14 ATR-FTIR Spectroscopy and the Skin Barrier: Evaluation of Penetration-Enhancement Effects

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14.1 Introduction and Theoretical Background

A prerequisite for dermal and transdermal drug delivery is to overcome the skin barrier. To this end, a thorough knowledge of the skin, its complex organisation and its barrier properties is essential. A plethora of biophysical methods have been employed to study the skin's main barrier, the stratum corneum. Among them, Fourier transform infrared (FTIR) spectroscopy was found to be a particularly promising approach. FTIR spectroscopy is a highly sensitive method to analyse molecule vibrations after excitation with the radiation of the infrared range. The functional principle of this technique is well known and described in the respective literature (Markovich and Pidgeon 1991; Naik and Guy 1997; Guenzler and Gremlich 2003). In contrast to traditional infrared spectrometers, FTIR instruments feature a Michelson interferometer and offer several distinct advantages (Perkins 1987). The signal-to-noise ratio is improved when compared to traditional IR devices, and thus higher sensitivity can be achieved (Hesse et al. 2012). Moreover, due to the exact definition of the mirror position, wavenumbers can be determined more precisely. Last but not least, the simultaneous recording of all frequencies renders FTIR spectroscopy a time-saving and convenient technique (Guenzler and Gremlich 2003). A further modification of this method is attenuated total

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reflection (ATR)-FTIR spectroscopy, which is a non-invasive technique to characterise organic samples rapidly and with high sensitivity (Yadav et al. 2009; Obata et al. 2010). Furthermore, in vivo sampling is possible with this internal technique (Naik and Guy 1997). A crystal that consists of material with a higher refractive index than the sample is required, such as zinc selenide or diamond. The gaseous, liquid, or solid sample is placed directly on the crystal during analysis. A beam of infrared light is sent through the crystal and is repeatedly reflected (Guenzler and Gremlich 2003); thus, the beam can penetrate the sample up to a depth of about 1 µm. This penetration depth corresponds to approximately 1–1.5 cell layers of the stratum corneum. Therefore, recorded spectra contain information about the surface of the sample. In order to characterise skin samples, the stratum corneum may remain on the intact skin; it is not necessary to separate it from underlying tissue (Rodriguez et al. 2010). Conveniently, the non-invasive nature of this technique offers the possibility to perform further experiments such as tape-stripping on the same skin sample (Caussin et al. 2009; Klang et al. 2012a). By correlating the peak intensity at a defined frequency with the concentration of an applied substance, even quantitative IR analysis is possible. In this case, the use of deuterated drugs and excipients is advantageous for a precise distinction from endogenous substances (Cotte et al. 2004; Dias et al. 2008; Schwarz et al. 2013).

Having summarised the basic principles of FTIR spectroscopy and the possibilities offered by this technique, the next section will provide an overview of experimental findings that elucidate the characteristic skin barrier properties through FTIR analysis. In this context, typical skin bands in ATR-FTIR spectra are discussed. Furthermore, the influence of selected permeation enhancers and dermal delivery systems on the respective skin bands as demonstrated in many studies is discussed. The importance of ATR-FTIR spectroscopy in skin science is well documented by the large number of recent publications using this technique to gather new information on the molecular level. This article represents a non-exhaustive overview of recent findings of interest in the field; selected data are presented, and the conclusions obtained in the respective contributions are discussed.

14.2 ATR-FTIR Spectroscopy and Dermal Drug Delivery

14.2.1 Characterisation of the Stratum Corneum by ATR-FTIR Spectroscopy

ATR-FTIR experiments have been widely employed to characterise human skin samples in vivo (Puttnam and Baxter 1962; Sakuyama et al. 2010; Gorcea et al. 2012) and in vitro (Boncheva et al. 2008; Hasanovic et al. 2011). Obtaining human skin samples for such experiments often involves significant organisational and legislative issues. Therefore, animal models such as the bovine udder skin model (Heise et al. 2002) are frequently employed as a substitute for human skin. Other types of skin that have been investigated by ATR-FTIR include porcine skin (Hasanovic et al. 2011), rat skin (Obata et al. 2010), guinea pig skin (Bello et al. 2006) and snake skin (Wonglertnirant et al. 2012). Minor differences held aside, the porcine ear skin model represents a highly adequate in vitro substitute for human skin (Jacobi et al. 2007; Hasanovic et al. 2011; Klang et al. 2012b). Figure 14.1 shows a typical ATR-FTIR spectrum of porcine ear skin with its characteristic skin bands. The latter will be discussed in the following section. These skin bands can be determined by ATR-FTIR with high reproducibility.

