Trends observed in NMF levels between the groups were not statistically significant. With respect to ceramides and fatty acids, statistical significance was found only in the higher levels in sensitive skin compared to atopic skin. Overall, the study served to show that sensitive skin was not a subclinical form of atopic dermatitis. The skin barrier of sensitive skin was shown to be unmodified with respect to SC thickness and water, NMF, and lipid content.

55.5 DRUG PENETRATION AND PENETRATION ENHANCEMENT STUDIES

CRM is a useful tool to follow label-free and simultaneously the distribution of an active drug and vehicle components such as penetration enhancers, as well as to record modifications of endogenous skin components arising from the application of a given product. CRM allows studying whether or not an active drug and vehicle components have similar distribution patterns in the skin. Conversely, it allows one to control for possible adverse effects due to the formulation. This is, for instance, of interest in the study of sunscreens, which are supposed to remain on the skin surface. CRM is also helpful for efficacy purposes. Detection of the active agent and quantification of metabolites help to gain insight into the protective potential of novel formulations and to reevaluate existing ones. Moreover, this method is also useful to establish in vitro/in vivo correlations.

The penetration of various drugs spanning a range of chemical structures and physicochemical properties has been studied (Table 55.2 and Table 55.3) insofar as these drugs possess a specific Raman signature allowing their identification within the skin. In general, penetration profiles of compounds as a function of depth inside SC consist of qualitative or semi-quantitative data (Zsikó et al. 2019). The methodology of Caspers et al. involves fitting a reference spectrum of the compound of interest to the spectra of skin onto which the compound has been applied. Since the collected Raman signal weakens with increasing depths into the skin due to scattering in the tissue, the compound signal is normalized by the signal of endogenous keratin, whose concentration in the skin is assumed constant as a function of depth in the skin (Pudney et al. 2007). This assumption of constant keratin concentration as a function of depth, and the general methodology based upon it, have recently been challenged by Darvin et al. (2019).

Recently, Caspers et al. (2019) introduced a new methodology yielding fully quantitative skin penetration–depth profiles from in vivo CRM, that is, in terms of mass of compound penetrated per cm^2 of skin. Their method, illustrated by the tracking of retinol in the skin of volunteers, is reviewed in the next section.

55.5.1 Methodologies to Assess Drug Skin Penetration

Similarly to classical skin penetration experiments, CRM lends itself to the tracking of chemicals from solutions or more complex formulations (with or without penetration enhancers) in ex vivo human and animal skin. Studies on skin explants may be conducted to define methodological parameters in view of performing in vivo CMR studies.

Caffeine has been selected as a model hydrophilic drug in a number of several CRM studies (Franzen et al. 2013, 2014, 2015; Tfaili et al. 2013, 2014; Alonso et al. 2018). Widely used in cosmetics as an active substance, it is also one of the most frequently used compounds in transdermal delivery studies. This hydrophilic molecule is not metabolized in the skin and has interesting physicochemical properties, with a low octanol/water partition coefficient ($\log P = -0.07$) and low molar mass ($M_W = 194$ g·mol^{-1}).

Caffeine penetrates the skin rapidly (Bolzinger et al. 2008). However, it is difficult to track with CRM because it does not accumulate in the upper layers of skin. Tfaili et al. (2013) followed caffeine penetration in human skin samples. Solutions of caffeine at two concentrations, 2.57×10^{-1} mg·mL^{-1} and 5.15×10^{-2} mg·mL^{-1}, were applied on human skin samples. Raman spectra were collected every half-hour from the surface to a depth of 50 µm in a 6-µm increment. The total duration was 4 hours after application. Raman analysis was not possible with the most concentrated solution