Trends in Analytical Chemistry 156 (2022) 116709

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Monitoring dermal penetration and permeation kinetics of topical products; the role of Raman microspectroscopy

S. Bielfeldt [a], F. Bonnier [b], H.J. Byrne [c, *], I. Chourpa [b], Y. Dancik [b, d, k], M.E. Lane [e], D.J. Lunter [f], E. Munnier [b], G. Puppels [g, h], A. Tfayli [i], E. Ziemons [j]

[a] proDERM Institut GmbH, Kiebitzweg 2, 22869 Schenefeld, Hamburg, Germany
[b] Université de Tours, EA 6295 Nanomédicaments et Nanosondes, 31 avenue Monge, 37200, Tours, France
[c] FOCAS Research Institute, TU Dublin, City Campus, Camden Row, Dublin 8, Ireland
[d] Le STUDIUM Institute of Advanced Studies, 1 rue Dupanloup, 45000, Orléans, France
[e] School of Pharmacy, University College London, 29-39 Brunswick Square, London, WC1N 1AX, United Kingdom
[f] Pharmaceutical Technology, Eberhard Karls Universitaet Tuebingen, Auf der Morgenstelle 8, 72076, Tuebingen, Germany
[g] RiverD International B.V., Rotterdam, the Netherlands
[h] Center for Optical Diagnostics and Therapy, Department of Dermatology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands
[i] Lipides: systèmes analytiques et biologiques, Université Paris-Saclay, 92296 Châtenay-Malabry, France
[j] University of Liege (ULiege), CIRM, Vibra-Sante Hub, Laboratory of Pharmaceutical Analytical Chemistry, Avenue Hippocrate 15, 4000 Liège 1, Belgium
[k] Certara UK Ltd., Simcyp Division, Level 2-Acero, 1 Concourse Way, Sheffield, S1 2B1, United Kingdom

ARTICLE INFO

Article history:
Received 28 January 2022
Received in revised form
31 May 2022
Accepted 4 June 2022
Available online 14 June 2022

Keywords:
Human skin
Penetration and permeation kinetics
Stratum corneum
Mathematical models
Franz cell diffusion
Tape stripping
Raman microspectroscopy

ABSTRACT

The study of human skin represents an important area of research and development in dermatology, toxicology, pharmacology and cosmetology, in order to assess the effects of exogenous agents, their interaction, their absorption mechanism, and/or their toxicity towards the different cutaneous structures. The processes can be parameterised by mathematical models of diffusion, of varying degrees of complexity, and are commonly measured by Franz cell diffusion, *in vitro*, and tape stripping, *in vitro* or *in vivo*, techniques which are recognised by regulatory bodies for commercialisation of dermally applied products. These techniques do not directly provide chemically specific measurement of the penetration and/or permeation of formulations *in situ*, however.

Raman microspectroscopy provides a non-destructive, non-invasive and chemically specific methodology for *in vitro*, and *in vivo* investigations, *in-situ*, and can provide a powerful alternative to the current gold standard methods approved by regulatory bodies.

This review provides an analysis of the current state of art of the field of monitoring dermal penetration and permeation kinetics of topical products, *in vitro* and *in vivo*, as well as the regulatory requirements of international guidelines governing them. It furthermore outlines developments in the analysis of skin using Raman microspectroscopy, towards the most recent demonstrations of quantitative monitoring of the penetration and permeation kinetics of topical products *in situ*, for *in vitro* and *in vivo* applications, before discussing the challenges and future perspectives of the field.

1. Introduction

The study of human skin represents an important area of research and development in dermatology, toxicology, pharmacology and cosmetology, in order to assess the effects of exogenous agents, their interaction, their absorption mechanism, and/or their toxicity towards the different cutaneous structures [1]. Topically applied formulation products and transdermal formulations are commonly used to carry active pharmaceutical (API) or cosmetic (ACI) ingredients to the skin and the underlying tissue, or through the skin for systemic action. They offer advantages such as avoidance of hepatic first pass metabolic effects, which is a serious effect of oral administration, as well as targeted delivery for local treatment of skin pathologies, controlled drug release, non-invasive drug delivery, and improved patient compliance [2]. Understanding the composition and properties of human skin is also of

* Corresponding author.
E-mail address: hugh.byrne@tudublin.ie (H.J. Byrne).

https://doi.org/10.1016/j.trac.2022.116709

significant importance for glucose sensor development, given the increased prevalence of diabetes [3–5].

The *stratum corneum* (SC) of the skin, although only 10–30 μm in thickness in most anatomical sites [6], forms a very effective barrier to the penetration of topically applied products, and SC-penetration and permeation are the rate limiting steps in dermal and transdermal drug delivery. The regulatory guidelines for topically applied products highlight the importance of sampling their distribution across, and transport through the SC, while maintaining its integrity [7]. However, current "gold standard" methods that are recommended for testing new products and for demonstrating the equivalence of generic products to the original product, do not visualise or monitor the penetration or permeation of the product in the skin, *in situ*. *In vivo* SC sampling protocols require sequential "stripping" of layers of the SC to determine the distribution profile of the API, while *in vitro* techniques monitor the permeated flux of material through excised skin samples. Clearly, a method which can noninvasively and quantitatively determine the concentration profile of a drug in the skin, *in situ*, is highly desirable.

Raman spectroscopy (RS) is a non-destructive, non-invasive and chemically specific optical technique, with demonstrated potential for *in vitro*, *ex vivo* and *in vivo* investigations [8,9]. Performed in confocal microscopic mode, with near infrared lasers as source, it can characterise the biochemical profile of the skin, and screen the distribution of chemical entities applied topically, *in situ*, with high spatial resolution, up to ~100 μm below the skin surface. Measurements can be performed *in vivo*, or *in vitro* in excised tissue or tissue models, *en face* or in cross-sections.

This paper provides a short introduction to skin penetration and permeation, the current gold standard methods for their analysis, and the regulatory guidelines governing them. It then explains the principles of confocal Raman microspectroscopy (CRM), describes its application to the field of research of skin and the topically applied products, using examples of recent advances, and makes the case for the acceptance of the methodology for routine monitoring of penetration and transdermal permeation kinetics of API and ACI, particularly for equivalence testing of generics.

2. The physical barrier function of skin

Anatomically, the skin is a heterogeneous organ, containing several biologically different layers, as well as appendages such as pilosebaceous units (hair follicles, sebaceous glands and the erector pili muscle), eccrine sweat glands and, in some body parts, apocrine glands [10]. Histologically, it is a squamous, keratinised stratified epithelial tissue. The main layers are the SC, the *stratum germinativum* or viable epidermis (VE) (collectively termed the epidermis), the dermis and the hypodermis or subcutaneous tissue.

The understanding of human skin physiology has led to the identification of four different types of barrier functions. These are the physical, the chemical, the immunological and the antimicrobial barriers [11]. However, in general, it is the physical barrier, mainly performed by the proteinaceous and lipidic composition of the SC, that is considered in topical and transdermal chemical transport studies.

Healthy adult SC ranges in thickness from <20 μm on the cheeks, buttocks, and forearms to ~200 μm on the palms of the hands [6,12,13], although it can vary as a function of age and in diseased skin (reviewed in Ref. [10]). The SC is composed of a "bricks and mortar" cellular phase of closely packed corneocytes, embedded within a dense lipidic matrix. Healthy SC owes its barrier function largely to the lipid composition and structure, which has been studied and reviewed in depth (e.g., Refs. [14–17]). At and close to the surface of healthy SC, the mass fraction of water, lipids and proteins is approximately 30, 20 and 50%, respectively [18]. The proportion of water increases with depth into the SC, such that it represents about 70% of SC mass near the SC-VE interface [19]. In addition to, for example, the water/keratin filled corneocytes, the natural hydration level of healthy SC is dependent on the presence of natural moisturising factor (NMF), a term encompassing an ensemble of components derived from the precursor filaggrin, including free amino acids, pyrrolidone carboxylic acid and lactates, [20,21]. Chemicals that are absorbed into, and penetrate through, the SC primarily do so primarily via the inter-cellular spaces, by passive diffusion within the lipid pathway surrounding the corneocytes [22]. The second possible pathway is the intra- (*trans*-) cellular pathway, involving diffusion within the lipid domain, partitioning from it across the protein envelope of the corneocytes, diffusion within the corneocyte intra-cellular space and partitioning back into the lipid domain. A number of studies have also suggested the follicular pathway to be a potential route of penetration into the viable skin for lipophilic compounds, *via* the sebum-filled infundibulum [23–29].

A variety of dermatological diseases as well as exposure to toxicants modify the SC composition and structure, potentially leading to a defective SC barrier function. Causes and effects of skin diseases on the permeability barrier have been extensively reviewed [10,15,30–32]. Reviews of the effects of exogenous compounds such as surfactants and permeation enhancers may be found in Refs. [10,33,34]. Loss-of-function mutation in the profilaggrin gene, resulting in reduced levels of NMF in the SC, as well as corneodesmosome dysfunction, are also known to be related to epidermal barrier diseases [35].

3. Skin penetration and permeation

The Organisation for Economic Co-operation and Development (OECD) defines the percutaneous/dermal absorption process according to three steps [7]:

- Penetration: the uptake of a substance into a particular layer or structure, such as an exogenous compound into the SC;
- Permeation: the penetration through one layer into another, which is both functionally and structurally different from the first layer;
- Resorption: the uptake of a substance into the vascular system (lymph and/or blood vessel), which acts as the central compartment.

Permeation across the SC is considered the rate determining step, and once the compound has passed this stage, ideally it rapidly passes through the remainder of the epidermis and dermis, under so-called "sink conditions". To aid in this process, a test compound, API or ACI, is often loaded in a vehicle, and/or the formulation can contain permeation enhancers. A number of factors can influence the processes of Penetration, Permeation and Resorption of chemical compounds in skin; (i) the Test compound (Physical state, Molecular size, Lipid/water partition coefficient) (ii) Skin (Species, Anatomical site, Hydration of SC, Damage to SC) (iii) Vehicle (Solubility, Distribution in SC, Excipients, Effect on SC pH) (iv) Application dose (Concentration, Finite and infinite), Total contact area, Exposure Duration [36]. Understanding, monitoring and quantifying these multiple influencing factors is the principal challenge to dermal absorption and penetration studies.

3.1. Mathematical models of skin penetration and permeation: steady-state transport modelling

Mathematical representation of the dermal absorption of exogenous agents can help to not only visualise, but also to

parameterise the processes of penetration, permeation, and resorption, enabling comparison of experimental methodologies, and validation of novel analytical techniques. In its most idealised form, the SC is modelled as a homogeneous membrane, lacking any of the biological complexity described in Section 2 (Fig. 1). Further simplifications are:

- the test compound or "donor" (permeant + vehicle) is modelled as a large, well-stirred reservoir, implying that the dose of permeant on the SC surface remains "infinite", or undepleted, over the course of the experiment;
- the permeant is rapidly cleared into the VE at the lower SC interface, i.e. "sink" conditions.

In this representation, the two physical processes governing transport of a donor across the SC are equilibrium partitioning across its interfaces (e.g. donor/SC partitioning coefficient $K_{D/SC}$) and passive diffusion (co-efficient D_{SC}) within the SC. Under steady state conditions, the gradient of donor concentration (C_D) across the SC (height h_{SC}) is constant, and diffusion can be modelled using Fick's 1st and 2nd laws [37], enabling definition of the steady state diffusion flux (penetration or absorption rate, (mol/m^2.s)):

$$J_{SS} = \frac{K_{D/SC} D_{SC}}{h_{SC}} C_D = K_P C_D \quad (1)$$

and the often reported steady-state permeability coefficient:

$$K_p = \frac{K_{D/SC} D_{SC}}{h_{SC}} \quad (2)$$

The use of the maximum flux at steady-state, $J_{MAX,SS}$, is advocated, occurring for a pure solid or a saturated solution of permeant in its vehicle, under sink conditions [38].