Although it represents a very basic and simplified model of the human skin barrier, the bricks and mortar model by Elias is still a valid approach to depict the skin structure (Elias and Friend 1975). While the bricks represent the stratum corneum proteins, that is, the cornified cells termed corneocytes, the mortar depicts the intercellular lipid matrix within the stratum corneum. The molecular organisation of the stratum corneum lipids is essential for the barrier function of the skin (Boncheva et al. 2008). The lipids are

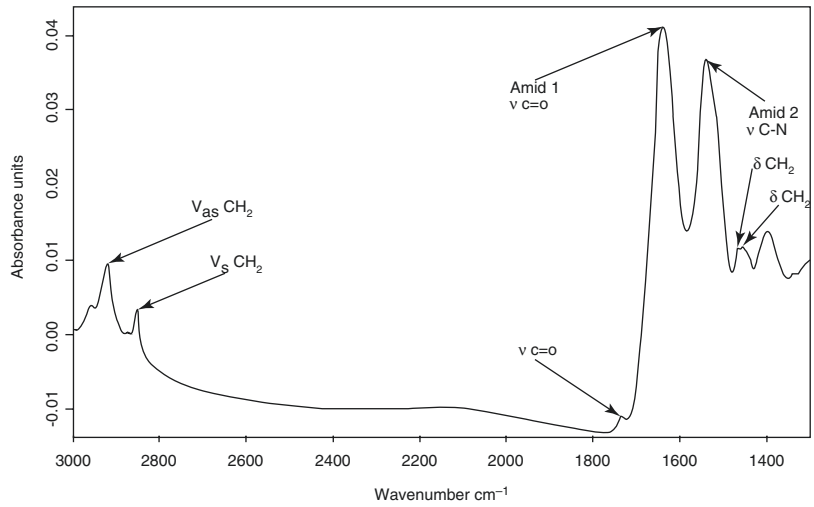

Fig. 14.1 ATR-FTIR spectrum of porcine ear skin recorded at 32 °C, showing the typical skin bands

arranged in two suborganisational patterns: the lamellar organisation, that is, the arrangement in layers parallel to the skin surface, and the lateral organisation, which describes the lipid arrangement in the lipid lamellae themselves. Regarding the lamellar organisation, long-periodicity phases and short-periodicity phases are present (Caussin et al. 2009; Obata et al. 2010; Groen et al. 2011). Recent findings suggest that the long-periodicity phase is essential for the skin barrier function (Caussin et al. 2009; Groen et al. 2011). Regarding the lateral lipid organisation, the domain mosaic model is consistent with most experimental data. This model proposes that the lipids are organised in ordered domains and connected by lipids in a disordered phase (Forslind 1994). The ordered domains, also referred to as gel phase, contain lipids in the orthorhombic or hexagonal phase. The disordered domain consists of lipids in the liquid-crystalline phase (Boncheva et al. 2008; Rodriguez et al. 2010; de Sousa Neto et al. 2011). Lipids in the orthorhomic phase are tightly packed, and the alkyl chains are organised in an all-trans conformation. The packing in the hexagonal phase is less tight, and the lipids have more rotational freedom. The lipids in the liquid-crystalline phase have the greatest freedom of movement and their alkyl chains exhibit a high percentage of gauche conformation (Bouwstra and Ponec 2006; Boncheva et al. 2008; Rodriguez et al. 2009). The lipid organisation is an important factor to determine skin permeability (Boncheva et al. 2008; de Sousa Neto et al. 2011). In addition to lipid composition, temperature and skin hydration, solvents or penetration enhancers can likewise influence the lateral packing of the stratum corneum lipids and thus the skin barrier (Naik and Guy 1997).

Regarding the stratum corneum, the most informative lipid absorbances in the IR spectrum originate from the hydrophobic alkyl chains (Potts and Francoeur 1993). In particular, the carbon–hydrogen stretching vibrations with peaks at 2850 and 2920 cm^{-1}, that is, the asymmetric and symmetric stretching modes, provide information about the conformational order of the hydrocarbon lipid chains of the horny layer (Naik and Guy 1997; Babita et al. 2006; Hathout et al. 2011). Although both bands describe the three-dimensional arrangement of the lipids, the symmetric stretching mode is more susceptible to changes (Moore et al. 1997; Gooris and Bouwstra 2007; Rodriguez et al. 2010). The peak width as well as its wavenumber allow a classification of the lipids. The symmetric stretching band ν_s appears at a wavenumber of about 2849–2850 cm^{-1} if the majority of the lipids are arranged in an orthorhombic pattern. During the transformation to a more fluid structure and a

mainly hexagonal lipid arrangement, the peak width increases and is shifted to higher wavenumbers (2851–2852 cm^{-1}). When most lipids are arranged in the liquid-crystalline phase, the symmetric stretching peak appears at a wavenumber of about 2853–2854 cm^{-1} (Boncheva et al. 2008; Caussin et al. 2008).

