to relate the data obtained with that obtained using conventional Franz diffusion cells.

Acknowledgments

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