The change in C_D in the SC as a function of depth and time is described by

$$C(x,t) = K_{D/SC} C_D \left[1 - \frac{x}{h_{SC}} - \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{1}{n} sin\left(n\pi \frac{x}{h_{SC}} \right) exp\left(-\frac{t}{t_D} \pi^2 n^2 \right) \right] \quad (3)$$

where $t_D = h_{SC}^2 / D_{SC}$ is the diffusion time in the SC.

Under steady-state conditions, the non-linear summation term can be ignored, yielding:

$$C(x) = K_{D/SC} C_D \left[1 - \frac{x}{h_{SC}} \right] \quad (4)$$

Rather than a concentration profile, experimental studies of topical or transdermal delivery often report the cumulative amount of donor permeated through the skin as a function of time which, under steady-state, simplifies to Ref. [37]:

$$Q_{SS}(t) = A J_{SS}(t - t_{LAG}) = A K_P C_D (t - t_{LAG}) \quad (5)$$

Equation (5) is commonly fitted to the linear portion of experimentally obtained cumulative amount *vs.* time curves (Fig. 2(a)). J_{SS} is the slope of the linear phase, whereas t_{LAG} is the intercept of the linear portion with the abscissa. Although simplistic, such a mathematical description of the SC barrier function identifies parameters to quantify the penetration and permeation of topically applied compounds as: the partition coefficient ($K_{D/SC}$), the diffusion constant (D_{SC}), the diffusion flux (J), permeability coefficient (K_p) and the cumulative amount of active permeated through the skin Q(t), and the time lag (t_{LAG}). Notably, however, the idealised conditions of infinite dose are not achievable in practice, leading to a deviation from the model behaviour, due to dose depletion (Fig. 2(a)) [39].

3.2. *In vivo* measurement of skin penetration and permeation

In vivo methods for measurement of transdermal resorption use a physiologically and metabolically intact system, allowing the determination of the penetration into the systemic circulation. As it is not feasible to perform extensive testing on humans, animal models, most commonly rat, are widely employed. The test substance, usually radiolabelled, is applied to the clipped skin at a single or a range of dose levels, after which they are euthanised, blood is collected and the application site removed for analysis, while the carcass is analysed for any unexcreted material. The samples are assayed by appropriate means and the degree of percutaneous absorption is estimated.

For penetration studies, SC sampling of human volunteers by "Tape stripping" is commonly used [41,42]. After exposure of an area of skin to a chemical for a fixed period, layers of the SC (~1 μm) of the exposed site are removed by successive application of adhesive tape. The mass of SC removed per tape should be determined using a gravimetric method by weighing the tapes strips before and after stripping. The drug is then extracted from the tapes then quantified in the extraction solvent(s) [43]. The technique is used to determine the concentration profile of a chemical in the SC as a function of depth and mathematical fitting enables the parameterisation of the process in terms of, for example, $K_{D/SC}$, J and Q(t) (Fig. 2(b) [40]). However, this method is unreliable and usually needs both an accurate estimate of the strip weight and the *trans*-epidermal water loss rate following the removal of the strip. The

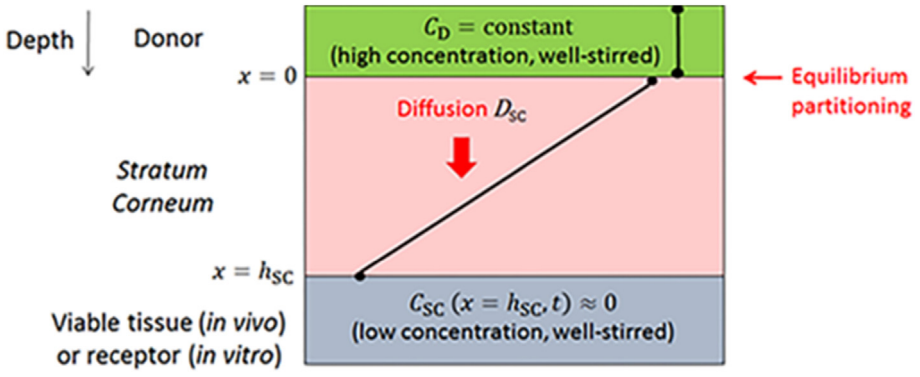

Fig. 1. Schematic representation of transport into and through the *stratum corneum* (SC) following application of an infinite dose of donor, C_D.

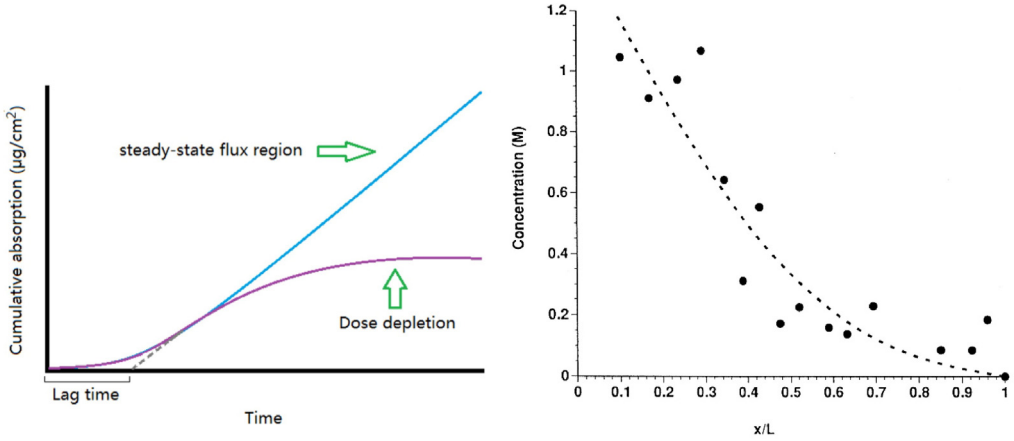

Fig. 2. (a) Graphical representation of cumulative permeation obtained after infinite dosing, indicating regions of steady-state flux (J_{SS}) and the lag time (t_{LAG}). Also shown is the deviation from the steady-state behaviour under finite dosing conditions (reproduced with permission from Ref. [39]). (b) Concentration profile of 4-cyanophenol transport across the depth (x/L) of the human SC *in vivo* (reproduced from Ref. [40], Copyright (1997) National Academy of Sciences).

number of strips needed to remove a given fraction of the SC may vary with the type of tape, the pressure applied and the peeling force, as well as the anatomical site and the age, sex and ethnicity of the subject. Nevertheless, tape stripping has the obvious advantage that it permits determination of SC penetration in humans *in vivo*.

3.3. *In vitro* measurement of skin penetration and permeation

The most frequently used model for *in vitro* skin permeation studies is the Franz diffusion cell [44], although the Saarbruecken model [45], Hamburg model [46] and enhancer cells [47] are also used. The Franz cell contains a donor compartment, to which the formulation is applied, and a receptor compartment, containing an appropriate receptor medium. The skin sample is fixed between the two, in direct contact with the formulation and the receptor medium. Extracts from the receptor medium are taken at predefined intervals, drug content is analysed and Q(t) is plotted over time. In accordance with the analysis of Equation (5), Jss is derived from the slope (Fig. 2(a)). Different formulations and/or the impact of permeation enhancers can then be quantitatively compared.

In addition to permeation studies, penetration can also be monitored *in vitro*. To this end, excised skin is incubated in Franz (or other) cells, after which the skin may be segmented by either tape stripping, heat separation or cryo-segmentation [48–50], although *in vitro* tape stripping obviously suffers from the same drawbacks as *in vivo* tape stripping [51].

4. Legislative considerations

The Organisation for Economic Co-operation and Development (OECD) "Guidance Notes On Dermal Absorption" [7] provide practical guidance to facilitate harmonised interpretation of experimental data from specific dermal absorption studies, primarily focussed on establishing appropriate dermal absorption values for occupational health and public health risk assessment, and are generally applicable to dermal absorption studies. Specifically, they provide additional technical background to both the *in vivo* and *in vitro* methods for skin absorption of chemicals, as described in Test Guidelines 427 [52] and 428, respectively [53].

The OECD Guideline for the Testing of Chemicals, Skin Absorption: *in vitro* Method (Test Guideline 428) [53], endorsed, for example, by the EU [54], specifically refers to studies which "measure the diffusion of chemicals into and across the skin to a fluid reservoir and can utilise non-viable skin to measure diffusion only, or fresh, metabolically active skin to simultaneously measure diffusion and skin metabolism". The recommended methodology is that of the (Franz) diffusion cell. When infinite dose conditions of exposure are used, skin absorption may be expressed using receptor fluid data alone, which permit the calculation of a permeability constant (or co-efficient) (K_p). The Guideline recommends the parallel analysis of relevant reference chemicals, preferably of a lipophilicity close to the test substance.

Post publication of the OECD Test 428, Franz et al. performed an evaluation of the kinetics of drug absorption through excised human skin, to demonstrate the suitability of the technique for studies of the bioequivalence of topical products [55]. Using generic topical drug products, the percutaneous absorption as measured using a Franz diffusion cell was compared to that of their corresponding reference products during preclinical development and to clinical data, post regulatory approval. The validity of the *in vitro* technique, based on excised skin, was further explored in the review of Lehman et al. [56], which examined the scientific literature on compounds whose percutaneous absorption had been measured in human skin both *in vitro* and *in vivo*. The review supported the validity of the correlation, but highlighted the importance of harmonisation of measurement protocols.

The European Medicines Agency (EMA) has published a draft guideline on quality and equivalence of topical products [43], which details equivalence testing protocols for:

- *in vitro* release
- *in vitro* human skin permeation
- *in vivo* SC sampling (tape stripping)
- *in vivo* vasoconstriction assay for corticosteroids

Such equivalence testing is applicable, and may be required, in the context of marketing authorisation applications relying on the dossier of an existing medicinal product, and in the case of changes to a product during pharmaceutical development or post-regulatory approval, which could have a potentially significant impact on the safety, quality or efficacy of the product.

Recommended permeation kinetics studies are *in vitro* excised human skin permeation, *in vivo* SC sampling (tape stripping) of human volunteers and pharmacokinetic bioequivalence. Where possible, pharmacodynamic studies are recommended, including the *in vivo* vasoconstriction assay for corticosteroids and *in vivo* microbial decolonisation studies for antiseptics, undertaken on human volunteers.

Although not explicitly referenced, the prescribed study design of the *in vitro* permeation test (Appendix I) is based on a diffusion

test, using excised (*ex vivo*) adult human skin, similar to that of the OECD Test 428 [53]. The protocol gives specific detail of; Choice and preparation of skin membrane, receptor medium, Number of sampling time points and Recommended dosing amount. Pseudo-infinite dosing regimens are recommended, and the method and device of administration should be similar. Relevant permeation parameters identified are, J_{max} and total amount permeated at the end of experiment (Q_{total}) for the test and comparator products, and the acceptance criteria are detailed. The time of maximal rate of absorption (t_{max}) and lag times, should also be reported. The mass balance should be determined, in accordance with OECD Test 428 [53].

The protocol for *in vivo* SC Sampling (Tape Stripping) for equivalence testing is detailed in Appendix III of the EMA draft guideline [43]. Notably, the United States Food and Drug Administration [57] proposed similar guidelines for determination of bioequivalence of topically applied products. A plot of drug content profile in the SC versus SC depth should be presented (Fig. 2(b)). For the comparison of products, the equivalence parameters: mass of drug recovered from the uptake (M_{uptake}) and clearance ($M_{clearance}$) sites, should be statistically compared, according to the Guideline on the Investigation of Bioequivalence [58].