In addition, the CH_2-scissoring band at about 1460 cm^{-1} characterises the lateral packing of the lipid chains in the stratum corneum. Depending on the arrangement of the lipid domain, either one or two peaks are visible in the skin spectrum (Moore et al. 1997; Boncheva et al. 2008; Rodriguez et al. 2010). When two separate bands appear in the spectrum with a distance of approximately 10 wavenumbers, the orthorhombic phase dominates. These two separate peaks are caused by the interaction between closely packed lipid chains, and appear between 1460 and 1470 cm^{-1} (Hasanovic et al. 2011). Moreover, as shown by Boncheva and coworkers, the scissoring bandwidth, especially when consulting second derivative spectra, provides a suitable measure for the presence and extent of the orthorhombic and hexagonal phases (Boncheva et al. 2008). The more lipids pass into the hexagonal phase, the more the distance between the two peaks decreases, until they merge (Caussin et al. 2008). The C=O-stretching band represents the lipid ester carbonyl and is mainly indicative of the presence of sebum in and on the SC (Machado et al. 2010).

While around 1650 cm^{-1}, the stretching band of the C=O group of keratin (amide 1) is present in the spectrum, the stretching band of the C–N bond of the amino group of keratin appears at around 1550 cm^{-1} (amide 2) (Moore et al. 1997; Babita et al. 2006; Rodriguez et al. 2009; He et al. 2009). The amide 1 band describes the secondary structure of keratin (He et al. 2009). Wavenumbers 1615–1638 cm^{-1} suggest a β-sheet arrangement, 1638–1645 cm^{-1} a random coil structure, 1645–1662 cm^{-1} α-helix arrangement and 1662–1695 cm^{-1} β-turning structure (He et al. 2009). Excipients or penetration enhancers can affect the secondary structure of keratin. They may induce a larger degree of freedom and may thus enhance the penetration of co-applied drugs (He et al. 2009). Moreover, it is possible to analyse the absorption of water within the stratum corneum by analysing the amide 1 and 2 bands. While the intensity of the amide 2 peak remains stable, the intensity of the amide 1 band increases upon water absorption. By calculating the ratio of these intensities, the water content in the skin can be determined (Prasch et al. 2000; He et al. 2009).

14.2.2 Influence of Selected Penetration Enhancers and Vehicles on the Skin Barrier

The characterisation of the skin barrier by biophysical methods is constantly exploring new levels as sophisticated methods of analysis are continuously evolved and refined. Among other methods, ATR-FTIR spectroscopy has successfully been employed to determine the influence of substances such as solvents, penetration enhancers, excipients, or even of complex drug delivery systems on the stratum corneum (Babita et al. 2006). With this method, the enhancer efficacy can be evaluated, while the mode of action can be elucidated as well (Naik and Guy 1997). In the following section, examples of recent ATR-FTIR studies of interest are discussed in which the penetration enhancement effect and mechanism of certain excipients and vehicles were investigated.

Regarding the stratum corneum lipids, there are two main effects that can be observed when the skin is treated with penetration enhancers. Some enhancers combine both modes of action, while others only make use of one of them. On the one hand, a fluidisation of the stratum corneum lipids can be induced and observed most prominently on the CH_2-stretching peaks in the spectra. In this case, the lateral packing of the lipids becomes less tight, and substances can penetrate the stratum corneum more easily. On the other hand, penetration enhancers can also cause a lipid extraction. Due to the lower amount of lipids in the stratum corneum, the barrier is weakened (Naik and Guy 1997). Furthermore, penetration enhancers may also affect the skin

barrier by changing the secondary structure of keratin. This leads to a loose accumulation of stratum corneum proteins with a larger degree of carbon movement (He et al. 2009).

The effect of ethanol, one of the most prominent permeation enhancers and a simple vehicle, on the skin barrier has been investigated by numerous groups (Kurihara-Bergstrom et al. 1990; Bommannan et al. 1991; Krill et al. 1992; Panchagnula et al. 2001). An in vitro study on human skin showed that ethanol induced lipid extraction and could thereby enhance the flux of salicylate (Kurihara-Bergstrom et al. 1990). These results were confirmed in vivo (Bommannan et al. 1991). Interestingly, in this study as well as in another study employing the stratum corneum of hairless mouse skin, a shift of the symmetric CH_2-stretching band to lower wavenumbers was observed. These findings indicate an increase in lipid order after treatment with ethanol, which represents a rather surprising observation. Based on these findings, ethanol seems to enhance drug penetration by stratum corneum lipid extraction and does not, even at high concentrations, fluidise the lipids (Babita et al. 2006).