Both *in vivo* and *in vitro* protocols are known to be prone to significant variability and error, and neither measures the actual penetration or permeation of the exogenous agent *in situ*. Methodologies to evaluate skin penetration, *in vitro*, have recently been reviewed by Zsikó et al. [59]. Techniques such as electron microscopy [60], Small Angle X-ray Scattering [60,61], Confocal Laser Scanning Microscopy (CLSM) [62,63], and Two Photon Microscopy (TPM) [64] have been explored to examine the penetration profiles and the interactions of exogenous molecules and substances with the skin. While electron microscopy and X-ray scattering techniques provide a visualisation of the structures of the SC, neither provide chemically specific information. In the case of CLSM and TPM, while the skin autofluorescence can be imaged [64], additional chemically specific information is provided by fluorescent probes [62,63] or labels [64]. Guidelines for the validation of new bioanalytical methods applied to measure drug concentrations in biological matrices associated with animal toxicokinetic studies and all phases of clinical trials have also been provided by the EMA [65], focusing on the provision of quantitative concentration data, as the basis for pharmacokinetic and toxicokinetic parameter determinations. The main objective of the validation is to demonstrate the reliability of a particular method for the determination of an analyte concentration in a specific biological matrix, such as blood, serum, plasma, urine, or saliva. The main characteristics of a bioanalytical method that are essential to ensure the acceptability of the performance and the reliability of analytical results are: selectivity, carry-over, lower limit of quantification, the response function and calibration range (calibration curve parameters), accuracy, precision, matrix effects, stability of the standard(s) and analyte(s), alone and in the biological matrix.

5. Raman microspectroscopy of skin

Raman spectroscopy (RS) provides a label free, fingerprint of the chemical composition of a sample. Similar CLSM [66], in its confocal microscopic mode, confocal Raman microspectroscopy (CRM) can spatially profile the chemical composition of samples in the lateral or axial directions, *in situ*, with resolution of the order of microns, or even submicron, depending on the wavelength of the source. In spatial mapping, each pixel contains a Raman spectrum, which is a complex superposition of the spectral features of each of the constituent biochemical. Increasingly, multivariate statistical analysis has been employed to, for example, spatially cluster spectra of similar profiles, reduce the dimensionality according to principle components to cluster and differentiate spectral groups within a dataset, and/or correlate systematic variations of features by regressing against external variables such as time, distance or concentration [67].

5.1. Raman spectroscopic analysis of the biochemistry of skin

Early RS studies were performed with the (non microscopic) Fourier Transform Raman technique, and enabled identification of the spectral features of the constituent biomolecular components of SC of post-mortem skin samples [68–70]. The advent of highly sensitive charged coupled device (CCD) detectors in the mid-1990s resulted in a shift to dispersive Raman microscopic instrumentation [71], enabling high spatial resolution of biochemical substructures of the skin, which were more easily accessible in sections of excised skin. Fig. 3 shows the example of a k-means cluster analysis (KMCA) of a human cadaver skin epidermal cross-section, profiled by CRM [72]. The spectra associated with each cluster represent the combination of contributions of all constituent components of the skin layers, clearly showing the characteristic differences. Caspers et al., in 1998, performed a similar CRM analysis of excised human skin sections, confirming that, while the dermis is dominated by signatures of collagen, the SC is dominated by contributions from keratin and lipids [8]. Notably, the study also demonstrated that CRM could be used to perform depth profiling, or "optical sectioning" of the biochemical composition of skin, *in vivo* (Fig. 4), opening up new prospects for non-invasive, *in situ*/*in vivo* monitoring of dermatological processes [8]. The use of optical clearing fluids has more recently been explored to reduce scattering and improve light penetration [73,74], and the state of the art of CRM for probing the physiological structure of the SC has recently been reviewed by Darvin et al. [75].

In the context of quantitative *in vivo* analysis, Caspers et al. proceeded to explore the determination of molecular concentration profiles in the skin [76]. The spectra of each region can be (least squares) fitted with weighted combinations of the spectra of the raw constituent components, and thus the composition of each region determined. The Raman scattering efficiency of each compound can differ significantly, however, and thus relative contributions are most readily determined. The water concentration profile in the SC was determined by evaluating the ratios of the characteristic water (3390 cm^{-1}) and protein (2935 cm^{-1}) bands, weighted by a proportionality constant determined by measurements of pure proteins and water. The study similarly determined the profiles of the major constituent components of NMF and the sweat constituents, lactate and urea. A correction factor was introduced to normalise the spectral response of water and protein relative to their mass ratio [19], enabling quantification of the % hydration over the range of analysis.

Depth profiling of the hydration level and NMF (Fig. 5(i)) enables calculation of the thickness of the SC [6,77–79], which is required for the mathematical description of penetration and permeation processes (Fig. 1, Equation (1)) [13,79]. In the methodology of Crowther et al., the upper 'levelling off point' of the hydration profile (Fig. 5(ii)) was taken to represent the SC depth [78]. The methodology was validated by comparison of SC thicknesses from a number of different anatomical sites, obtained directly by Optical Coherence Tomography (OCT), albeit with relatively less spatial resolution. Alternatively, Bielfeldt et al. fitted the %Hydration profiles with two separate straight lines, one to the rapid increase in the stratum corneum, the other to the plateau in the deeper stratum granulosum and epidermis. The intersection of the lines was used to define the lower boundary of the SC [79]. The technique was validated by comparison of SC thickness of the volar forearm,

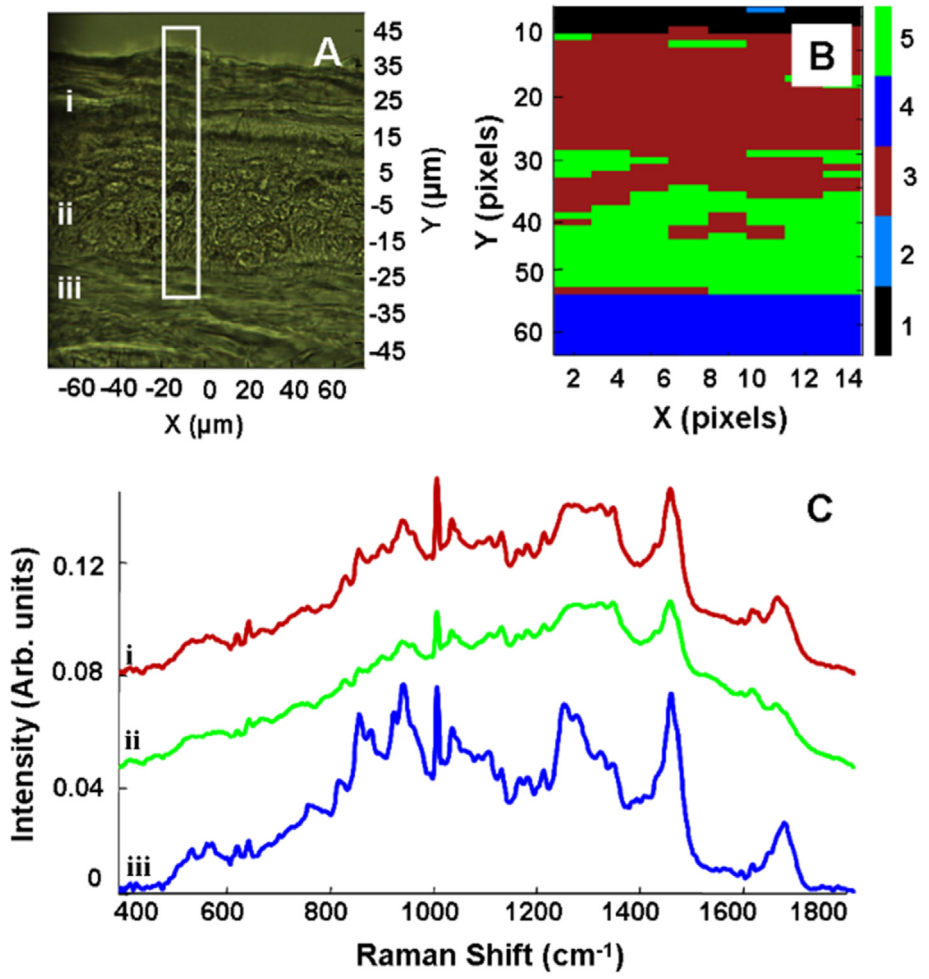

Fig. 3. (A) Optical image of human hand skin tissue section; (B) KMCA of Raman spectral map; (C) KMCA mean Raman spectra (i) cluster 3, stratum corneum (ii) cluster 5, intermediate epithelial layer (iii) cluster 4, dermis (reproduced from Ref. [72]).

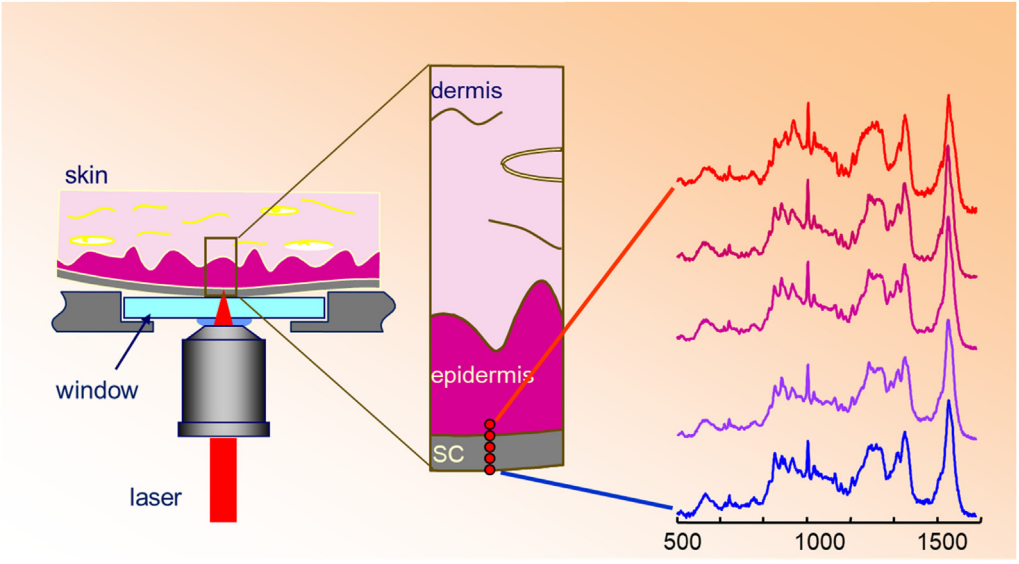

Fig. 4. Schematic representation of *in situ*, depth dependent Raman microspectroscopic profiling of the SC of skin.

lower leg and cheek, determined by *in vivo* CRM, and in *vivo* CLSM [6]. Recognising the emergence of an increasing number of approaches to determination of SC thickness [13,78,79], in 2012, Hancewicz et al. proposed a consensus approach for determination of SC thickness as measured by CRM [80]. The approach entails the application of multiple different models and methods of calculating the SC thickness to each individual CRM profile. The most likely, least error, solution is chosen from the distribution of solutions. The

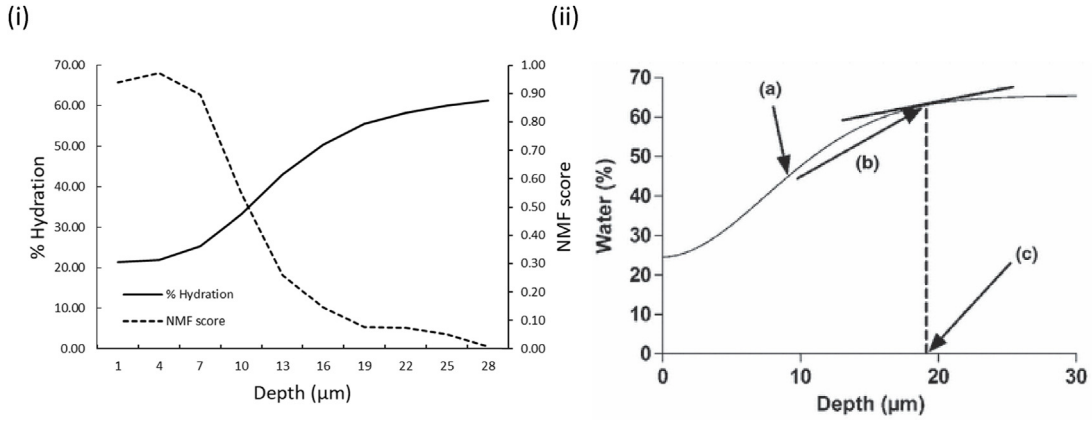

Fig. 5. (i) Comparison of hydration and NMF profiles measured at the same point on the volar forearm (ii) Calculation of SC thickness from hydration curve (reproduced with permission from Ref. [77]). The algorithm calculates the point where the gradient equals 0.5, working from the middle of the curve (*a*) inwards (i.e. deeper in the tissue) (*b*). The depth at this point corresponds to the base of the SC (*c*).

methodology was validated by correlation with reflectance confocal microscopy measurements of SC thickness.

5.2. Raman microspectroscopic analysis of skin penetration and permeation: in vitro

CRM of sections of excised skin can also be employed to monitor the penetration and permeation of active agents, as demonstrated for example by Tfayli et al. [2], who profiled the penetration of metronidazole in sections of human skin samples. A number of studies have utilised caffeine as a model permeant to demonstrate the potential of CRM as a tool for routine quantitation of skin permeation and penetration [39,82–87]. A notable challenge is the identification and extraction of the signature of the active agent from that of the background signal of skin. In a similar CRM analysis of sections of a reconstructed *in vitro* skin model, Stella et al. compared the approaches of least squares analysis with that of multiple curve resolution – alternating least squares (MCR-ALS) to establish the penetration depth profiles, after different exposure times, of the commercially available slimming ACI, Delipidol® (Fig. 6) [81,88]. While both approaches successfully profiled the distribution of the active within the cross-section, *in situ*, it was noted that, without *a priori* knowledge of the absolute strengths of the Raman scattering efficiencies of the ACI and the constituent components of the skin, the analysis was at best semi-quantitative. However, for the comparison of products, the equivalence parameters of drug uptake and clearance, could conceivably be compared, *in situ*. As an example, Essendoubi et al. used MCR-ALS for the analysis of CRM penetration profiles of free and an encapsulated form of retinol in sections of frozen and living tissue, demonstrating a higher permeation efficiency of the encapsulated form [89], and a number of further studies have used CRM to monitor the penetration of nanoformulations [90,91].

Bonnist et al. utilised a modified, Bronaugh-type diffusion cell [92] to monitor the *in vitro* penetration of the skin sensitiser, *trans*-cinnamaldehyde, in different delivery vehicles, over a 24 h period [93]. The study demonstrated the ability of the technique to simultaneously profile the penetration of the active as well as the delivery vehicle and highlighted important differences between the rate of delivery of the sensitiser in the vehicles absolute ethanol, 50% aqueous ethanol, propylene glycol and a 4:1 acetone:olive oil mixture, chosen because they are commonly used in *in vivo* skin sensitisation protocols [94].

A comparison of the techniques of CRM and Franz diffusion cell penetration was conducted by Lunter and Daniels [95]. The Franz diffusion cell measurements found the permeation rate of procaine hydrochloride to be similar for three hydrogel formulations: one without penetration enhancer, one with propylene glycol and one with POE-23-laurylether. Using cross sections of the skin samples exposed for 14 h, the CRM penetration of the formulations were then profiled using a combination of K-means clustering and classical least squares fitting analysis (Fig. 7). Comparing the results of CRM and Franz diffusion cell analysis, partition coefficients and amounts of drug penetrated were found to be similar. However, for the formulation with POE-23-lauryl ether [96], whereas enhancement ratios were 2.3 for CRM, no enhancement effect was found for the Franz diffusion cell flux values. It was thus concluded that, whereas CRM gives similar results to Franz diffusion cell experiments on procaine penetration, it allows a more direct representation of the most relevant parameters, analysis of penetrated drug amount and penetration depth, as it is capable of giving more detailed information on the location of the drug inside the skin than is possible with conventional Franz diffusion cell methods. This was further demonstrated in the *in situ* study of caffeine permeation in porcine skin, using a similar diffusion cell [97], and a number of further studies has used CRM to examine the effects of permeation enhancers [98–100].

5.3. Raman microspectroscopic analysis of skin penetration and permeation: in vivo

Since the initial studies of Caspers et al., CRM has been

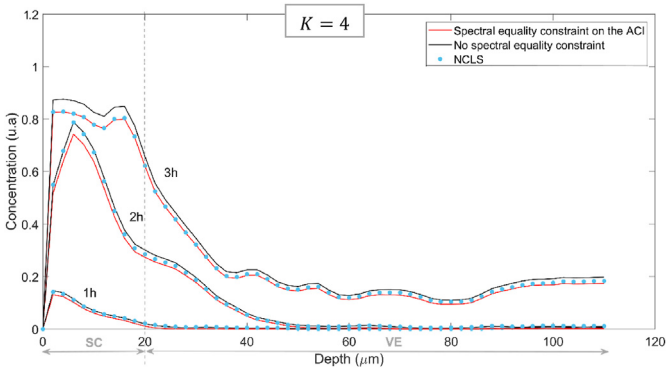

Fig. 6. Average penetration profiles of the Delipidol® for 1 h, 2 h and 3 h of exposure time obtained by MCR-ALS applied with (red) versus without (black) spectral equality constraints on the ACI and using 4 contributing spectral components (K = 4). The average concentrations obtained by NCLS are illustrated by blue points (Reproduced from Ref. [81]).

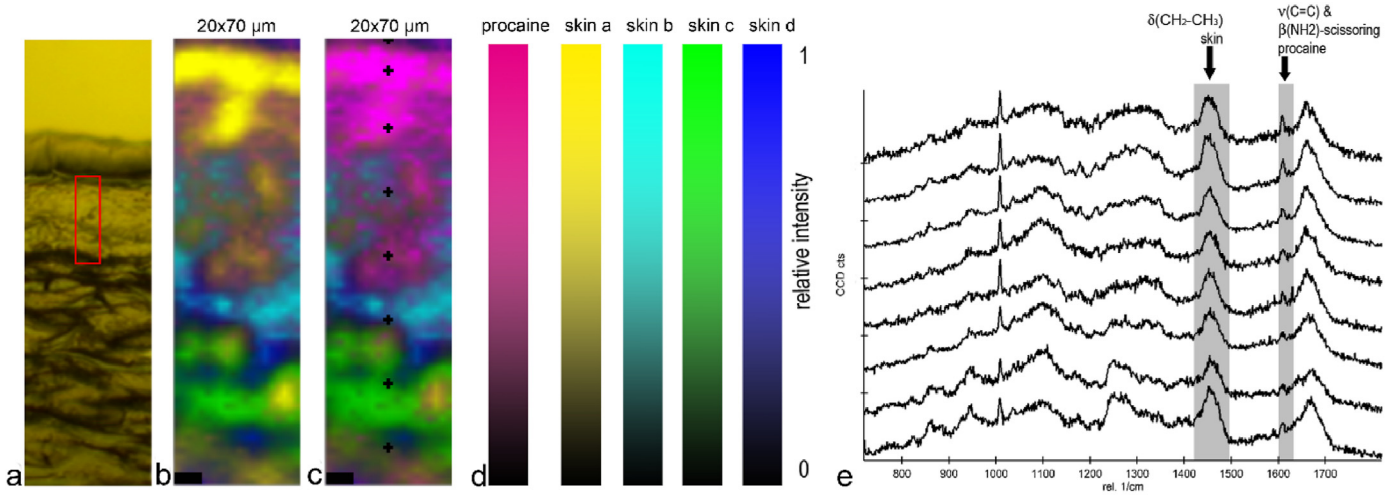

Fig. 7. Representative images of *ex vivo* cross sections of skin incubated with a formulation that contained procaine HCl and POE-23-lauryl ether (a) microscopic image; the box indicates the area from which the Raman scan was made (b) color-coded CRM image [top layer: skin(b)] (c) color-coded CRM image (top layer: procaine) (d) color scale bars (d) spectra extracted from the spots indicated in (c) (reproduced from Ref. [95]).

extensively explored to analyse human skin *in vivo* [8,9], for example to study the effects of moisturising factors [77,78,101–103]. Temporal and spatial variations of the penetration-enhancer, dimethyl sulfoxide in the SC have been studied [104], and the technique was used to track phospholipid permeation into the skin [105]. Wascotte et al. [106] used CRM to

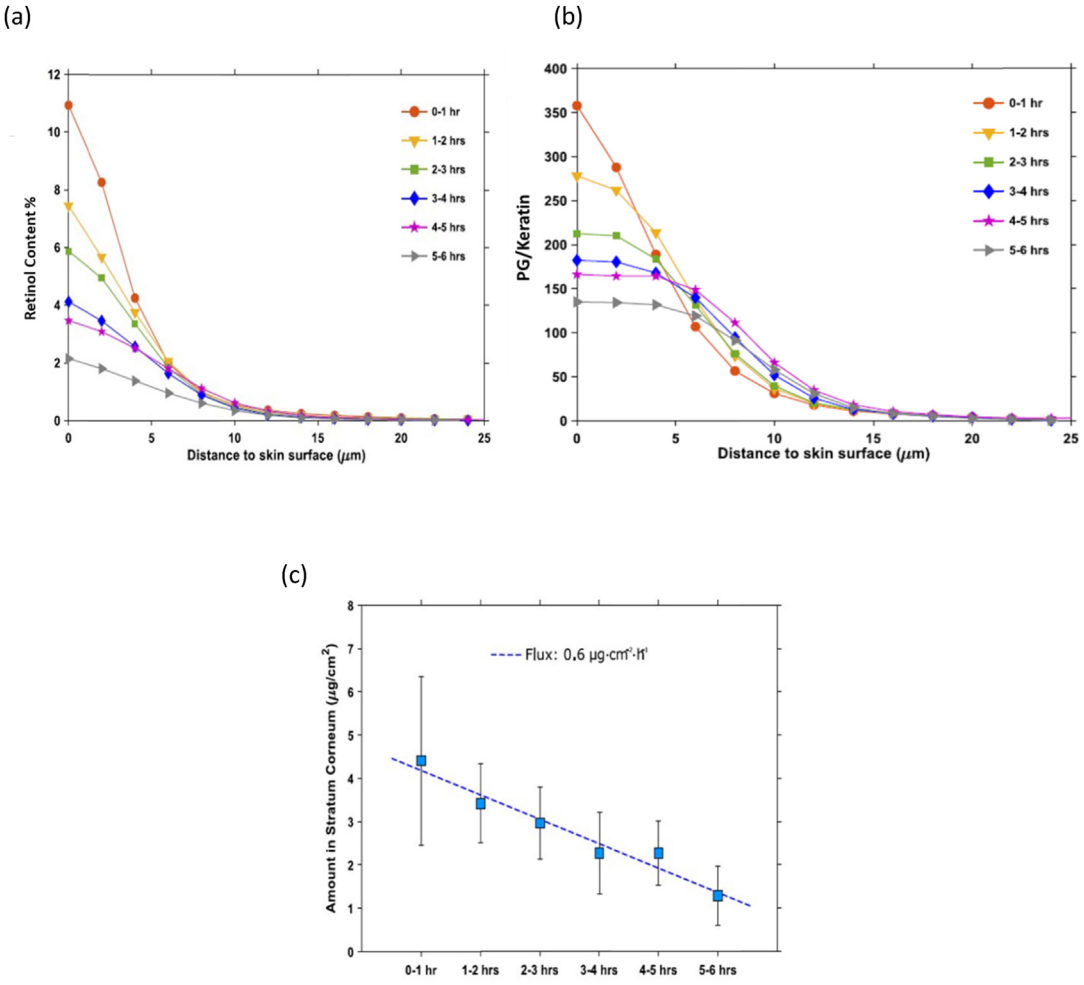

Fig. 8. Concentration profiles of (a) *trans*-retinol (%) and (b) propylene glycol in the volar forearm over 6 h [107]. (c) Quantitative analysis of the concentration and total amount of retinol in the skin of the volar forearm after topical application of a 0.3% solution of retinol in 30% PG:70% ethanol - Total amount in µg/cm^2 skin area. The error bars show the SD of the eight repeated measurements at different locations within the treated skin area. The dashed line is a linear fit to determine the flux of PG [110]. *In vivo* data were reused from Pudney et al. [107]. (Reproduced from Ref. [110] under Creative Commons Licence).

assess the "skin reservoir" of urea. Pudney et al. undertook a CRM study of the delivery of trans retinol to the skin, *in vivo* [107], and the study was extended to explore the effects of permeation enhancers [108]. A least squares fitting of the spectra of the constituent components of skin, as well as that of retinol was employed to establish the percentage retinol and propylene glycol penetration profiles as a function of SC depth and exposure time (Fig. 8(a and b)). Notably, Raman signals were normalised to the depth profile of keratin, to account for loss of signal due to scattering [76]. The study also profiled the relative concentrations of NMF and water content, assuming that the SC is probed when the water contribution is less than 55% and the NMF signal is greater than 30% of the maximum NMF signal measured in the SC, enabling an estimation of the SC thickness, and therefore the profile of the active relative to it [107].