Fatty acids are natural constituents of the stratum corneum lipids. Therefore, compounds of this group, such as oleic acid, have a natural ability to intercalate within the lipid bilayer and thus modulate the barrier function (Babita et al. 2006). Oleic acid is a penetration enhancer that is widely used in the field of transdermal drug delivery. Regarding its mode of action, it is assumed that the molecules create a highly permeable, fluid-like phase that coexists with the endogenous stratum corneum lipids, but disrupts their tight packing (Ongpipattanakul et al. 1991; Babita et al. 2006; Boncheva et al. 2008). This hypothesis was supported by experimental data which showed a shift of the symmetric CH_2-stretching vibration to higher wavenumbers (Mak et al. 1990; Boncheva et al. 2008). Moreover, the scissoring bandwidth confirmed that oleic acid forms a separate phase within the stratum corneum lipids (Boncheva et al. 2008).

Propylene glycol, a vehicle for penetration enhancers and a cosolvent in many dermal drug delivery systems, interacts mostly with keratin and does not alter the stratum corneum lipid organisation (Mak et al. 1990). It does not show an effect on the symmetric CH_2-stretching band, which indicates a preservation of the conformational order of the lipids (Panchagnula et al. 2001; Boncheva et al. 2008, 42). However, a decrease in height and area of the CH_2-stretching peak suggests a mild extraction of stratum corneum lipids (Babita et al. 2006).

Dimethyl sulfoxide (DMSO) is a well-known, but rather aggressive, penetration enhancer. Results of ATR-FTIR studies suggested lipid extraction, protein denaturation and lipid fluidisation to be among the enhancer's mode of action (Naik and Guy 1997; Babita et al. 2006). DMSO effectively enhances the skin permeation of both hydrophilic and lipophilic drugs, but concentrations of more than 60 % w/w are required for this effect. However, such high concentrations lead to erythema on skin, and thus DMSO is rarely used nowadays (Babita et al. 2006). In lower concentration, DMSO may be employed as a drug solubiliser in the stratum corneum (Remane et al. 2006).

The analysis of the effect of dermal or transdermal drug delivery systems on the skin barrier properties is a rather complex task. Where possible, employing deuterated compounds is advantageous to distinguish the skin bands from the formulation peaks in the spectra (Naik and Guy 1997). Otherwise, very careful data interpretation is necessary. However, reliable results can be obtained, especially if control spectra are recorded. For instance, the influence of natural sucrose esters on the stratum corneum was analysed by ATR-FTIR. Sucrose oleate and laurate showed no effect on the skin bands or drug permeation when applied in aqueous solutions (Ayala-Bravo et al. 2003). However, a permeation-enhancing effect could be determined when the sucrose esters were dissolved in Transcutol. Especially sucrose laurate induced a decreased absorbance and a frequency shift to higher wavenumbers of the CH_2-stretching bands (Ayala-Bravo et al. 2003). Another group investigated the effect of sucrose laurate on the permeation of ibuprofen from a hydrogel (Csizmazia et al. 2012). Besides an increase in drug permeation and a more pronounced skin-hydrating effect as shown

by the amide bands, the sucrose laurate-loaded hydrogel resulted in the same slight lipid extraction and fluidisation as the control hydrogel. The latter effects were reversible, and thus, sucrose laurate may be used as a mild penetration and hydration enhancer (Csizmazia et al. 2012). In another study, microemulsions based on natural surfactants, namely sucrose laurate, lecithin and alkylpolyglucoside, were investigated (Schwarz et al. 2012a). In skin diffusion experiments, lecithin showed the strongest skin-enhancing properties for the model drugs flufenamic acid and fluconazole. Accordingly, the CH_2-stretching indicated that the stratum corneum lipids had undergone a phase transition to liquid-crystalline organisation. Also, the CH_2-scissoring and the amide bands suggested a more permeable skin structure (Schwarz et al. 2012a). In contrast, the application of solid lipid nanoparticles and nanostructured lipid carriers based on alkylpolyglucosides seemed to exert a barrier-strengthening effect on the stratum corneum (Schwarz et al. 2012b). Both the CH_2-stretching and CH_2-scissoring bands indicated a strictly orthorhombic organisation of the lipid chains.

Conclusion

In summary, ATR-FTIR spectroscopy provides an excellent tool for in vivo and in vitro characterisations of penetration-enhancement effects on the stratum corneum. Besides its high sensitivity, the non-destructive nature of this technique as well as the short duration of the experiments are convenient and advantageous features of this method. As the knowledge about the barrier function of the skin is continuously being expanded, more accurate predictions about penetration-enhancement properties of substances as well as their skin-irritation potential will become possible.

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