Using similar quantification techniques, Mateus et al. undertook a direct comparison of *in vivo* CRM and the tape stripping technique to profile the permeation of ibuprofen, prepared in propylene glycol and propylene glycol/water solutions, applied to the volar aspect of the forearm of human volunteers [109]. The CRM analysis was performed based on profiles quoted relative to the highest ibuprofen concentration in the skin. A comparison of diffusion parameters, (D_{SC}/h^2), determined by a fitting of the results of each technique (Equation (3)), indicated a good correlation between the CRM measurement and the legislative "gold-standard". The SC thickness was determined using the CRM profile of water, enabling calculation of the diffusion flux, J (Equation (1)).

In 2019, Caspers et al. demonstrated an *in vivo* CRM methodology to quantify the penetration profiles of products topically applied to the skin in absolute terms [110]. Based on *in vitro* concentration dependent calibration of the Raman spectrum of the active in a suitable solvent, and of the solvent and SC protein, the mass ratio of the material to protein in the SC can be determined, which can be translated to absolute concentrations of the material, knowing the protein content of the SC. For the examples of *trans*-retinol and propylene glycol (PG), the absolute concentration profiles in the skin at different timepoints after topical application were determined, enabling calculation of the flux, J, of materials into the skin (Fig. 8(c)).

More recently, Iliopoulos et al. undertook a comparative study of the permeation of niacinamide from various formulations in human skin *in vitro*, using conventional Franz cells, and *in vivo*, using a quantitative CRM method under finite dose conditions [111]. The formulation vehicles were chosen to encompass a significant range of physicochemical properties. The *in vitro*/*in vivo* comparative study showed an excellent correlation of the cumulative amounts of niacinamide permeated *in vitro* and the amounts measured *in vivo* with CRM at 2 μm depth. A similarly good correlation was observed between the total cumulative *in vitro* permeation of niacinamide and the total amount that penetrated the SC per unit of surface area *in vivo*.

6. Challenges and future perspectives

It has been clearly demonstrated that CRM is an effective tool to profile the chemical composition of skin, both *in vitro* and *in vivo*, and to monitor the penetration and permeation of topically-applied exogenous molecules/agents. The chemical specificity enables the unambiguous identification and localisation of the agent in the skin, and more detailed analysis of the spectral profiles can even be used to monitor transformation of the API/ACI by, for example, crystallisation or metabolisation [84]. The ability to determine the penetration and permeation parameters *in situ*, in skin models, excised skin, and *in vivo* has enabled favourable comparison of CRM with the legislatively accepted methodologies like Franz cells diffusion or tape stripping, strengthening the argument for the legislative acceptance of the CRM-based methodology for validation of topically applied pharmaceutical and cosmetic products.

It should be noted, however, that the CRM technique does not necessarily monitor processes of follicular penetration. Although molecules or particles that accumulate within the infundibulum could remain trapped there (depending on size for instance), they may be released at a later stage, due to sebum flow towards the skin surface or hair growth [112]. The hair follicle is traditionally investigated as a pathway which bypasses the SC barrier. However, it should be kept in mind that it is still potentially a physical barrier to the deeper penetration of exogenous compounds.

A further potential application of the technique is for glucose monitoring in skin, which has become increasingly important given the increasing prevalence of diabetes [113]. Quantitative analysis of glucose in blood serum has been demonstrated using CRM [114]. Quantitative *in vivo* glucose measurements have been demonstrated using SERS substrates subcutaneously implanted in a rat model [115–117]. Pandey et al. have discussed the challenges to the direct measurement of glucose in skin, including in interstitial fluids [118], but more recently, Kang et al. have reported the direct observation of the glucose spectroscopic fingerprint using *in vivo* Raman analysis of pig skin [119].

In terms of legislative validation of the technique, reliable quantification of concentrations of the active constituents of the applied formulation is critical. In this respect, it is important to note that CRM is an optical technique, and the penetration depth of the light in the skin is dependent on the source laser wavelength, and the absorption and refractive index of the skin at that wavelength. Near infrared wavelengths are normally chosen, for maximum transparency of the skin [120]. Comparisons of *in vitro* and *in vivo* depth profiling have demonstrated the mismatch between the real and apparent depths, and the requirements to account for it [121–124]. However, this effect can be accounted for by use of an oil immersion objective, the oil being index matched to the skin and the window on which it rests. As an optical measurement, CRM is also impacted by scattering of the source laser as it penetrates the skin, as well as the Raman signal itself, as it emerges. Quantification studies have accounted for this by normalising to the simultaneously measured keratin content at identical location and depth. After water, keratin is the most abundant molecule in the SC, so that this normalisation is similar to a normalisation to dry weight of skin [107,109,110]. Scattering occurs due to localised changes in the refractive index associated with the heterogeneous structure and composition of the skin, and may depend on, for example, the degree of hydration. Hydration levels, and the permeation and transport properties themselves, can also change as a result of topically applied agents, in a dynamic fashion [77], and so correction for such effects may not be trivial.

Computational models of the penetration and permeation processes across the SC can add insight to better understand, describe and quantify studies of permeation kinetics [125,126]. They can potentially be used to establish quantitative structure activity relationships to guide formulation design [127] and equivalence testing [128], and to establish and validate novel bioanalytical methods to monitor skin penetration and permeation [129]. The reality of *in vitro* and *in vivo* experimental observations can be significantly more complex that presented in Section 3.1, however, such that simple analytical solutions such as Equation (3) can no longer be derived. In most practical scenarios, the donor is depleted over the course of the experiment (finite dose application), or even removed before measurement, the API/ACI can crystallise or be metabolised in the SC, which itself can undergo changes due to the physiological process of desquamation [130,131]. The mathematics describing these nonhomogeneous boundary value problems can

be reduced in complexity through application of the Laplace transform, which reduces the partial differential equations in the time domain to ordinary differential equations in the Laplace domain, which are easier to solve. Comprehensive reviews of the mathematical process of Laplace transformation and numerous examples pertaining to skin penetration are provided in Refs. [37,130].

An alternative approach is that of numerical modelling, by which, in principle, an unlimited degree of complexity can be introduced into the model. In the compartmentalised approach of Guy and Hadgraft [132], the pharmacokinetics of percutaneous absorption can be described by a series of one dimensional rate equations describing the transport between successive compartments, and such an approach was employed, for example, to simply model loss of applied sample by surface evaporation [132]. Depending on the complexity, the parameters of the model have been interpreted in the context of penetration and permeation parameters, as reviewed for example by McCarley and Bunge [133].

Such a modelling approach has been extended to physiologically-based pharmacokinetic (PBPK) modelling and simulation to predict *in vitro* or *in vivo* penetration of chemicals, and has been recognised by the US FDA as a core technology for the future of generic drug products [134]. PBPK models incorporate a detailed structural description of the skin layers (SC, viable epidermis, dermis) with a 2D microscopic description of the SC, transport equations and partition and diffusion coefficient estimation based on empirical correlations and can simulate, for example, evaporation of volatile chemicals, different exposure scenarios, the effect of skin surface temperature and airflow, and even incorporate and simulate the effects of open and blocked hair follicles [126–128]. As a future perspective, the application of simple and more complex models to guide analysis of CRM profiles of skin penetration may be important, and indeed CRM may add molecular level information to help guide and validate such models, particularly for example to visualise metabolisation of products.

While modelling approaches can help interpret results and even guide experimental protocols, verification requires the application of the models to experimental observations. In order to progress the field of CRM analysis of dermal absorption, it is important that such protocols are standardised to better enable comparability between measurements, different instrumentation, users and laboratories. Notably, Binder et al. have recently undertaken an evaluation of inter-individual variability and inter-laboratory comparability, of skin penetration profiles in samples of three different donors, as measured by two different confocal Raman microspectroscopic instruments, concluding that comparable estimations of the skin penetration of procaine were achieved [135]. In all studies, the rigour of legislative protocols should be applied. Ultimately, as a demonstration of the appropriateness and validity of the technique, equivalence studies should be performed, ideally on a patented original and a generic which has been approved by alternative protocols.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stephan Bielfeldt reports a relationship with proDERM Institut GmbH that includes: employment. Yuri Dancik reports a relationship with Certara UK Limited that includes: employment. Gerwin Pupples reports a relationship with RiverD International B.V. that includes: board membership, employment, and equity or stocks.

Acknowledgements

This review was prepared within the framework of the LE STUDIUM RESEARCH CONSORTIUM and the ARD Centre Val de Loire - Project MINIONs (2020−00141275). Y.D. thanks Le STUDIUM Institute of Advanced Studies for the research fellowship award and the ARD Cosmetosciences program for financial support.

References

[1] A. Baroni, E. Buommino, V. De Gregorio, E. Ruocco, V. Ruocco, R. Wolf, Structure and function of the epidermis related to barrier properties, Clin. Dermatol. 30 (2012) 257−262. https://doi.org/10.1016/j.clindermatol.2011.08.007.

[2] A. Tfayli, O. Piot, F. Pitre, M. Manfait, Follow-up of drug permeation through excised human skin with confocal Raman microspectroscopy, Eur. Biophys. J. 36 (2007) 1049−1058. https://doi.org/10.1007/s00249-007-0191-x.

[3] W. Groenendaal, G. Von Basum, K.A. Schmidt, P.A.J. Hilbers, N.A.W. Van Riel, Quantifying the composition of human skin for glucose sensor development, J. Diabetes Sci. Technol. 4 (2010) 1032−1040. https://doi.org/10.1177/193229681000400502.

[4] S. Ullah, F. Hamade, U. Bubniene, J. Engblom, A. Ramanavicius, A. Ramanaviciene, T. Ruzgas, In-vitro model for assessing glucose diffusion through skin, Biosens, Bioelectron 110 (2018) 175−179. https://doi.org/10.1016/j.bios.2018.03.039.

[5] S. Ramanavicius, A. Ramanavicius, Conducting polymers in the design of biosensors and biofuel cells, Polymers (Basel) 13 (2021) 1−19. https://doi.org/10.3390/polym13010049.

[6] A. Böhling, S. Bielfeldt, A. Himmelmann, M. Keskin, K.P. Wilhelm, Comparison of the stratum corneum thickness measured in vivo with confocal Raman spectroscopy and confocal reflectance microscopy, Skin Res. Technol. 20 (2014) 50−57. https://doi.org/10.1111/srt.12082.

[7] OECD, Guidance document for the conduct of skin absorption studies. https://doi.org/10.1787/9789264078796-en, 2004.

[8] P.J. Caspers, G.W. Lucassen, R. Wolthuis, H.A. Bruining, G.J. Puppels, In vitro and in vivo Raman spectroscopy of human skin, Biospectroscopy 4 (1998) S31−S39. https://doi.org/10.1002/(sici)1520-6343, 1998)4:5+<s31::aid-bspy4>3.0.co;2-m.

[9] M. Förster, M.A. Bolzinger, G. Montagnac, S. Briançon, Confocal Raman microspectroscopy of the skin, Eur. J. Dermatol. 21 (2011) 851−863. https://doi.org/10.1684/ejd.2011.1494.

[10] Y. Dancik, P.L. Bigliardi, M. Bigliardi-Qi, What happens in the skin? Integrating skin permeation kinetics into studies of developmental and reproductive toxicity following topical exposure, Reprod. Toxicol. 58 (2015) 252−281. https://doi.org/10.1016/j.reprotox.2015.10.001.

[11] H. Niehues, J.A. Bouwstra, A. El Ghalbzouri, J.M. Brandner, P.L.J.M. Zeeuwen, E.H. van den Bogaard, 3D skin models for 3R research: the potential of 3D reconstructed skin models to study skin barrier function, Exp. Dermatol. 27 (2018) 501−511. https://doi.org/10.1111/exd.13531.

[12] J. Sandby-Møller, T. Poulsen, H.C. Wulf, Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits, Acta Derm. Venereol. 83 (2003) 410−413. https://doi.org/10.1080/00015550310015419.

[13] M. Egawa, T. Hirao, M. Takahashi, In vivo estimation of stratum corneum thickness from water concentration profiles obtained with Raman spectroscopy, Acta Derm. Venereol. 87 (2007) 4−8. https://doi.org/10.2340/00015555-0183.

[14] M.J. Choi, H.I. Maibach, Role of ceramides in barrier function of healthy and diseased skin, Am. J. Clin. Dermatol. 6 (2005) 215−223. https://doi.org/10.2165/00128071-200506040-00002.

[15] P.M. Elias, Skin barrier function, Curr. Allergy Asthma Rep. 8 (2008) 299−305. https://doi.org/10.1007/s11882-008-0048-0.

[16] K. Vavrova, A. Kovačik, L. Opalka, Ceramides in the skin barrier, Eur. Pharmaceut. J. 64 (2017) 28−35. https://doi.org/10.1515/afpuc-2017-0004.

[17] J. van Smeden, M. Janssens, G.S. Gooris, J.A. Bouwstra, The important role of stratum corneum lipids for the cutaneous barrier function, Biochim. Biophys. Acta - Mol. Cell Biol. Lipids. 1841 (2014) 295−313. https://doi.org/10.1016/j.bbalip.2013.11.006.

[18] M. Boncheva, J. de Sterke, P.J. Caspers, G.J. Puppels, Depth profiling of stratum corneum hydration in vivo: a comparison between conductance and confocal Raman spectroscopic measurements, Exp. Dermatol. 18 (2009) 870−876. https://doi.org/10.1111/j.1600-0625.2009.00868.x.

[19] P.J. Caspers, G.W. Lucassen, H.A. Bruining, G.J. Puppels, Automated depth-scanning confocal Raman microspectrometer for rapid in vivo determination of water concentration profiles in human skin, J. Raman Spectrosc. 31 (2000) 813−818. https://doi.org/10.1002/1097-4555(200008/09)31:8/9<813::AID-JRS573>3.0.CO.

[20] J.A. Bouwstra, H.W.W. Groenink, J.A. Kempenaar, S.G. Romeijn, M. Ponec, Water distribution and natural moisturizer factor content in human skin equivalents are regulated by environmental relative humidity, J. Invest. Dermatol. 128 (2008) 378−388. https://doi.org/10.1038/sj.jid.5700994.

[21] P. Kwan, G.J. Sills, M.J. Brodie, Understanding the role of NMF in skin

hydration, Pract. Dermatology (2012) 21–34. http://www.ncbi.nlm.nih.gov/pubmed/11448723.
[22] O.G. Jepps, Y. Dancik, Y.G. Anissimov, M.S. Roberts, Modeling the human skin barrier - towards a better understanding of dermal absorption, Adv. Drug Deliv. Rev. 65 (2013) 152–168. https://doi.org/10.1016/j.addr.2012.04.003.
[23] S. Valiveti, G.W. Lu, Diffusion properties of model compounds in artificial sebum, Int. J. Pharm. 345 (2007) 88–94. https://doi.org/10.1016/j.ijpharm.2007.05.043.
[24] S. Valiveti, J. Wesley, G.W. Lu, Investigation of drug partition property in artificial sebum, Int. J. Pharm. 346 (2008) 10–16. https://doi.org/10.1016/j.ijpharm.2007.06.001.
[25] A. Abe, M. Saito, W.R. Kadhum, H. Todo, K. Sugibayashi, Establishment of an evaluation method to detect drug distribution in hair follicles, Int. J. Pharm. 542 (2018) 27–35. https://doi.org/10.1016/j.ijpharm.2018.02.034.
[26] A. Lauterbach, C.C. Müller-Goymann, Applications and limitations of lipid nanoparticles in dermal and transdermal drug delivery via the follicular route, Eur. J. Pharm. Biopharm. 97 (2015) 152–163. https://doi.org/10.1016/j.ejpb.2015.06.020.
[27] A. Patzelt, W.C. Mak, S. Jung, F. Knorr, M.C. Meinke, H. Richter, E. Rühl, K.Y. Cheung, N.B.N.N. Tran, J. Lademann, Do nanoparticles have a future in dermal drug delivery? J. Contr. Release 246 (2017) 174–182. https://doi.org/10.1016/j.jconrel.2016.09.015.
[28] E. Abd, H.A.E. Benson, M.S. Roberts, J.E. Grice, Minoxidil skin delivery from nanoemulsion formulations containing eucalyptol or oleic acid: enhanced diffusivity and follicular targeting, Pharmaceutics 10 (2018) 1–12. https://doi.org/10.3390/pharmaceutics10010019.
[29] E. Abd, H.A.E. Benson, M.S. Roberts, J.E. Grice, Follicular penetration of caffeine from topically applied nanoemulsion formulations containing penetration enhancers: in vitro human skin studies, skin pharmacol, Physiol. 31 (2018) 252–260. https://doi.org/10.1159/000489857.
[30] P.M. Elias, R. Gruber, D. Crumrine, G. Menon, M.L. Williams, J.S. Wakefield, W.M. Holleran, Y. Uchida, Formation and functions of the corneocyte lipid envelope (CLE), Biochim. Biophys. Acta - Mol. Cell Biol. Lipids. 1841 (2014) 314–318. https://doi.org/10.1016/j.bbalip.2013.09.011.
[31] M. Haftek, Epidermal barrier disorders and corneodesmosome defects, Cell Tissue Res. 360 (2015) 483–490. https://doi.org/10.1007/s00441-014-2019-1.
[32] A.S. Halling-Overgaard, S. Kezic, I. Jakasa, K.A. Engebretsen, H. Maibach, J.P. Thyssen, Skin absorption through atopic dermatitis skin: a systematic review, Br. J. Dermatol. 177 (2017) 84–106. https://doi.org/10.1111/bjd.15065.
[33] M.E. Lane, Skin penetration enhancers, Int. J. Pharm. 447 (2013) 12–21. https://doi.org/10.1016/j.ijpharm.2013.02.040.
[34] Y. Liu, D.J. Lunter, Systematic investigation of the effect of non-ionic emulsifiers on skin by confocal Raman spectroscopy—a comprehensive lipid analysis, Pharmaceutics 12 (2020). https://doi.org/10.3390/pharmaceutics12030223.
[35] G.K. Menon, G.W. Cleary, M.E. Lane, The structure and function of the stratum corneum, Int. J. Pharm. 435 (2012) 3–9. https://doi.org/10.1016/j.ijpharm.2012.06.005.
[36] WHO | draft environmental health criteria (EHC): dermal exposure (n.d.), https://www.who.int/ipcs/methods/dermal_exposure/en/.
[37] Y.G. Anissimov, O.G. Jepps, Y. Dancik, M.S. Roberts, Mathematical and pharmacokinetic modelling of epidermal and dermal transport processes, Adv. Drug Deliv. Rev. 65 (2013) 169–190. https://doi.org/10.1016/j.addr.2012.04.009.
[38] M.S. Roberts, Solute-vehicle-skin interactions in percutaneous absorption: the principles and the people, Skin Pharmacol. Physiol. 26 (2013) 356–370. https://doi.org/10.1159/000353647.
[39] L. Luo, M.E. Lane, Topical and transdermal delivery of caffeine, Int. J. Pharm. 490 (2015) 155–164. https://doi.org/10.1016/j.ijpharm.2015.05.050.
[40] F. Pirot, Y.N. Kalia, A.L. Stinchcomb, G. Keating, A. Bunge, R.H. Guy, Characterization of the permeability barrier of human skin in vivo, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 1562–1567. https://doi.org/10.1073/pnas.94.4.1562.
[41] S.J. Bashir, A.L. Chew, A. Anigbogu, F. Dreher, H.I. Maibach, Physical and physiological effects of stratum corneum tape stripping, Skin Res. Technol. 7 (2001) 40–48. https://doi.org/10.1034/j.1600-0846.2001.007001040.x.
[42] H. Löffler, F. Dreher, H.I. Maibach, Stratum corneum adhesive tape stripping: influence of anatomical site, application pressure, duration and removal, Br. J. Dermatol. 151 (2004) 746–752. https://doi.org/10.1111/j.1365-2133.2004.06084.x.
[43] Quality and Equivalence of Topical Products | European Medicines Agency, (n.d.). https://www.ema.europa.eu/en/quality-equivalence-topical-products.
[44] T.J. Franz, Percutaneous absorption. On the relevance of in vitro data, J. Invest. Dermatol. 64 (1975) 190–195. https://doi.org/10.1111/1523-1747.ep12533356.
[45] H. Wagner, K.H. Kostka, C.M. Lehr, U.F. Schaefer, Drug distribution in human skin using two different in vitro test systems: comparison with in vivo data, Pharm. Res. 17 (2000) 1475–1481. https://doi.org/10.1023/A:1007648807195.
[46] M.E. Herbig, P. Houdek, S. Gorissen, M. Zorn-Kruppa, E. Wladykowski, T. Volksdorf, S. Grzybowski, G. Kolios, C. Willers, H. Mallwitz, I. Moll, J.M. Brandner, A custom tailored model to investigate skin penetration in porcine skin and its comparison with human skin, Eur. J. Pharm. Biopharm. 95 (2015) 99–109. https://doi.org/10.1016/j.ejpb.2015.03.030.
[47] P. Pharmacokinetics, Office of training and communications division of communications management drug information branch, Tel. http://www.fda.gov/cder/guidance.htm, 1999. (Accessed 16 January 2022).
[48] Y.N. Kalia, Ion mobility across human stratum corneum in vivo, J. Pharm. Sci. 87 (1998) 1508–1511. https://doi.org/10.1021/js980247i.
[49] A.V. Rawlings, P.J. Matts, Stratum Corneum Moisturization at the Molecular Level: an update in relation to the dry skin cycle, J. Invest. Dermatol. 124 (2005) 1099–1110. https://doi.org/10.1111/j.1523-1747.2005.23726.x.
[50] H.-J. Weigmann, U. Jacobi, C. Antoniou, G.N. Tsikrikas, V. Wendel, C. Rapp, H. Gers-Barlag, W. Sterry, J. Lademann, Determination of penetration profiles of topically applied substances by means of tape stripping and optical spectroscopy: UV filter substance in sunscreens, J. Biomed. Opt. 10 (2005), 014009. https://doi.org/10.1117/1.1854683.
[51] L.K. Pershing, J.L. Nelson, J.L. Corlett, S.P. Shrivastava, D.B. Hare, V.P. Shah, Assessment of dermatopharmacokinetic approach in the bioequivalence determination of topical tretinoin gel products, J. Am. Acad. Dermatol. 48 (2003) 740–751. https://doi.org/10.1067/mjd.2003.175.
[52] OECD, OECD Guidleines 427 Skin Absorption: in Vivo Method, OECD, 2004. https://doi.org/10.1787/9789264071063-EN.
[53] OECD Guideline for Testing of Chemicals. Test Guideline 428: Skin Absorption: in Vitro Method, 8, 2004. https://doi.org/10.1787/20745788.
[54] Scientific Committee on Consumer Safety, Basic Criteria for the in Vitro Assessment of Dermal Absorption of Cosmetic Ingredients, 2010.
[55] T.J. Franz, P.A. Lehman, S.G. Raney, Use of excised human skin to assess the bioequivalence of topical products, Skin Pharmacol. Physiol. 22 (2009) 276–286. https://doi.org/10.1159/000235828.
[56] P.A. Lehman, S.G. Raney, T.J. Franz, Percutaneous absorption in man: in vitro-in vivo correlation, Skin Pharmacol, Physiol. 24 (2011) 224–230. https://doi.org/10.1159/000324884.
[57] U.S. FDA, Draft guidance for industry on topical dermatological drug product NDAs and ANDAs-in vivo bioavailability, bioequivalence, in: Vitro Release and Associated Studies, Withdrawal, 2002.
[58] J.A. Morais, Mr Lobato, The New EMEA Guideline on the Investigation of Bioequivalence, 2009.
[59] S. Zsikó, E. Csányi, A. Kovács, M. Budai-Szűcs, A. Gácsi, S. Berkó, Methods to evaluate skin penetration in vitro, Sci. Pharm. 87 (2019) 19. https://doi.org/10.3390/scipharm87030019.
[60] H.E.J. Hofland, J.A. Bouwstra, H.E. Bodde, F. Spies, H.E. Junginger, Interactions between liposomes and human stratum corneum in vitro: freeze fracture electron microscopial visualization and small angle X-ray scattering studies, Br. J. Dermatol. 132 (1995) 853–866. https://doi.org/10.1111/j.1365-2133.1995.tb16940.x.
[61] J.A. Bouwstra, M.A. de Vries, G.S. Gooris, W. Bras, J. Brussee, M. Ponec, Thermodynamic and structural aspects of the skin barrier, J. Contr. Release 15 (1991) 209–219. https://doi.org/10.1016/0168-3659(91)90112-Q.
[62] J.A. Veiro, P.G. Cummins, Imaging of skin epidermis from various origins using confocal laser scanning microscopy, Dermatology 189 (1994) 16–22. https://doi.org/10.1159/000246752.
[63] L.D. Swindle, S.G. Thomas, M. Freeman, P.M. Delaney, View of normal human skin in vivo as observed using fluorescent fiber-optic confocal microscopic imaging, J. Invest. Dermatol. 121 (2003) 706–712. https://doi.org/10.1046/j.1523-1747.2003.12477.x.
[64] B. Yu, K.H. Kim, P.T.C. So, D. Blankschtein, R. Langer, Visualization of oleic acid-induced transdermal diffusion pathways using two-photon fluorescence microscopy, J. Invest. Dermatol. 120 (2003) 448–455. https://doi.org/10.1046/j.1523-1747.2003.12061.x.
[65] EMA, Committee for Medicinal Products for Human Use, Guideline on bioanalytical method validation, 2012. EMEA/CHMP/EWP/192217/2009.
[66] F. Cristina, L. Vieira, M.V.L. Badra Bentley, Confocal laser scanning microscopy as a tool for the investigation of skin drug delivery systems and diagnosis of skin disorders, in: Confocal Laser Microsc. - Princ. Appl. Med. Biol. Food Sci., InTech, 2013. https://doi.org/10.5772/55995.
[67] H.J. Byrne, P. Knief, M.E. Keating, F. Bonnier, Spectral pre and post processing for infrared and Raman spectroscopy of biological tissues and cells, Chem. Soc. Rev. 45 (2016) 1865–1878. https://doi.org/10.1039/c5cs00440c.
[68] B.W. Barry, H.G.M. Edwards, A.C. Williams, Fourier transform Raman and infrared vibrational study of human skin: assignment of spectral bands, J. Raman Spectrosc. 23 (1992) 641–645. https://doi.org/10.1002/jrs.1250231113.
[69] A.C. Williams, H.G.M. Edwards, B.W. Barry, Fourier transform Raman spectroscopy a novel application for examining human stratum corneum, Int. J. Pharm. 81 (1992). https://doi.org/10.1016/0378-5173(92)90022-T. R11–R14.
[70] G. Scanlon, P. Connell, M. Ratzlaff, B. Foerg, D. McCartney, A. Murphy, K. O'Connor, J. Loughman, Macular pigment optical density is lower in type 2 diabetes, compared with type 1 diabetes and normal controls, Retina 35 (2015) 1808–1816. https://doi.org/10.1097/IAE.0000000000000551.
[71] S.C. Denson, C.J.S. Pommier, M.B. Denton, The impact of array detectors on Raman spectroscopy, J. Chem. Educ. 84 (2007) 67–74. https://doi.org/10.1021/ed084p67.
[72] S.M. Ali, F. Bonnier, H. Lambkin, K. Flynn, V. McDonagh, C. Healy, T.C. Lee, F.M. Lyng, H.J. Byrne, A comparison of Raman, FTIR and ATR-FTIR micro spectroscopy for imaging human skin tissue sections, Anal. Methods 5 (2013) 2281–2291. https://doi.org/10.1039/c3ay40185e.
[73] A. Jaafar, R. Holomb, A.Y. Sdobnov, Z. Ocskay, Z. Jakus, V.V. Tuchin, M. Veres,

Ex vivo confocal Raman microspectroscopy of porcine dura mater supported by optical clearing, J. Biophot. (2021), e202100332. https://doi.org/10.1002/jbio.202100332.
[74] A. Jaafar, M.H. Mahmood, R. Holomb, L. Himics, T. Váczi, A.Y. Sdobnov, V.V. Tuchin, M. Veres, Ex-vivo confocal Raman microspectroscopy of porcine skin with 633/785-NM laser excitation and optical clearing with glycerol/water/DMSO solution, J. Innov. Opt. Health Sci. 14 (2021) 2021–2035. https://doi.org/10.1142/S1793545821420037.
[75] M.E. Darvin, J. Schleusener, J. Lademann, C.-S. Choe, Current views on non-invasive in vivo determination of physiological parameters of the stratum corneum using confocal Raman microspectroscopy, Skin Pharmacol. Physiol. (2022). https://doi.org/10.1159/000521416.
[76] P.J. Caspers, G.W. Lucassen, E.A. Carter, H.A. Bruining, G.J. Puppels, In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles, J. Invest. Dermatol. 116 (2001) 434–442. https://doi.org/10.1046/j.1523-1747.2001.01258.x.
[77] J.M. Crowther, A. Sieg, P. Blenkiron, C. Marcott, P.J. Matts, J.R. Kaczvinsky, A.V. Rawlings, Measuring the effects of topical moisturizers on changes in stratum corneum thickness, water gradients and hydration in vivo, Br. J. Dermatol. 159 (2008) 567–577. https://doi.org/10.1111/j.1365-2133.2008.08703.x.
[78] J.M. Crowther, P.J. Matts, J.R. Kaczvinsky, Changes in stratum corneum thickness, water gradients and hydration by moisturizers, in: Treat. Dry Ski. Syndr. Art Sci. Moisturizers, Springer-Verlag Berlin Heidelberg, 2012, pp. 545–560. https://doi.org/10.1007/978-3-642-27606-4_38.
[79] S. Bielfeldt, V. Schoder, U. Ely, A. van der Pol, J. de Sterke, K.-P. Wilhelm, Assessment of human stratum corneum thickness and its barrier properties by in-vivo confocal Raman spectroscopy, Int. J. Cosmet. Sci. 31 (2009) 479–480. https://doi.org/10.1111/j.1468-2494.2009.00532_2.x.
[80] T.M. Hancewicz, C. Xiao, J. Weissman, V. Foy, S. Zhang, M. Misra, A consensus modeling approach for the determination of stratum corneum thickness using in-vivo confocal Raman spectroscopy, J. Cosmet. Dermatological Sci. Appl. 2 (2012) 241–251. https://doi.org/10.4236/jcdsa.2012.24046.
[81] A. Stella, F. Bonnier, A. Tfayli, F. Yvergnaux, H.J. Byrne, I. Chourpa, E. Munnier, C. Tauber, Raman mapping coupled to self-modelling MCR-ALS analysis to estimate active cosmetic ingredient penetration profile in skin, J. Biophot. 13 (2020). https://doi.org/10.1002/jbio.202000136.
[82] L. Franzen, J. Anderski, M. Windbergs, Quantitative detection of caffeine in human skin by confocal Raman spectroscopy - a systematic in vitro validation study, Eur. J. Pharm. Biopharm. 95 (2015) 110–116. https://doi.org/10.1016/j.ejpb.2015.03.026.
[83] C. Alonso, V. Carrer, C. Barba, L. Coderch, Caffeine delivery in porcine skin: a confocal Raman study, Arch. Dermatol. Res. 310 (2018) 657–664. https://doi.org/10.1007/s00403-018-1854-4.
[84] S. Tfaili, G. Josse, J.F. Angiboust, M. Manfait, O. Piot, Monitoring caffeine and resveratrol cutaneous permeation by confocal Raman microspectroscopy, J. Biophot. 7 (2014) 676–681. https://doi.org/10.1002/jbio.201300011.
[85] S. Tfaili, C. Gobinet, G. Josse, J.F. Angiboust, A. Baillet, M. Manfait, O. Piot, Vibrational spectroscopies for the analysis of cutaneous permeation: experimental limiting factors identified in the case of caffeine penetration, Anal. Bioanal. Chem. 405 (2013) 1325–1332. https://doi.org/10.1007/s00216-012-6512-7.
[86] Y. Liu, R. Krombholz, D.J. Lunter, Critical parameters for accurate monitoring of caffeine penetration in porcine skin using confocal Raman spectroscopy, Int. J. Pharm. 607 (2021). https://doi.org/10.1016/j.ijpharm.2021.121055.
[87] J. Bakar, R. Michael-Jubeli, R. El Khoury, S. Hamla, A. Assi, A. Baillet-Guffroy, A. Tfayli, Assessment of the skin barrier function in the reconstructed human epidermis using a multimodal approach at molecular, tissue and functional levels, Analyst 146 (2021) 4649–4658. https://doi.org/10.1039/d1an00465d.
[88] L. Miloudi, F. Bonnier, A. Tfayli, F. Yvergnaux, H.J. Byrne, I. Chourpa, E. Munnier, Confocal Raman spectroscopic imaging for in vitro monitoring of active ingredient penetration and distribution in reconstructed human epidermis model, J. Biophot. 11 (2018). https://doi.org/10.1002/jbio.201700221.
[89] M. Essendoubi, F. Alsamad, P. Noël, M. Meunier, A. Scandolera, J. Sandré, M. Manfait, C. Gobinet, R. Reynaud, O. Piot, Combining Raman imaging and MCR-ALS analysis for monitoring retinol permeation in human skin, Skin Res. Technol. 27 (2021) 1100–1109. https://doi.org/10.1111/srt.13069.
[90] M. Choimet, A. Tourrette, O. Marsan, G. Rassu, C. Drouet, Bio-inspired apatite particles limit skin penetration of drugs for dermatology applications, Acta Biomater. 111 (2020) 418–428. https://doi.org/10.1016/j.actbio.2020.05.010.
[91] L. Van Gheluwe, E. Munnier, H. Kichou, K. Kemel, F. Mahut, M. Vayer, C. Sinturel, H.J. Byrne, F. Yvergnaux, I. Chourpa, F. Bonnier, Confocal Raman spectroscopic imaging for evaluation of distribution of nano-formulated hydrophobic active cosmetic ingredients in hydrophilic films, Molecules 26 (2021) 7440. https://doi.org/10.3390/molecules26247440.
[92] R.L. Bronaugh, R.F. Stewart, Methods for in vitro percutaneous absorption studies IV: the flow-through diffusion cell, J. Pharm. Sci. 74 (1985) 64–67. https://doi.org/10.1002/jps.2600740117.
[93] E.Y.M. Bonnist, J.P. Gorce, C. MacKay, R.U. Pendlington, P.D.A. Pudney, Measuring the penetration of a skin sensitizer and its delivery vehicles simultaneously with confocal Raman spectroscopy, Skin Pharmacol. Physiol. 24 (2011) 274–283. https://doi.org/10.1159/000328729.
[94] R.U. Pendlington, H.J. Minter, L. Stupart, C. MacKay, C.S. Roper, D.J. Sanders, C.K. Pease, Development of a modified in vitro skin absorption method to study the epidermal/dermal disposition of a contact allergen in human skin, Cutan. Ocul. Toxicol. 27 (2008) 283–294. https://doi.org/10.1080/15569520802327005.
[95] D. Lunter, R. Daniels, Confocal Raman microscopic investigation of the effectiveness of penetration enhancers for procaine delivery to the skin, J. Biomed. Opt. 19 (2014), 126015. https://doi.org/10.1117/1.jbo.19.12.126015.
[96] E. Touitou, V.M. Meidan, E. Horwitz, Methods for quantitative determination of drug localized in the skin, J. Contr. Release 56 (1998) 7–21. https://doi.org/10.1016/S0168-3659(98)00060-1.
[97] R. Krombholz, D. Lunter, A new method for in-situ skin penetration aAnalysis by confocal Raman microscopy, Molecules 25 (2020). https://doi.org/10.3390/molecules25184222.
[98] J. Ruan, C. Liu, H. Song, T. Zhong, P. Quan, L. Fang, Sustainable and efficient skin absorption behaviour of transdermal drug: the effect of the release kinetics of permeation enhancer, Int. J. Pharm. 612 (2022), 121377. https://doi.org/10.1016/j.ijpharm.2021.121377.
[99] M. Sigg, R. Daniels, Impact of alkanediols on stratum corneum lipids and triamcinolone acetonide skin penetration, Pharmaceutics 13 (2021). https://doi.org/10.3390/pharmaceutics13091451.
[100] Y. Liu, D.J. Lunter, Profiling skin penetration using PEGylated emulsifiers as penetration enhancers via confocal Raman spectroscopy and fluorescence spectroscopy, Eur. J. Pharm. Biopharm. 166 (2021) 1–9. https://doi.org/10.1016/j.ejpb.2021.04.027.
[101] L. Chrit, P. Bastien, G.D. Sockalingum, D. Batisse, F. Leroy, M. Manfait, C. Hadjur, An in vivo randomized study of human skin moisturization by a new confocal Raman fiber-optic microprobe: assessment of a glycerol-based hydration cream, Skin Pharmacol. Physiol. 19 (2006) 207–215. https://doi.org/10.1159/000093116.
[102] L. Chrit, P. Bastien, B. Biatry, J.T. Simonnet, A. Potter, A.M. Minondo, F. Flament, R. Bazin, G.D. Sockalingum, F. Leroy, M. Manfait, C. Hadjur, In vitro and in vivo confocal Raman study of human skin hydration: assessment of a new moisturizing agent pMPC, Biopolymers 85 (2007) 359–369. https://doi.org/10.1002/bip.20644.
[103] V.K. Tippavajhala, T.D. Magrini, D.C. Matsuo, M.G.P. Silva, P.P. Favero, L.R. De Paula, A.A. Martin, In vivo determination of moisturizers efficacy on human skin hydration by confocal Raman spectroscopy, AAPS PharmSciTech 19 (2018) 3177–3186. https://doi.org/10.1208/s12249-018-1143-8.
[104] P.J. Caspers, A.C. Williams, E.A. Carter, H.G.M. Edwards, B.W. Barry, H.A. Bruining, G.J. Puppels, Monitoring the penetration enhancer dimethyl sulfoxide in human stratum corneum in vivo by confocal Raman spectroscopy, Pharm. Res. 19 (2002) 1577–1580. https://doi.org/10.1023/A:1020481305420.
[105] C. Xiao, D.J. Moore, M.E. Rerek, C.R. Flach, R. Mendelsohn, Feasibility of tracking phospholipid permeation into skin using infrared and Raman microscopic imaging, J. Invest. Dermatol. 124 (2005) 622–632. https://doi.org/10.1111/j.0022-202X.2004.23608.x.
[106] V. Wascotte, P. Caspers, J. De Sterke, M. Jadoul, R.H. Guy, V. Préat, Assessment of the “skin reservoir” of urea by confocal Raman microspectroscopy and reverse iontophoresis in vivo, Pharm. Res. 24 (2007) 1897–1901. https://doi.org/10.1007/s11095-007-9314-4.
[107] P.D.A. Pudney, M. Mélot, P.J. Caspers, A. Van Der Pol, G.J. Puppels, An in vivo confocal Raman study of the delivery of trans-retinol to the skin, Appl. Spectrosc. 61 (2007) 804–811. https://doi.org/10.1366/000370207781540042.
[108] M. Mélot, P.D.A. Pudney, A.M. Williamson, P.J. Caspers, A. Van Der Pol, G.J. Puppels, Studying the effectiveness of penetration enhancers to deliver retinol through the stratum cornum by in vivo confocal Raman spectroscopy, J. Contr. Release 138 (2009) 32–39. https://doi.org/10.1016/j.jconrel.2009.04.023.
[109] R. Mateus, H. Abdalghafor, G. Oliveira, J. Hadgraft, M.E. Lane, A new paradigm in dermatopharmacokinetics-Confocal Raman spectroscopy, Int. J. Pharm. 444 (2013) 106–108. https://doi.org/10.1016/j.ijpharm.2013.01.036.
[110] P.J. Caspers, C. Nico, T.C. Bakker Schut, J. Sterke, P.D.A. Pudney, P.R. Curto, A. Illand, G.J. Puppels, Method to quantify the in vivo skin penetration of topically applied materials based on confocal Raman spectroscopy, Transl. Biophotonics 1 (2019). https://doi.org/10.1002/tbio.201900004.
[111] F. Iliopoulos, M.E. Lane, P.J. Caspers, G.J. Puppels, Franz cell diffusion testing and quantitative confocal Raman spectroscopy: in vitro-in vivo correlation, Pharmaceutics 12 (2020) 1–12. https://doi.org/10.3390/pharmaceutics12090887.
[112] A. Patzelt, J. Lademann, Recent advances in follicular drug delivery of nanoparticles, Expet Opin. Drug Deliv. 17 (2020) 49–60. https://doi.org/10.1080/17425247.2020.1700226.
[113] K. Ogurtsova, J.D. da Rocha Fernandes, Y. Huang, U. Linnenkamp, L. Guariguata, N.H. Cho, D. Cavan, J.E. Shaw, L.E. Makaroff, IDF Diabetes Atlas: global estimates for the prevalence of diabetes for 2015 and 2040, Diabetes Res. Clin. Pract. 128 (2017) 40–50. https://doi.org/10.1016/j.diabres.2017.03.024.
[114] D.R. Parachalil, C. Bruno, F. Bonnier, H. Blasco, I. Chourpa, M.J. Baker, J. McIntyre, H.J. Byrne, Analysis of bodily fluids using vibrational spectroscopy: a direct comparison of Raman scattering and infrared absorption techniques for the case of glucose in blood serum, Analyst 144 (2019) 3334–3346. https://doi.org/10.1039/c9an00125e.
[115] D.A. Stuart, C.R. Yonzon, X. Zhang, O. Lyandres, N.C. Shah, M.R. Glucksberg,

J.T. Walsh, R.P. Van Duyne, Glucose sensing using near-infrared surface-enhanced Raman spectroscopy: gold surfaces, 10-day stability, and improved accuracy, Anal. Chem. 77 (2005) 4013–4019. https://doi.org/10.1021/ac0501238.
[116] D.A. Stuart, J.M. Yuen, N. Shah, O. Lyandres, C.R. Yonzon, M.R. Glucksberg, J.T. Walsh, R.P. Van Duyne, In vivo glucose measurement by surface-enhanced Raman spectroscopy, Anal. Chem. 78 (2006) 7211–7215. https://doi.org/10.1021/ac061238u.
[117] K. Ma, J.M. Yuen, N.C. Shah, J.T. Walsh, M.R. Glucksberg, R.P. Van Duyne, In vivo, transcutaneous glucose sensing using surface-enhanced spatially offset Raman spectroscopy: multiple rats, improved hypoglycemic accuracy, low incident power, and continuous monitoring for greater than 17 days, Anal. Chem. 83 (2011) 9146–9152. https://doi.org/10.1021/ac202343e.
[118] R. Pandey, S.K. Paidi, T.A. Valdez, C. Zhang, N. Spegazzini, R.R. Dasari, I. Barman, Noninvasive monitoring of blood glucose with Raman spectroscopy, Acc. Chem. Res. 50 (2017) 264–272. https://doi.org/10.1021/acs.accounts.6b00472.
[119] J.W. Kang, Y.S. Park, H. Chang, W. Lee, S.P. Singh, W. Choi, L.H. Galindo, R.R. Dasari, S.H. Nam, J. Park, P.T.C. So, Direct observation of glucose fingerprint using in vivo Raman spectroscopy, Sci. Adv. 6 (2020). https://doi.org/10.1126/sciadv.aay5206.
[120] R.R. Anderson, J.A. Parrish, The optics of human skin, J. Invest. Dermatol. 77 (1981) 13–19. https://doi.org/10.1111/1523-1747.ep12479191.
[121] N.J. Everall, Confocal Raman microscopy: why the depth resolution and spatial accuracy can be much worse than you think, Appl. Spectrosc. 54 (2000) 1515–1520. https://doi.org/10.1366/0003702001948439.
[122] N.J. Everall, Modeling and measuring the effect of refraction on the depth resolution of confocal Raman microscopy, Appl. Spectrosc. 54 (2000) 773–782. https://doi.org/10.1366/0003702001950382.
[123] K.J. Baldwin, D.N. Batchelder, Confocal Raman microspectroscopy through a planar interface, Appl. Spectrosc. 55 (2001) 517–524. https://doi.org/10.1366/0003702011952190.
[124] J.L. Bruneel, J.C. Lassègues, C. Sourisseau, In-depth analyses by confocal Raman microspectrometry: experimental features and modeling of the refraction effects, J. Raman Spectrosc. 33 (2002) 815–828. https://doi.org/10.1002/jrs.915.
[125] FDA, FY2015 Regulatory Science Research Report: Physiologically-Based Absorption and Pharmacokinetic Models, 2015.
[126] Y. Dancik, M.A. Miller, J. Jaworska, G.B. Kasting, Design and performance of a spreadsheet-based model for estimating bioavailability of chemicals from dermal exposure, Adv. Drug Deliv. Rev. 65 (2013) 221–236. https://doi.org/10.1016/j.addr.2012.01.006.
[127] L. Chen, L. Han, O. Saib, G. Lian, In silico prediction of percutaneous absorption and disposition kinetics of chemicals, Pharm. Res. 32 (2015) 1779–1793. https://doi.org/10.1007/s11095-014-1575-0.
[128] P. Kattou, G. Lian, S. Glavin, I. Sorrell, T. Chen, Development of a two-dimensional model for predicting transdermal permeation with the follicular pathway: demonstration with a caffeine study, Pharm. Res. 34 (2017) 2036–2048. https://doi.org/10.1007/s11095-017-2209-0.
[129] G. Smith, European medicines agency guideline on bioanalytical method validation: what more is there to say? Bioanalysis 4 (2012) 865–868. https://doi.org/10.4155/bio.12.44.
[130] Y.G. Anissimov, A. Watkinson, Modelling skin penetration using the laplace transform technique, Skin Pharmacol. Physiol. 26 (2013) 286–294. https://doi.org/10.1159/000351924.
[131] L. Simon, A. Goyal, Dynamics and control of percutaneous drug absorption in the presence of epidermal turnover, J. Pharm. Sci. 98 (2009) 187–204. https://doi.org/10.1002/jps.21408.
[132] R.H. Guy, J. Hadgraft, Percutaneous absorption kinetics of topically applied agents liable to surface loss, J. Soc. Cosmet. Chem. Japan. 35 (1984) 103–113.
[133] K.D. Mccarley, A.L. Bunge, Pharmacokinetic models of dermal absorption, J. Pharm. Sci. 90 (2001) 1699–1719. https://doi.org/10.1002/jps.1120.
[134] FDA, Regulatory Science Research Report, Narrow therapeutic index drugs. https://www.fda.gov/industry/generic-drug-user-fee-amendments/fy2015-regulatory-science-research-report-narrow-therapeutic-index-drugs, 2015.
[135] L. Binder, C. Valenta, D. Lunter, Determination of skin penetration profiles by confocal Raman microspectroscopy: evaluation of interindividual variability and interlab comparability, J. Raman Spectrosc. 51 (2020) 1037–1043. https://doi.org/10.1002/jrs.5871.